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Review

Theoretical aspects of water-holding in meat ☆

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ABSTRACT

As myofibrils consist of a three-dimensional network of long, solid protein particles with the shortest dimension of less than 20 nm, the theoretical foundations of water-holding in meat should be studied from a colloid or surface chemistry point of view. The classical hypotheses for water-holding in meat are based on electrostatic forces or osmotic forces, which cause the swelling of the myofibrils. The more recent research adds to those the structure of water, whether it is low density water induced by kosmotropic effects dominating in the system, or high density water induced by chaotropes, respectively. The phenomena in the one to three molecules thick water layers on protein surfaces do not, however, explain the bulk water-holding. The interactions of ions and non-polar kosmotropes with water and proteins have a relevant effect on water-holding. The chaotropic/kosmotropic effects of different ions will be of importance especially when reducing sodium contents in meat-based foods.

Rough estimates of the surface areas of different constituents of the myofibrils showed that transverse elements have larger contact surfaces with the liquid phase than longitudinal. Therefore, more attention should be paid to heavy meromyosin, Z-line and other elements of molecular size or colloidal size. Short range surface forces seem to dominate theories of water–protein interactions, and the theoretical foundations of bulk water-holding are still lacking. Irrespective of the lack of theoretical explanation on the mechanism of water-holding in meat, the meat industry is able to control the macroscopic behaviour of meat-based ingredients rather well.

Contents

☆ Dedicated to Professor Fritz P. Niinivaara (1919–2010).

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Nomenclature

Chaotrope Water-structure breaker
Colloidal system Two-phase heterogeneous system consisting of a dispersed phase (colloid; size between 1 nm and 1000 nm) and dispersion medium
DLVO theory Theory of the stability of lyophobic colloids (named after Derjaguin, Landau, Verwey and Overbeek)
HD Water high density water
Hydrophilic Water-preferring
Hydrophobic Water-rejecting
Kosmotrope Water-structure maker
LD water Low density water
Lyophobic Solvent rejecting
Water-holding The ability of a particle hold own or added water against a force or an effect

1. Introduction

Water is most important as a natural or added constituent of almost all foods. Meat science has always been most interested in practical applications and macroscopic effects of internal/external factors in relation to water-holding. The research has been motivated by technological and sensory aspects, both finally linked to economical benefits. A theoretical approach has not been particularly common.

Meat structure is very complex. The myofibrillar protein system has developed to perform very fast and highly specific repetitive movements. Water is in the muscle fibre as a lubricant, as well as a medium to transport metabolites in the fibre. The water content should be rather constant for well organized and rapid functions, but on the other hand, water must be able to move to other places in the sarcomere during contraction, within a fraction of a millisecond (Lampinen & Noponen, 2005). Hydrophilic and hydrophobic areas in the proteins must be well organized to allow rapid translocations, as well as structural elements should provide optimal filament distances at each level of contraction. Therefore, one should not expect the myofibrillar protein system to follow only one simple model of water-holding.

This review deals with the basic theories of water-holding in meat. The number of original research papers dealing with different practical or technological aspects of water-holding *capacity* in meat numbers thousands, and thus, are not discussed here. These aspects have been thoroughly discussed in several previous reviews over the years (e.g. Chen & Sun, 2008; Hamm, 1972; Huff-Lonergan, 2009; Huff-Lonergan & Lonergan, 2005; Fennema, 1990; Offer & Knight, 1988; Ruusunen & Puolanne, 2005). This review will focus on phenomena at the myofibrillar and myofilamental level. The review does not stress whether the system is fresh or cooked, salted etc., as it is anticipated that the fundamental aspects are the same, irrespective of circumstances. Very thorough and extensive theoretical discussions on mechanisms of water-holding in meat have been presented by Hamm (1972) and Offer and Knight (1988) in particular, and also by Offer and Trinick (1983), Hermansson (1986) and Millman (1998). Basic research has also been done by Bertram, Purslow, and Andersen (2002) using NMR on how and why water is divided into different compartments and its motility in muscle. These contributions are very valid and still useful today.

2. Current hypotheses on water-binding in meat

While the theoretical approach in chemistry and biology concentrates on surface phenomena between proteins and the first one to three layers of water, meat scientists have been more interested in the holding of bulk water in the filamental lattice, whole muscle and gels. Consequently, these two approaches will be treated separately here, and unfortunately, we will not be able to provide an inclusive theory to cover the entire water-holding phenomenon in meat. The following hypotheses presented within meat science have been widely known for decades.

2.1. Electrostatic forces

In 1972, Hamm presented his extensive book *Kolloidchemie des Fleisches* on water-binding, summarizing his research and that of his collaborators, and discussing the literature published on the field up to August 1970. He concluded that water-holding is caused by electrostatic repulsion between the myofibrillar proteins (myofilaments), which results in a swelling of myofibrils, or in some cases (e.g. with salts or at very low or high pH) even a partial solubilisation of filaments, the latter being due to the repulsions between individual molecules. The various cross-bridges (Z-lines, actomyosin cross-bridges and intermediate filaments) between the myofilaments, prohibit the unlimited swelling of the myofibrils. Polar groups of the side chains of the amino acids (ca. 76–80%; Hamm, 1972) bind water molecules on their surfaces by van der Waals forces. The water molecules, being polar, then orientate themselves, so that in the case of a negative ionic group, the positive part (hydrogen atoms) will orientate towards the ionic group and the negative part (oxygen) will point to the solution, and vice versa with positive groups. Additional water molecule layers will be formed on this, so-called, monomolecular layer with similar orientations. Hamm (1972) also discusses the Donnan equilibrium and osmotic effects in muscle and in gels, but concludes that it is relevant only in a living muscle and that the electrostatic effects dominate in meat. Thus, Hamm (1972) claims that all the water molecules are more or less influenced by the pulling forces caused by the polar groups of the proteins in a three-dimensional network.

Contrary to polar side chains, non-polar side chains of amino acids push the polar water molecules causing an arched-like structure around the non-polar group. The combined effect is that water molecules are pulled (polar groups) and pushed (non-polar groups) between the filaments creating tension which forces the water molecules to adopt an ice-like form in the protein network of filaments and transverse elements. This idea, originating from Ling (1965), is an early thought of the kosmotropic effect (see Section 6). The major

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factors inhibiting unlimited swelling of myofibrils are the actomyosin cross-bridges between the filaments and Z-lines; on the fibre level also other structural elements, like costameres, have an effect (Clark, McElhinny, Beckerle, & Gregorio, 2002). According to Hamm (1972) the amount of the water bound is determined by the net charge of the proteins causing a repulsion that increases the binding, and by the number and strength of cross-bridges that limit the binding.

This explanation sounds reasonable, as the distances between the filament surfaces are about 15–20 nm (actin to myosin and actin to actin) or 25–30 nm (myosin to myosin), or less, which translates to layer thicknesses of about 60 to 90 water molecules. The hypothesis explains the effects of salt content and pH, as well as the role of cross-bridges and consequently how water is retained in meat. However, the hypothesis does not take into account the effect of counter-ions, e.g. sodium ions, in case of the selective binding of chloride ions that increase the negative net charge (see next paragraph). In addition, Offer and Knight (1988) also claim that the distances between the filaments are too long to establish a repulsive force that would be strong enough to generate water-binding.

Hamm (1972) postulated that the effect of sodium chloride on meat proteins is most probably caused by the fact that the chloride ion is more strongly bound to the proteins than the sodium ion. This causes an increase in the negative charges of proteins. The effect of NaCl on different proteins in meat is very complex, and this complexity increases if different concentrations of added NaCl, KCl and phosphates act simultaneously (Hamm, 1972; Offer & Knight, 1988. See also Section 6). Concisely, the solubility of myosin increases as the NaCl concentration increases from 0.04 to 0.5 M. After the initial aggregation and formation of filaments, the structures start to dissociate at salt contents higher than 0.25 M. The swelling of myofibrils begins at 0.5 M, without added phosphates, and at 0.4 M, with added phosphates, where an extensive extraction of myosin also occurs (Offer & Knight, 1988).

The degree of swelling depends on pH which is causing changes in the net charge of the protein network (Hamm, 1972; Millman, 1998; Offer & Knight, 1988). Without salt, swelling has a maximum at pH 3.0, a minimum at pH 5.0 (the average isoelectric point of meat proteins) and from there a constant increase within the physiological pH range of 6.4–7.2 (Hamm, 1972). Due to the selective binding of ions, salts move the isoelectric point. At 2% NaCl the isoelectric point and swelling minimum are at pH 4.0 (Hamm, 1972). Wilding, Hedges, and Lillford (1986) found that hypertonic salt solutions (KCl and KI) induce fibre shrinkage at pHs below the isoelectric point of myofibrillar proteins (pH 5.0), which means that these salts also lower the isoelectric point. With NaCl there is a maximum in swelling (Hamm, 1972) as well as in heated gel strength at pH 6.0 (Ishioroshi, Samejima, & Yasui, 1979) or at pH 6.2 (Puolanne, Ruusunen, & Vainionpää, 2001). This has been believed to be due to the increased sodium ion binding to the negatively charged myofilaments, and the simultaneous weakening of the binding of chloride ions because of electrostatic forces. Hamm (1972) also stresses the relationship of Hofmeister series with water-holding.

Conclusively, it can be said that the above hypothesis is well in line with the more recent explanations given below.

2.2. Osmotic forces

Offer and Knight (1988) reviewed the literature up to the late 1980s and brought a physicochemical approach and structural aspects into focus. They also like Hamm (1972) supported strongly the electrostatic swelling mechanism, but stressed particularly the swelling of filamental lattice in myofibrils. They suggested an alternative hypothesis also based on the selective binding of chloride ions to the myofibrillar proteins. According to Offer and Knight (1988), the selective binding does not cause a marked repulsion between the filaments but between the molecules of myosin filaments breaking down the shaft of the filament. This effect will cause a loosening of myofibrillar lattice. If phosphate is not used, the S1 (Fig. 1) units of heavy meromyosin would still be attached to actin filaments. Offer's collaborators Knight and Parsons (1988) postulate that the swelling occurs by an entropic mechanism driven by the free light meromyosin (LMM) parts bound to actin filaments.

Offer and Knight (1988) also present another aspect that is close to the hypothesis of Hamm (1972). They start with the same selective binding of chloride ions to myosin filaments as Hamm, but because the structural proteins are solid in meat and cannot move, electrical forces pull the counter-ions (sodium ions) very close to the filament surfaces thus creating an uneven distribution of ions in the water phase. The concentration differences establish an osmosis-like force (pressure) within the filament lattice, which in turn pulls water molecules into the system. The pressure would cause unlimited swelling, but again, the cross-bridges cause an opposite force, that Offer and Knight call 'elastic pressure'. In any case, osmotic pressure created by the uneven distribution of ions and the elastic pressure are equal at any moment. According to Offer and Knight (1988), the osmotic pressure π is

$$\pi = \frac{RT\left({}^{1}/_{2}-\chi_1\right)}{v_1 q^2} \tag{1}$$

elastic pressure P is

$$P = \frac{RT\left(\frac{v_e}{V_0}\right)}{q^{\frac{1}{3}}} \tag{2}$$

and as $\pi = P$, the degree of swelling q_m is approximately

$$q_m^{\frac{5}{3}} = \frac{V_0}{v_e v_1}\left({}^{1}/_{2}-\chi_1\right) \tag{3}$$

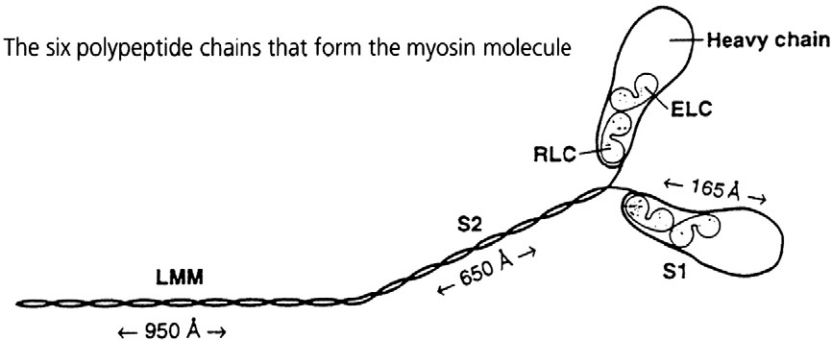

Fig. 1. Myosin molecule (Rayment & Holden, 1994).

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where χ_1 is the Flory–Huggins interaction constant of polymer with a solvent ($\chi_1 < ½$ for a good solvent and $\chi_1 > ½$ for a poor solvent) (Flory, 1941), R is the gas constant, T is the absolute temperature, q is the amount of swelling, ν_1 is the specific volume of the solvent, and ν_e is the number of moles of cross-linked units in the unswollen volume V_0. It can be seen that the only relevant variables are the interaction constant χ_1 and the density of cross-bridges ν_e. These variables also explain the effects of salt content, cross-bridges, pH and the denaturation effects with the resulting shortening of myosin S1–S2 complex. Increasing the osmotic force (interaction between proteins and solution) and eliminating the elastic pressure by disrupting or decreasing the strength of cross-bridges will lead to an increased water-binding, and vice versa. Similar aspects, but using different terminologies, are seen in Hamm's hypothesis. The main difference is that Offer and collaborators stress the structural aspects, while Hamm (1972) concentrates on the electrostatic forces.

2.3. Capillary forces

Offer and Trinick (1983), and based on their findings, Hermansson (1986), suggested the hypothesis of capillary forces. Offer and Trinick conclude that surface tension forces in a capillary with a diameter equal to the interfilamental spacing would support a water column of 300 m in height, and therefore it seems reasonable to suppose that water is held in meat by capillarity, the majority of that being in the interfilamental spaces within the myofibrils, but a substantial part in the extracellular space and spaces between myofibrils. Interestingly enough, Offer does not return to the conclusion in his later papers (except in relation to drip formation; Offer and Knight, 1988), but suggests the osmotic hypothesis with the interaction constant χ of protein polymer and solution (see Section 2.2. above). Actually, these hypotheses are closely related.

The equation of capillary force is

$$h = \frac{2\gamma\cos\theta}{r\Delta\rho g} \quad (4)$$

where h is the height to which liquid is drawn, γ is the surface tension, is the contact angle, r is the capillary radius, $\Delta\rho$ is the density of liquid-density of vapour and g is the strength of the gravitational field (Hermansson, 1986).

The capillary force seems to require a gas/solution interface and a vector along the gravitational field (g), but these are not necessarily relevant. Under normal circumstances, air is a strong hydrophobic element in the system as it contains only non-polar molecules, but there are also hydrophobic and hydrophilic areas on the surfaces of the meat proteins (Hamm, 1972). Although Offer and Trinick (1983) and Hermansson (1986) supported by Tornberg (2005) strongly stressed the importance of capillarity in water-holding, the actual proving of the hypothesis has not been done. The radius of the capillaries will decrease *post-mortem*, which, according to Eq. (4), should lead to a higher capillary force and consequently to an increase in water-holding capacity, however, in fact, as the filamental lattice shrinks, the water-holding capacity decreases (Offer & Knight, 1988). This stresses the structural aspects of filamental lattice in water-holding, but a change in surface tension can be involved. It is also difficult to define the actual pore radius in a network of protein filaments. The protein/solution interactions may be more relevant than small changes in pore radius, as the former may be related with, for example, pH or salt.

When studying the photos in the paper of Offer and Trinick (1983) regarding the swelling of myofibrils totally embedded in salt solutions, it is difficult to relate the mechanisms of water-holding, or its changes, to capillary forces. Hamm (1972) claimed that at low humidity, capillary effects may be relevant, but at higher humidities (when there is high moisture in meat) the effect may not be relevant anymore. It is clear that structural aspects, like actomyosin cross-bridges or denaturation (which usually lead to a shortening of filaments or cross-bridges), also have an influence on capillarity, which Offer and Trinick also stress. The free energy aspects related to protein surface/solution, solution to solution, protein surface/air and solution/air may have a contribution, and capillary forces, as calculated classically by the height of the water column it would support, may not be that relevant. The validity of the capillary force hypothesis is has yet to be investigated.

3. Basic hypotheses on water in biological systems

In a review, Fennema (1999) presented a description of the properties of water. The water molecule itself is very unique. The diameter of a non-hydrogen-bonded water molecule is about 0.32–0.33 nm, while that of hydrogen-bonded water is 0.27–0.28 nm (Fennema, 1999; Graziano, 2004). The water molecule is V-shaped. The electronegative oxygen atom pulls the electrons from the covalent bond between the oxygen atom and the hydrogen atoms leaving each hydrogen atom with a partial positive charge, and the oxygen atom with a partial negative charge. Therefore, water molecules have a small size, a high dipole moment, an ability to form hydrogen bonds and a very high dielectric constant.

Collins and Washabaugh (1985) introduced an extensive review on the Hofmeister effect and the behaviour of water at interfaces. These principles can be applied to protein network, i.e. protein–water interactions. They conclude that water near an isolated interface is divided into three monomolecular layers. They proposed that the solute determines the behaviour of the first interfacial layer (I_1), the bulk solution the behaviour of the third interfacial layer (I_3), and that I_1 and I_3 compete for hydrogen-bonding interactions with the intervening water layer (I_2, transition layer). Polar kosmotropes (water-structure makers, see Section 7) interact with I_1 more strongly than bulk water would in its place; chaotropes (water-structure breakers) interact with I_1 less strongly, respectively; and non-polar kosmotropes (water-structure makers) interact with I_1 much less strongly than bulk water would in its place. They introduced two postulates: (i) a water molecule will favour its strongest hydrogen-bonding interactions and will be less favourable with the other with which it cannot simultaneously maximise the interaction; (ii) strong kosmotrope–water interaction will transfer charge from polar kosmotropes to water. These aspects will result in not only tight binding (partial immobilization) of I_1 water molecules but also an attempt to distribute the charge transferred from the solute with several molecules. With cumulative charge transfer, the solvation layer can become up to 5 to 6 molecules thick. This approach closely resembles the theory of Ling (1965), adopted and further developed by Hamm (1972).

In a fundamental book on water in biological systems (Roos, Leslie & Lillford (Edit.), Water Management in the Design and Distribution of Quality Foods), Wennerström (1999) has thoroughly reviewed the fundamental mechanisms of water-holding in biological and colloidal (i.e., at least one of the three dimensions being between 1 and 1000 nm; Weast & Astle, 1981) systems. The complexity of biological systems is very well-known, but the foundations of water-holding must, however, be presented using more simplistic models. Wennerström (1999) suggests the following.

3.1. Surface force

Surface force, or disjoining pressure, is a force which is needed to keep two bodies at a fixed separation. The force is proportional to the area of the surfaces and expressed as a force per unit area, like pressure. It was suggested that the force will be treated thermodynamically as a derivative of free energy which can be divided into two contributions, enthalpy and entropy. It is also possible to relate the

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force not only between two planes but also between curved surfaces. This would then apply to e.g. myofilaments.

3.2. Electric double layer force

There will always be repulsion between two surfaces having similar net charges. The repulsion causes an uneven distribution of counter-ions in the bulk water between the surfaces, resulting in an osmotic pressure in the bulk water. The theoretical background goes back to the Poisson–Boltzmann equation (Chapman, 1913; Gouy, 1910) and the Debye-Hückel theory (Debye, 1929; Debye & Hückel, 1923). The mechanism behind the repulsion is that when the two surfaces approach, the counter-ions in the bulk water will have less available space causing a decrease in entropy and thus creating a repulsive force. Wennerstöm also stresses that the ion–ion interaction tends to a lower internal energy as the two surfaces approach. In colloidal systems (myofilaments are of colloidal size), the stability is built from competition between the attractive van der Waals forces promoting association and the repulsive double layer force. They have been incorporated into the so-called DLVO theory (see the list of acronyms), but not so far as to accurately describe the behaviour of biological substances at physiological or higher salt concentrations (Boström, Williams, & Ninham, 2001). However, we think that these aspects could be of importance when discussing the behaviour of the filamental lattice in relation to technological factors like pH, salts, heat etc.

3.3. Hydrophobic interaction

Hydrophobic interactions have a fundamental role in molecular organisation in aqueous systems. The molecular basis for hydrophobic interaction is the strong cohesion of bulk water molecules causing the expulsion of hydrophobic groups from water. The range of this effect is still under debate. However, when two hydrophobic surfaces (e.g. hydrophobic areas of proteins or oils) from large separation come into contact, there will be a substantial decrease in free energy. In conclusion, the hydrophobic effects on water-holding are indirect, as given by Hamm (1972), and later, e.g. by Collins and Washabaugh (1985), concerning the effects of water-structure.

3.4. Repulsive entropic forces

The free energy of a system ΔG is determined by enthalpy ΔH, entropy ΔS and temperature T (Eq. (5)):

$$\Delta G = \Delta H - T\Delta S \tag{5}$$

In a spontaneous reaction, the free energy tends to decrease ($\Delta G<0$) and entropy increase ($\Delta S>0$). Wennerström (1999) postulates that when two similar surfaces approach and the surface molecules start to lose their free energy due to the decrease of the number of allowed configurations, the consequent decrease of entropy will always cause a repulsive interaction. There is typically a compensating decrease in energy due to the increased monomer–monomer contacts, but in a good solvent (in terms of the surface molecules rendering their contacts minimal) the entropic term will win out over enthalpy. We see here an analogy with myosin and actin filaments. The myofilaments will therefore not approach, and e.g. the molecules in myosin filament tend to disintegrate in salt solution (a good solvent) as a result of entropic force. Offer and Knight (1988), Millman and Nickel (1980) and Knight and Parsons (1988) have also discussed the effects of the configurational entropy and electrostatic forces in relation to the swelling of myofibrils following the swelling of myosin filaments.

4. Charge distribution in filaments

To understand the basic mechanisms of water and ion binding of the myofibrillar system, it is necessary to go deeper into the details of its structure. Thus a short description of actin and myosin molecules/filaments as well as some other relevant proteins is presented.

4.1. Myosin molecule/thick filament

The myosin molecule found in striated muscles belongs to type II of the myosin family of the 24 different myosin classes found so far (Foth, Goedecke, & Soldati, 2006). Type II myosin (conventional myosin) is the first of the myosin family ever discovered and the only one which forms bipolar filaments. It contains 4500 amino acid residues and has a molecular weight of about 500 kD. It has a head and a neck (heavy meromyosin) as well as a tail (light meromyosin) (Fig. 1). The tail (125–150 kD) consists of two polypeptide chains, and is an α-helical coiled coil. It contains 1094 amino acids and has a regular 28 residue charge pattern: many aliphatic, apolar residues (leucine, $-CH_2CH(CH_3)_2$, valine, $-CH(CH_3)_2$, alanine, $-CH_3$), some negatively charged amino acids and quite a few positively charged side chains (arginine ($-(CH_2)_3NHC(NH_2)NH_3^+$) and lysine ($-(CH_2)_4NH_3^+$)) (McLachlan & Karn, 1982, 1983). The tail has a high density of charged amino acids (both positive and negative side chains) clustered on the outer surface and contains most of the net charge of the myosin molecule (Miroshnichenko, Balanuk, & Nozdrenko, 2000). At about pH 6.5 it has a high negative charge which remains negative at pH 5.5 (Offer & Knight, 1988). It has dimensions of 2 nm × 160 nm.

One myosin molecule possesses two 'heads' (Fig. 2). The 'head' or 'motor domain' (S1) (95–110 kD) can be divided to 3 subregions: 1) 25 kD NH_2-terminal (N-terminal) subregion, which contains the ATP binding region also called the ATPase pocket/nucleotide binding pocket /catalytic site; 2) 50 kD central section which consists of a lower 50 kD region containing the actin binding region/cleft and an upper 50 kD region, and 3) 20 kD section (converter domain).

The structure of S1 has been determined by x-ray diffraction, and it can be obtained as a pdb-file (Protein Data Bank) from protein libraries (Rayment et al., 1993). The polarity of S1 is pH dependent. At about pH 6.5 it has been claimed to possess no net charge, while at approx. pH 5.5 it has a weak positive charge (Offer & Knight, 1988). S1 has the dimensions of 17 × 7 × 4 nm. The distance between the ATP binding region and the actin binding region is about 5–6 nm (Dos Remedios, Miki, & Barden, 1987). In actin binding, the closing of the actin binding cleft is structurally coupled to the opening of the nucleotide binding cleft (Holmes, Angert, Kull, Jahn, & Schröder, 2003).

The myosin filament (thick filament) is bipolar, and it contains about 200–300 myosin molecules that are in the centre of sarcomere (Fig. 3). It is a right-handed 3-stranded helix that has a repeat of 42.9 nm (Squire, 2009). The dimensions are 16 × 1600 nm, and there is a 150−200 nm long bare zone in the middle that contains no myosin heads. The non-polar amino acid side groups are orientated inwards and the polar groups outwards with respect to the bulk water. Various models for the packing of thick filaments have been suggested (Chew & Squire, 1995; Craig & Woodhead, 2006; Miroshnichenko et al., 2000; Skubiszak & Kowalczyk, 2002; Squire, 2009; Squire et al., 1998). It has been proposed that the interactions between the tails are mainly ionic (electrostatic) due to the highly regular charge pattern of the myosin molecule tail (Miroshnichenko et al., 2000). Thus, it has been suggested that myosin filament is held together largely by electrostatic forces (McLachlan & Karn, 1982). However, according to a contrary view, hydrophobic interactions are dominant (Goodman, 2008). The core of the filament is most probably hollow with a radius of about 2 nm (Squire et al., 1998).

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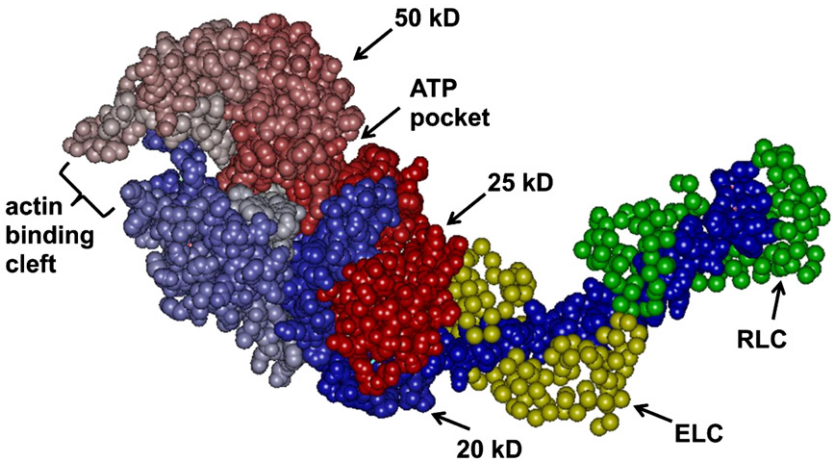

Fig. 2. S1 unit of a myosin molecule (Rayment et al., 1993).

Unfortunately, the detailed structure and packing of the filament remain unknown.

The myosin filament is bound by three proteins (C, M and H). Both C-(~140 kD) and H-proteins (~58 kD) appear as striated bands which are 43 nm apart at the long axis of the thick filament. The molecular weight of M-protein is about 165 kD.

4.2. Actin molecule/thin filament

The actin molecule (G-actin) contains 375 amino acids, and it has a molecular weight of 41—42 kD (Fig. 4). It is formed of two 5-stranded β-discs, and it can be divided into two domains, a small and a large one. The small domain consists of two subdomains. Subdomain 1 has a net negative charge: it contains 11 negatively charged residues (aspartic acid, $-CH_2COO^-$, glutamic acid, $-CH_2CH_2COO^-$) and only one positively charged residue (lysine) (Kinoshita & Suzuki, 2009). It is involved in the binding of the myosin heads. Subdomain 2, on the other hand, has many positive charges (Suzuki et al., 2004). The large domain consists of subdomains 3 and 4. G-actin has 21% negatively charged and 12% positively charged amino acids, so the surface polarity is negative (Wiggins, 1990). There is a cleft (pocket) between the two main domains, which contains the nucleotide (ATP) binding site (between subdomains 1 and 3), as well as a two-valent ion binding site. The four domains are held together by a nucleotide (ATP/ADP) and salt bridges. G-actin has dimensions of ~5.5 nm × 5.5 nm × 3.5 nm (Wong et al., 2000).

Actin is the main component of thin filaments (Fig. 5). The actin filament (F-actin) contains about 400 actin molecules, and it consists of two twisted super-helices. Its polarity has been determined from actin filaments bound to myosin-S1 fragments, which have an arrowhead appearance in electron micrographs. The pointed end has a negative charge, and it interdigitates with the thick filaments in the A-band. The barbed end has a positive charge, and it is towards the Z-disc. F-actin growth and dissociation at the positive ends is about 8–9 times faster than at the negative ends (Chu & Voth, 2005). The actin filament can be considered as a double helix of negative charges which extends into the solvent (Ouropov, Knull, & Thomasson, 1999). The filament dimensions are ~10 × 1000 nm and it has a repeat of 13 monomers. The subunit repeat (axial rise) is about 5.5 nm and the helical repeat about 37 nm. According to molecular modelling of actin applying the coarse graining method, the main interactions in a trimer (3 G-actins) are hydrophobic contacts (Chu & Voth, 2005). The structure and properties of F-actin strongly depend on the bound ligands (ATP/ADP) and the types of ions in the solution. Unfortunately, the detailed atomic structure of F-actin also remains unknown, as there are problems with crystallisation of the sample (Kudryashov et al., 2005). α-Actinin is responsible for transverse bundling of G-actins.

Capping proteins stabilise the ends of actin filaments: tropomodulin (~40 kD) stabilises the pointed, slow-growing end, while Cap-Z (α ~ 36 kD, β ~ 32 kD) stabilises the fast-growing, barbed end (Fischer

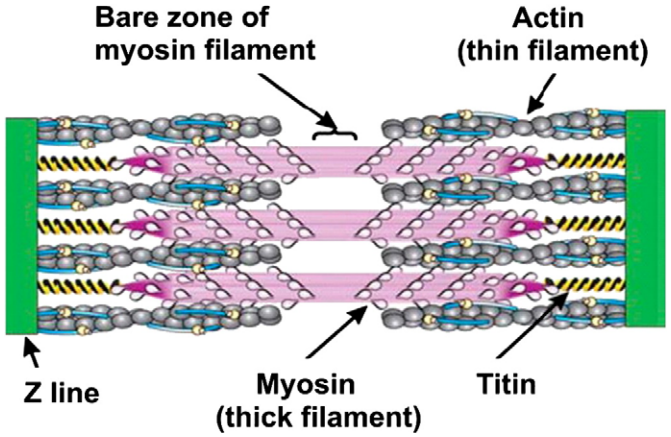

Fig. 3. Sarcomere (Plotnikov, Millard, Campagnola, & Mohler, 2006).

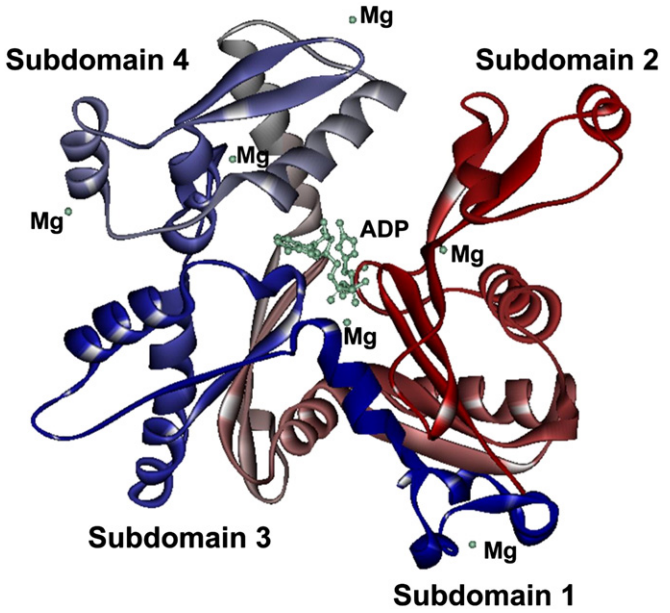

Fig. 4. G-actin subdomains (Geeves, Fedorov, & Manstein, 2005).

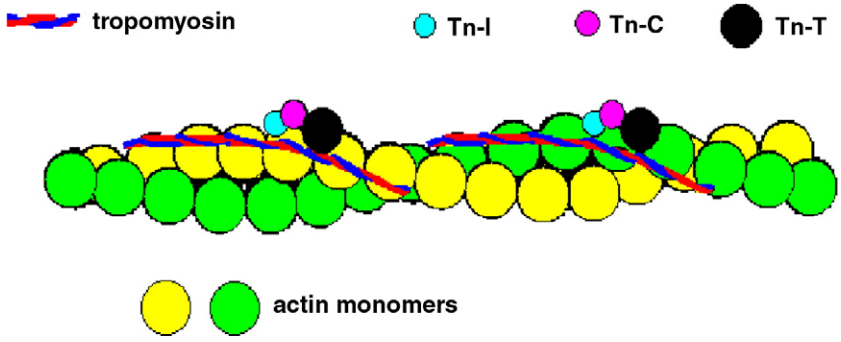

Fig. 5. Actin filament (F-actin) (http://www.cryst.bbk.ac.uk/PPS2/course/section11/assembli.html).

& Fowler, 2003; Littlefield, Almenar-Queralt, & Fowler, 2001). The latter is localised in Z-discs where it binds α-actinin, which, in turn, crosslinks anti-parallel actin filaments of neighbouring sarcomeres in Z-discs. The actin filaments are surrounded by tropomyosin and troponin. The relation of G-actin:tropomyosin:troponin is 7:1:1. Tropomyosin contains 284 amino acid residues and has a molecular weight of about 37 kD. The dimensions are about 40 × 2 nm. It is formed from two α-helical chains, has a coiled coil structure, and, with actin, is thought to form a supercoil of about 38.5 × 4 nm. Its function is to regulate the interaction between thin and thick filaments as well as to stabilise thin filaments (Clark et al., 2002). Tropomyosin slows actin depolymerisation/polymerisation at pointed ends. It is highly negatively charged, and its relative charge is much greater than that of myosin (McLachlan & Karn, 1982). On the other hand, tropomyosin is considered much more hydrophobic than myosin (McLachlan et al., 1982; Miroshnichenko et al., 2000). When the muscle is inactivated, tropomyosin shields, at least partly, the double helix of negative charges of F-actin (Diaz Baños, Bordas, Lowy, & Svensson, 1996). Troponin is a co-operative complex of three proteins: troponin-T (~30 kD), troponin-I (~20 kD) and troponin-C (~18 kD).

4.3. Actomyosin

The contact sites between myosin-S1 and G-actin are 1) positive lysine residues of S1 at the 20–50 kD junction and negatively charged amino acid residues of G-actin subdomain 1; 2) the hydrophobic residues of S1 (lower 50 kD) and G-actin subdomain 1 (and some of subdomain 3); and 3) some loops of S1 (upper 50 kD) and G-actin subdomain 2. Unfortunately, the high resolution structure of actomyosin is not yet available (Geeves, Fedorov, & Manstein, 2005).

Actin is mainly negatively charged in the myosin binding site, while myosin is mainly positively charged in the actin binding site (Agbulut et al., 2007). However, the hydrophobic interaction between S1 and G-actin is stronger than the electrostatic.

During the development of rigor mortis, the pH decreases to 5.5–5.8. The solubility, as well as the water-holding of myofibrillar proteins, decrease. Proteins are strongly bound by hydrogen bonds and salt bridges in the rigor complex (Oplatka, 1994). Formation of rigor cross-bridges between actin and myosin filaments has been proposed to disturb structured water bound to them (Yamada, 1998).

4.4. Macromolecular crowding

As the centre-to-centre distance between actin and myosin filaments, two actin filaments and two myosin filaments are about 27 nm, 18.5 nm and 32 nm, respectively (the distance between relaxed S1 and actin is 2–5 nm), we begin to enter the world of nanochemistry and nanofluidics (Eijkel & van der Berg, 2005). As a matter a fact, both actin and myosin have been mentioned in several reports as promising biological nanomaterials. They both have the attractive property of self-assembly (Barral & Epstein, 1999; Miroshnichenko et al., 2000).

To make the problem even more complicated, the third most abundant protein in the sarcomere (about 8%) happens to be largest protein known: titin (Linke & Grützner, 2008). It contains 34,000–38,000 amino acid residues and has a molecular weight of 3000–4000 kD. I-band titin has elastic properties and acts as a sarcomere stabiliser. It connects the Z-disc to the thick filaments and prevents over-stretch of a muscle/sarcomere. The A-band titin molecules are located at the surface of the thick filament. The titin filament has a bead-like substructure with 4 nm periodicity (Tskhovrebova & Trinick, 2003). In cardiac muscles, titin has been suggested to belong to main modulators of lattice spacing (Cazorla, Wu, Irving, & Granzier, 2001).

Macromolecular crowding must be taken into account when considering the sarcomeric environment (Despa, Orgill, & Lee, 2005; Grazi & Di Bona, 2006; Minton, 2006; Tskhovrebova, Houmeida, & Trinick, 2005). There are not only actin, myosin and titin filaments in the sarcomere but a whole bunch of other proteins (some of which have been mentioned earlier in this article) as well as enzymes etc. For instance, a change in sarcomere length affects the sarcomere diameter and thereby macromolecular crowding (Grazi & Di Bona, 2006). According to the excluded volume theory, even small changes in cellular hydration can cause drastic changes in the reactivity of macromolecules (Minton, 2006).

4.5. Polyelectrolytes

Intracellular proteins are highly charged, and F-actins as well as myosin filaments can be regarded as polyelectrolytes which have a net negative charge at physiological pH. Actin and myosin filaments are narrow and long, and they have a relatively high charge density. Each filament is surrounded by a diffuse cloud of counter-ions (Morel, 1985). According to the Manning counter-ion condensation model, when the charge density of a polyelectrolyte is high, it very strongly attracts counter-ions from the solution, which could lead to so-called counter-ion condensation on the polyelectrolyte surface (Manning, 1969). Counter-ion condensation, in turn, affects the charge distribution of the polyelectrolyte and may even lead to conformational changes of the polymer (Hinderberger, Jeschke, & Spiess, 2002).

5. Intracellular fluid

Muscle fibre is a multinucleate cell. The nature of intracellular water has been under debate. There have been claims that it is similar to bulk water (Israelachvili & Wennerström, 1996), but opposite views have also been proposed (Cameron, Kanal, Keener, & Fullerton, 1997; Ling & Peterson, 1977; Morel, 1985; Watterson, 1987). They have suggested that cellular water is highly structured and that a large fraction of it has perturbed osmotic and motional properties; sometimes it is even thought to resemble the glassy state (Pizzitutti

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& Marchi, 2007). Water viscosity in cells has been estimated to be about twice that of bulk water (Cooke & Kuntz, 1974). One of the most radical insights might be that of Chaplin (2004), who considers cell water organized as clathrates around K^+ ions.

6. Effect of salts/ions on water-structure: high and low density water

To understand the effects of the prevailing ion distribution of a sarcomere on the water-holding of a muscle, it is necessary to introduce two concepts, high and low density water. Experiments have shown that small or multivalent ions organise water, producing so-called low density (LD) water, while large monovalent ions disorder it, producing high density (HD) water (Fig. 6). In HD water, the water molecules have collectively moved together, and the hydrogen bonds are bent and very weak (Wiggins, 1990). It is very reactive and has a low viscosity. LD water, on the other hand, is inert, and its viscosity is higher. The increase in viscosity has been related to the lowering of water activity (Grazi & Di Bona, 2006). HD water has a lower freezing point than LD water, which means that during, for instance, the freezing of meat, the concentration of HD water increases, and as it is highly reactive, the freezing should be carried out as quickly as possible.

LD water is induced by so-called structure-making (kosmotropic) ions. Kosmotropic ions cause a positive hydration effect, meaning that as solutes, they enforce a hydrogen bound network of neighbouring water molecules which makes the water molecules less mobile and more structured than in bulk water. For instance, monovalent cations Na^+ and H^+, divalent cations Ca^{2+} and Mg^{2+}, divalent anions $HP0_4^{2-}$ and $S0_4^{2-}$, hydrophobic amino acids, glucose and some monovalent anions, like OH^-, are classified as kosmotropes (Wiggins, 1995). They stabilise the native conformations of many proteins (Dér et al., 2007). In high concentrations, anionic kosmotropes are excluded from protein–water interphases. Hydrocarbons in aliphatic and aromatic molecules, as well as most intracellular anions, including phosphates, sulphates, carboxylates and glutamates, belong to kosmotropes (Collins, 1997).

Chaotropic (structure-breaking) ions have an opposite, negative hydration effect which means that they weaken the hydrogen bonds of neighbouring water molecules, making the water molecules more mobile and less structured than in bulk water, i.e., they induce HD water (Fig. 6). Thus bulk water tends to repel chaotropes towards interphases (Lo Nostro et al., 2006). Chaotropes destabilise many proteins which have a closed native conformation and stabilise those which have an open conformation (Dér et al., 2007). For instance, K^+, Cl^- and Br^-, large monovalent ions NH_4^+ and $C(NH_3)_4^+$ as well as $H_2P0_4^-$, $HC0_3^-$ and HSO_4^- are classified as chaotropes (Hribar, Southall, Vlachy, & Dill, 2002). Stable charges of biomolecules are strong chaotropes and most intracellular monovalent cations are chaotropic (Collins, 1997). These include K^+ and lysine, arginine and histidine. Anionic chaotropes (e.g. Cl^-) accumulate in the protein–H_2O interphase, making them more hydrophilic (Dér et al., 2007). They are also known to be adsorbed to non-polar surfaces and interphases, which means that they might be adsorbed to the non-polar side chains of arginine, histidine and lysine (Collins, 1997; Collins, Neilson, & Enderby, 2007; Washabaugh & Collins, 1986). As will be seen in Section 8.4, the above-mentioned properties of chaotropes are essential in understanding the effect of added NaCl on meat.

The effect of anions on the structuring of water is stronger than that of cations due to the asymmetry of charges in the water molecule. Small, highly hydrated cations preferably accumulate in HD water, while bigger, monovalent cations accumulate in LD water (Wiggins, 1990). The methods of classification of ions as kosmotropes or chaotropes have been recently reviewed (Marcus, 2009). One of the most widely used is based on the sign of the so-called Jones–Dole viscosity β-coefficients, being positive for kosmotropic ions and negative for chaotropic ions (Collins, 1997; Hribar et al., 2002). It describes ion–water interactions and correlates with surface charge density. The β-value zero marks the strength of water–water interactions. According to the Jones–Dole viscosity β-coefficients, Mg^{2+} is a very strong kosmotrope ($\beta = 0.385$), Ca^{2+} ($\beta = 0.285$) and acetate, Ac^- ($\beta = 0.250$), somewhat weaker, while Na^+ is much weaker ($\beta = 0.086$) (Collins, 1997; Zhao, 2005). Cl^-, on the other hand, is a weak chaotrope ($\beta = -0.007$) like K^+ ($\beta = -0.007$). Omta, Kropman, Woutersen, and Bakker (2003), however, are of opposite view regarding that the effects of ions on water-structure as negligible, i.e., not extending beyond the first hydration shell of the ion.

7. Effect of proteins on water-structure/effect of water on protein structure

The vicinity of nearby protein surfaces strongly affects water dynamics. Water becomes ordered close to some macromolecules, and inside the cell an average distance between macromolecules can be only about 2 nm. Water has been claimed to possess remarkably different solvent properties as far as 2–3 nm from hydrophobic surfaces. Water tends to minimise the surface area with non-polar groups which leads to hydrophobic interaction. The origin of the long-range hydrophobic interaction is still not clear. The hydrophobic interaction can be interpreted as the growth of LD water on hydrophobic surfaces (Wiggins, 1996). Water structured by hydrophobes has also been suggested to increase effective forces between charged groups (Baldwin, 2002; Despa, Fernández, & Berry, 2004). Hydrophobic interphases decrease the dielectric permittivity of the surroundings (Despa et al., 2004).

The negatively charged amino acid side chains are strongly hydrated, while the positively charged are weakly-hydrated (Collins et al., 2007). The two amino acids that have the highest water-binding ability are aspartic acid and glutamic acid (Low, Hoffmann, Swezey, &

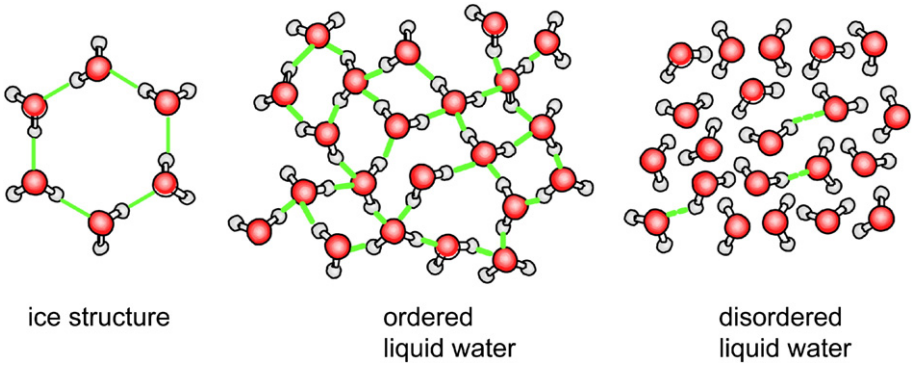

Fig. 6. Molecular forms of water (Moelbert, Normand, & De Los Rios, 2004).

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Somero, 1978). One amino acid of an ionic side chain has been claimed to bind 4–7 water molecules in aspartic acid, glutamic acid and lysine (Zayas, 1997). F-actin has both kosmotropic and chaotropic properties. The F-actin surface has plenty of negative charges, which together with its filamentous structure give a structural basis for generating hypermobile water which has a greater mobility than bulk water (Kabir, Yokoyama, Mihashi, Kodama, & Suzuki, 2003). The subdomain 1 of G-actin is fully accessible to water in the F-actin helix (Kinoshita & Suzuki, 2009). In filamentous F-actin, it has been observed that when the osmotic pressure of the protein rises, part of the intrafilamentous water is ejected, and the volume and diameter of the hydrated filament decrease (Magri, Cuneo, Trombetta, & Grazi, 1996).

The water-binding ability of myosin has been related to polar amino acids, especially the negatively charged aspartic acid and glutamic acid residues (Zayas, 1997). S1 contains many strongly bound water molecules (Lampinen & Noponen, 2005). It has been estimated that the number of bound water molecules per head is about 2000. The osmotic properties of myosin-S1 have been characterised. The amount of hypermobile water has been found to increase when actin and myosin filaments come into contact (Suzuki et al., 2004). There are implications that the formation of a rigor cross-bridge disturbs the binding of the structural water to myosin and actin filaments in muscle fibres (Yamada, 1998). The effect of osmotic pressure on the hydration of actomyosin has been studied and it was estimated that about 2500 water molecules are released when a myosin head binds to an actin filament (Yamada, 1998).

Water molecules can pass through the cell wall either by diffusing through a phospholipid double layer or through aquaporins (AQP) which are water channel proteins of the cell membrane that permit faster transport of water through cellular membranes. AQP4 has been found in skeletal muscles, especially in fast twitch muscles which undergo rapid volume changes under muscle contraction. However, it has been suggested that AQP4 does not seem to have an important role in the physiology of the muscle (Takata, Matsuzaki, & Tajika, 2004; Yang et al., 2000). There is an inversion of cell membrane polarity *post-mortem*, which releases extracellular ions into the sarcoplasm, and the sarcolemma becomes disrupted and leaky (Varriano-Marston, Davis, Hutchinson, & Gordon, 1976; Wu & Smith, 1987).

8. Effect of ion distribution on muscle protein system and its water-holding

8.1. Hofmeister series revisited

Hofmeister series has an effect on the ion distribution of actin and myosin filaments, their stability and water-holding. Hofmeister invented his famous series (Hofmeister/lyotropic series) in the 1880s when he examined the effects of ions on proteins and noted that some ions had stronger salting-out characteristics than others (Hofmeister, 1888). The series can be used to make conclusions about the stability of proteins. For instance, the order of effectiveness to stabilise proteins is $PO_4^{3-} > SO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^-$ for anions and $(CH_3)_4N^+ > NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$ for cations (Zhao, 2005). Consequently, optimal stabilisation of a biological macromolecule is obtained using a salt consisting of a kosmotropic anion and a chaotropic cation. Hofmeister effects have been demonstrated by numerous techniques (Collins & Washabaugh, 1985). Surprisingly, this series, even though recognised by scientists, was almost forgotten, but has come to the fore recently. The series has been lacking a theoretical explanation: why do different ions react differently with the proteins? Collins re-explained it with the 'Law of matching water affinities' (Collins, 1997).

The effect of ions on proteins follows the Hofmeister series: they are stabilised by strong chaotropic cations and kosmotropic anions and destabilised by kosmotropic cations and chaotropic anions (Zhao, 2005). The efficiency of anions and cations to promote water-holding capacity of meat also follows the Hofmeister series. Subsequently, it has been suggested that NaCl functions by disturbing the structure of protein constituents. According to NMR results, adding salt to meat causes more water to be tightly bound to the myofibrillar matrix while the rest becomes less tightly bound (Bertram et al., 2001).

The effectiveness of ions to structure water can also be derived from the Hofmeister series. The ions accumulate around filament charges, so that water will equilibrate by increasing its density, thus producing HD water around filament charges, and by decreasing it, producing LD water, around hydrophobic filament areas/between filaments (Wiggins, 1990). The Hofmeister salts change the protein–water inter-phase properties so that kosmotropes make them more hydrophobic and chaotropes more hydrophilic (Dér et al., 2007). According to experiments, anions seem to dominate these effects. Chaotropes destabilise many proteins, while kosmotropes stabilise them. Kosmotropes increase and chaotropes decrease protein–water interfacial tension at high salt concentrations (Dér et al., 2007). Anionic kosmotropes are excluded from interphases at high concentrations, while anionic chaotropes have the opposite tendency, especially when cations can approach the surface, which correlates with the water-structuring properties of kosmotropic anions and the structure-breaking properties of chaotropic anions (Manciu & Ruckenstein, 2003). Weakly-hydrated ions, like Cl^- and K^+, will be adsorbed to non-polar surfaces and interphases as water prefers water–water bonds to the weaker bonds with chaotropes (Collins et al., 2007).

It is worth mentioning that the effect of ions on the activity and stability of enzymes can also be explained by the Hofmeister series (Zhao, 2005). They are stabilised by chaotropic cations and kosmotropic anions and destabilised by kosmotropic cations and chaotropic anions.

Almost all Hofmeister ions salt-out non-polar groups and salt-in peptide groups (Baldwin, 1996). The basic mechanisms behind these opposite effects are still controversial. The salting-out coefficient increases as the number of carbon atoms increases in the aliphatic side chain of an amino acid because the hydrophobicity increases accordingly.

8.2. Law of matching water affinities

As already mentioned, muscle fibre is a multinucleate cell. According to Ling's association–induction hypothesis, intracellular K^+ is adsorbed to the β- and γ-carboxyl groups of cellular proteins, i.e. aspartic acid ($pK_R = 4.1$) and glutamic acid ($pK_R = 3.9$) residues, respectively (Ling, 1964, 1977). Thus K^+ would not be free and would have a minor effect on the osmotic activity of the cell. Ling claims that K^+ localises in the A-band and Z-line (Ling, 1977).

It has been suggested that K^+ has a higher affinity than Na^+ towards negatively charged polymeric surfaces of the cell, because more energy would be needed to remove the hydration layers of Na^+ which has a greater hydrated diameter than K^+ (Pollack, 2003). On the other hand, according to Chaplin (2004), Na^+ ions prefer to bind to weaker carboxylate groups ($pK_a < 4.5$), while K^+ ions prefer stronger acids ($pK_a < 3.5$). There is recent evidence that Na^+ has, in fact, a stronger affinity to protein surfaces than K^+, due to local ion-pairing of Na^+ ions with the negatively charged carboxylate groups in the side chains of aspartic acid and glutamic acid (Jagoda-Cwiklik, Vácha, Lund, Srebro, & Jungwirth, 2007; Uejio et al., 2008; Vrbka, Vondrášek, Jagoda-Cwiklik, Vácha, & Jungwirth, 2006). Carboxylate groups belong to the dominant anions of biological systems and they are known to be kosmotropic. In addition to this ion–ion interaction there is a weaker ion–dipole interaction between Na^+ ions and the protein backbone (mainly the amide carbonyl group), and an even weaker contribution from other side chains.

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Another view can be obtained using the 'Law of matching water affinities' that explains the Hofmeister series qualitatively (Collins, 1997). According to Collins, positive and negative ions that 'match' in size and have equal charge form contact ion pairs. In the Hofmeister series, the ions are arranged according to their surface charge density which correlates with their water affinity (Squire et al., 1998). If a protein contains kosmotropic side chains carrying a charge, it would preferably form an ion pair with another kosmotrope of opposite charge. The same would apply to chaotropic side chains of proteins and chaotropic ions in solution: like prefers like. The major intracellular anions (phosphates, sulphates, carboxylates, carbonates) are kosmotropic while the major cations (K^+, lysine, arginine, histidine) are chaotropic. Na^+ 'matches', for instance, with carboxylates. Collins ranks the strength of interactions in aqueous salt solutions in the following decreasing order: two kosmotropes>kosmotropes and water>two water molecules>chaotropes and water>two chaotropes (Collins et al., 2007). Ion-pairing affects the activity of the electrolyte as well as the osmotic pressure of the solution (Hess & van der Vegt, 2009). In Section 8.4 it is shown how the 'Law of matching water affinities' can be applied to explaining the effect of added NaCl on actin and myosin filaments.

The interaction between ions and proteins depends not only on the ion and protein in question but also on pH and temperature, which makes the overall system quite complicated. It should be mentioned that there are opposing views to the 'Law of matching water affinities' which emphasise the role of hydration water. According to them, the law does not take into account the possibility of solvent-shared ion pairs, instead of contact ion pairs, forming with anions of weak carboxylic acids (Hess & van der Vegt, 2009).

8.3. Pre- and post-mortem sarcomeric ionic species and ionic strengths

When considering meat, the concentrations of the ions are usually given with respect to their location in the muscle, i.e., either inside or outside the muscle fibre (intra- or extracellular). However, knowing the intracellular concentration does not necessarily give the precise ionic strength inside the sarcomere itself, which is the ionic strength that we are most interested in. The ionic strength, on the other hand, is directly connected to the electrostatic screening effect, where the free ions in solution screen and thus reduce the strength of electrostatic interactions between charged surfaces. As the concentration of the free ions increases, the screening of the ions also increases. Here the word 'free' must be stressed, as the ions paired with the protein filaments do not take part in the ionic strength and thus do not affect the screening.

In addition to differentiating between extra- and intracellular concentrations in a muscle, and in intra- and extrasarcomeric concentrations in a muscle fibre, another division should be made, namely *pre-* and *post-mortem* ionic concentrations and ionic strengths. The *pre-mortem* ionic strength (physiological ionic strength) of intracellular fluid is usually taken as 0.15 M, a value calculated, for instance, by Alberts and Ganong (Alberts et al., 1983; Ganong, 1983; Wu & Smith, 1987), while the *post-mortem* ionic strength has been estimated to be 0.19 M–0.26 M (Winger & Pope, 1980–81; Wu & Smith, 1987). The question is, how trustworthy these values are. They have either been calculated from individual ion concentrations, measured *in vitro*, or obtained by relating the freezing point depression of the *whole* muscle to the corresponding osmotic pressure. However, it would be essential to know the real ionic strength *inside the sarcomere*.

Due to the stable negative charges in myofilaments, the cations are concentrated and the soluble anions are largely excluded from myofibrils (Maughan & Godt, 1989). A substantially more negative potential has been found in the A-band than the I-band in ATP-free solutions, while a uniform charge distribution has been found in solutions containing ATP (Bartels & Elliott, 1981). On the other hand, according to another study, negative potential is lowest in the I-band, intermediate in the A-band and highest in the Z-discs, which has been interpreted as that the I-band having the highest concentrations of diffusible anions and the lowest concentrations of diffusible cations (Aldoroty & April, 1984).

One of the major differences between *pre-* and *post-mortem* ionic concentrations in muscle concerns Na^+ ions. The *pre-rigor* sodium contents are 0.142 M and 0.01 M in extracellular and intracellular space, respectively (Aberle, Forrest, Gerrard, & Mills, 2001). From rigor onwards, the ATP-driven Na/K-pump ceases and the concentrations will tend to equalise, resulting in an intracellular Na^+ concentration of 0.027 M (Offer & Knight, 1988). Na^+ ions are practically excluded from the living muscle fibre to prevent the precipitation of salts inside the cell (as suggested by the Hofmeister series). As mentioned before, the major intracellular anions are kosmotropic, and 'like prefers like', as the 'Law of matching water affinities' defines. Therefore, K^+ is the dominant intracellular cation (concentration about 0.15 M). Intracellular Ca^{2+} concentration is about 4×10^{-2}–10^{-4} times lower than the extracellular concentration, which is itself rather low (about 0.0025 M), and intracellular Mg^{2+} is highly complexed with ATP and other anions. On the other hand, the intracellular Cl^- ion concentration is low (about 0.003 M), as it competes with DNA for the positively charged binding sites of proteins (Collins, 1997).

After the fibre death, changes are taking place in the ion permeability and integrity of the sarcolemma (Varriano-Marston et al., 1976; Wu & Smith, 1987). As the fibre enters rigor, the semi-permeability of sarcolemma starts to disappear. Thus Na^+, Cl^- and Ca^{2+} ions will be able to diffuse into the sarcoplasm. However, even though the extracellular Na^+ and Cl^- concentrations are reasonably high (about 0.15 M and 0.1 M, respectively), the extracellular volume is only about one tenth of the whole volume of the muscle so that their concentrations will be substantially diluted inside the muscle fibre. It is still unknown whether all the ions that enter the sarcoplasm diffuse all the way into the sarcomeres. F-actin is surrounded by a counter-ion cloud which in physiological conditions consists mainly of K^+ ions (Gartzke & Lange, 2002). However, when Na^+ enters the sarcomere, there is probably competition between K^+ and Na^+ for the counter-ion cloud around F-actin. It has also been suggested that low molecular weight substances, especially inorganic electrolytes, are adsorbed to the various macromolecules in *pre-rigor* muscles, thus reducing the osmotic activity (Winger & Pope, 1980–81). Altogether, very little is known about the *post-mortem* behaviour of ions in meat. Yet, Millman (1998) has claimed that ionic strength affects the filament lattice more than electrostatic forces or filament charges.

8.4. Added salts

In experiments, researchers often try to mimic the physiological situation by producing the physiological ionic strength, estimated to be about 0.15 M. This is usually achieved by adding either NaCl or KCl (Collins et al., 2007). When curing meat NaCl may be partly substituted by KCl as NaCl is a health hazard. NaCl, however, behaves very differently compared with KCl, a fact which is often totally ignored. NaCl is formed from a kosmotropic cation and a chaotropic anion, while in KCl, both the cation and anion are chaotropic. Thus, according to the 'Law of matching water affinities', these two salts affect the cell protein structures in quite a different manner.

The curing of meat raises the ionic strength from the *post-mortem* values. Salt diffuses through the ultrastructure of meat and finally reaches the sarcomeres. The question is, how much does it influence the prevailing ionic strength inside the sarcomere? If most of the salt ions form ion pairs with the different protein groups, the effect might be marginal. Binding of ions reduces the osmotic pressure and also has an effect on pH measurements.

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Both myosin and actin are classified as salt-soluble proteins, however, their solubility characteristics are very different. When the ionic strength of the salt solution is above 0.25 M, thick filaments start to progressively dissociate into myosin molecules (Offer & Knight, 1988). At high concentrations (for instance 0.6 M KCl), salt dissolves myosin filament. The solubilisation of proteins increases the intracellular water viscosity. However, the actin filament does not dissolve, even at 2.7 M NaCl (~13% solution) or KCl concentrations at room temperature, while at 0 °C, 0.3–0.7 M KCl causes partial depolymerisation of actin (Offer & Knight, 1988). This supports the claim of Offer and Trinick (1983) that the addition of NaCl to meat causes depolymerisation of thick filaments to myosin molecules but keeps their cross-bridges attached to actin filaments which themselves do not depolymerise.

Elevated ionic strengths decrease actin–myosin interactions in relaxed, activated and rigor muscles (Kentish, 1984; Wu and Smith, 1987; Yu & Lee, 1986). In the sarcomere, salt has been reported to extract the A- and Z-bands, produce swelling of the I-segments and disrupt structures in the M-line. As proposed by Hamm (1972) and opposed by Offer and Knight (1988), it increases the electrostatic repulsion between myofilaments, which widens the lattice spacing and reduces the intermyofibrillar space.

When small pieces of meat are immersed in concentrated NaCl solutions, there is an initial shrinking followed by swelling which is restricted by the endomysium. At the myofibrillar level, the greatest swelling is at about 1 M NaCl (5.8%). Hamm (1960) noticed that NaCl causes swelling of the myofibrils but Na-acetate does not. Thus, Hamm (1972) concluded that Cl^- rather than Na^+ ions bind to the myofibril and induce swelling. Na-acetate increases the water-binding ability less than NaCl, as acetate anions bind to muscle proteins essentially more weakly than Cl^-.

The N-terminal of F-actin is highly negative and attracts cations (Chaplin, 2004). As mentioned earlier, F-actin is surrounded by a counter-ion cloud which in physiological conditions consists mainly of K^+ ions. The cations can move parallel to the F-actin axis but not perpendicularly. As the negative charges of F-actin are compensated by the counter-ion cloud, free anions are largely excluded. Divalent ions (e.g. Ca^{2+}) are bound more tightly to F-actin than monovalent ions. Ca^{2+} has been found to bind to the I-band (Legato & Langer, 1969).

The meat industry might be interested in manipulating the muscle fibre using different salts. It is important not to consider the F-actin and myosin filaments only as surfaces having a negative net charge but to go deeper into their amino acid composition to understand the effect of added salts on myofibrils. According to the Hofmeister series, optimal stabilisation of a biological macromolecule can be achieved using a salt consisting of a kosmotropic anion and a chaotropic cation. NaCl, on the contrary, consists of a kosmotropic cation and a chaotropic anion. At physiological pH, an increase of ionic strength has been claimed to leave the thin filament charge practically unaffected, while the thick filament charge increases rapidly to more negative values (Bartels, Cooke, Elliott, & Hughes, 1984; Millman & Irving, 1988). We suggest the following mechanism for the action of adding NaCl to meat. According to the 'Law of matching water affinities', Na^+ ions form ion pairs with the negatively charged aspartic acid and glutamic acid carboxylates of the side chains in myosin filaments. If contact ion pairs are formed between them, Na^+ ions neutralise the negative charges of the carboxylates. Some of the Na^+ ions may also be in a dipole–ion interaction with the protein chain backbone carbonyl groups. In addition, they form a counter ion cloud around the double helix of negatively charged actin monomers of F-actin, thus replacing the physiological K^+ ion cloud. Na^+ ions are structure-making, while the K^+ ions are structure-breaking. The accumulation of Na^+-ions around F-actin could thus increase the amount of LD water.

Cl^- has been suggested to be bound to myofilaments (Cl^- ions are hydrogen bound to the so-called Saroff sites which are networks of protein side chains) making them mainly negatively charged, which will lead to the repulsion of myosin molecules, swelling of myosin filaments and, consequently, the whole myofibrils (Regini & Elliott, 2001). It has been proposed that the Cl^- ions are released from the sites when they obtain enough thermal energy, and that they weaken salt linkages, thereby introducing swelling. Considering a myosin filament formed of myosin molecules, myosin rod (light meromyosin) has many positively charged side chains that could, in principle, be attacked by Cl^-, and also many negatively charged amino acids that could attract Na^+. Cl^- is a small, weakly-hydrated chaotrope, and it easily forms ion pairs with the positively charged chaotropic groups of proteins, like the positively charged arginine, histidine, and lysine residues of the myosin filament (Collins, 1997). In addition, as chaotropes are repelled by water and as anionic chaotropes will be adsorbed to non-polar surfaces, we suggest that Cl^- ions could also be adsorbed to hydrophobic amino acid side chains (leucine, valine and alanine) on the outer surface of the myosin filament, to the non-polar side chains of arginine, histidine, and lysine, or, as the myosin filament is probably hollow (Squire et al., 1998), also to the hydrophobic core of the inner surface. Thus, Na^+ would interact both with actin and myosin filaments while the effect of Cl^- would concern only myosin filaments. The net effect could be an increase of the negative net charge of the myosin filaments.

NaCl increases the protein solubility of meat products, which means that it decreases protein–protein interactions (Zayas, 1997). NaCl dissolves myosin filaments, which might be induced by the absorption of Cl^- ions to the hydrophobic amino acid chains of myosin filament. Cl^- belongs to the anionic chaotropes, and as mentioned earlier, chaotropes tend to make the protein–water inter-phase more hydrophilic, which could promote the dissolution. The dissolution of myosin filaments would expose the hydrophobic amino acids of the myosin filament core to water thus possibly increasing the amount of LD water. Simultaneously, as the filament decomposes to the corresponding molecules, the surface area increases (Cacace, Landau, & Ramsden, 1997). On the other hand, accumulation of Na^+-ions around F-actin would make the F-actin-water inter-phase more hydrophobic and thus further increase the amount of LD water.

Returning to the osmosis model proposed by Offer and Knight (1988), electrical forces are thought to pull the Na^+ ions very close to the filament surfaces and cause an osmotic force. The filament itself has not been specified in their model. However, according to our proposition contact ions would be formed by the carboxylates if it was a myosin filament, which would not affect the osmosis. An actin filament would, however, be surrounded by a Na^+ ion cloud, which means that Donnan-osmotic effects could play a role.

9. Surface forces

There seems to be indications that surface forces may play a role in the muscle system of colloidal proteins. To give a rough estimate of the surface area of proteins interacting with sarcoplasm in muscle fibre, the following calculations can be made. The first assumption is that the filaments are smooth cylinders, which they in reality are not. A practical tool to calculate water-accessible surface areas (ASA) for myofilaments does not exist, as the available programs are only for protein molecules of known composition, and not for protein aggregates. Also, the starting values below are averages, and they vary considerably in practical circumstances.

If the lattice spacing d (rigor state, pH 5.6, Offer & Knight, 1988) is 37.5 nm, it will give a unit cell area of 1.2×10^3 nm^2. Assuming that the myofibrillar volume of 1 dm^3 (ca 1 kg) muscle is 70% and sarcomere length 2.5 μm, there will be 2.3×10^{17} sarcomere unit cells in total. In each unit cell there is one myosin filament. Consequently, as the actin: myosin ratio is 4:1, the number of actin filaments in 1 kg muscle is 9.2×10^{17}. Using myosin filament smooth dimensions of 16×1600 n\m with 600 times $4\times7\times17$ nm S1 units/filament, and for actin

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6×1000 nm, the total surface area of actin and myosin filaments would be in the order of 102 000 m^2, from which myosin-S1 units alone are about 66,000 m^2. It seems to us that, when discussing the water-holding in meat, the effects of S1 units have been underestimated compared to myosin shaft and actin filament. One must especially note that their isoelectric point is around 6.5, i.e they are positively charged at normal meat pH range.

It should be emphasised that this is an oversimplification, as it is not known what the effective water-accessible surface area of a filament actually is. In addition, the areas of Z-line, other cytoskeletal proteins, filaments or components of filaments (like S2 units) were not calculated. If the graininess of filaments at the colloidal level, or even that caused by atoms, was taken into account, the water-accessible surface area can be estimated to be much larger. Whatever the actual area of the interface is, it must be very large.

The surface tension of pure water/air is 73 mJ/m^2. In their unpublished experiments, Starr and Offer (unpublished data, ref. Offer & Knight, 1988) showed that the surface tension of drip/air is 33 mJ/m^2. There is no data available on sarcoplasm/myofilament surface tension, but it must be lower than the previous values. Therefore, the free energy changes, in the order of mJ/m^2, caused by addition of salts, pH changes, denaturations etc., could be used as indicators of water-holding changes. For example, one unit of pH change, i.e. protons from 10^{-7} to 10^{-6} M (actually the amount of protons is 50 mmol/kg, due to the buffering capacity; Puolanne & Kivikari, 2000), or 20 g NaCl in the bulk sarcoplasm interacting with the interface (say, of 102 000 m^2), causes dramatic changes in the myofibrillar volume. Offer and Trinick (1983) showed that myosin filaments swell, and the light meromyosin units release from the thick filament backbone, which greatly increases the accessible surface areas of proteins. Yet, according to Offer and Trinick (1983), myosin molecules are still attached to the actin filaments, so the system at that stage could perhaps be considered as a, so-called, polyelectrolyte brush (Kumar & Seidel, 2005). It would be useful to study the surface tension values of myofibrillar bulk solution of different pHs, salts and salt contents, temperatures etc., and attempt to relate the information with protein surface data. Finally, little has been described in the literature about the chaotropic/kosmotropic effects of pH on different ions and ion pairs in relation to the muscle protein systems and their changes during processing. Muscle proteins themselves also have a role in the structuring of water.

The S1 units of different myosin molecules are about 10 nm apart, which means that there is a very dense network, perpendicular to the myofibrillar axis, in the sarcomere. As discussed in Section 4.1, the isoelectric point of isolated S1 units is 6.5, and therefore S1 units may carry a more positive local net charge than the other parts of the filament at meat pH. This property could enhance the affinity of chloride ions to S1 units (see Section 6), which may be a part of the explanation of the effects of chloride ions on water-binding.

Wennerström (1999) also discusses the hydrophobic effects when two hydrophobic surfaces are put together from a large separation in water. According to Wennerström, the hydrophobic forces, as such, are not relevant, but the energy comes from the increased electrostatic cohesion of water molecules when hydrophobic material associate.

In addition, it should be remembered that the myofibrillar system and gels formed of solubilised proteins as well are three-dimensional. In a myofibril the distance from myosin filament to actin filament equals less than the length of one hundred water molecules. There are also transverse bridges (e.g. Z-lines, actomyosin bridges, intermediate filaments etc.) increasing the porosity of the system. This creates an additional aspect, as surface forces explain the interactions between the solution (water) and proteins, but they do not explain the bulk water-holding. If, for example, one myosin filament is immersed in a solution, one cannot talk about water-holding. The structural aspects must also be taken into consideration. We suggest, however, that more attention should be paid to transverse effects of S1 + S2 units, in addition to the usual considerations dealing with myosin shaft–actin filament relationships.

10. Concluding remarks

On the macroscopic level, factors affecting water-holding of meat are well-known, and all relevant practical aspects can be controlled by reasonable means. Water-holding continues to be determined in a great number of studies, and therefore, there is an immense amount of data available on the subject. These studies, however, have not markedly increased our knowledge on the foundations of water-holding.

The classical hypotheses of water-holding in meat (Hamm, 1972 as well as Offer & Knight, 1988, with collaborators) are based similarly on solution/protein interactions, but there are differences in the structural aspects of the amount the bulk water in the system. The well-known macroscopic phenomena are quite well explained by the surface forces/interactions. We believe that the surface free energy could provide an essential force to keep the water in the muscle protein system.

However, we were not able to find a fundamental explanation for the bulk water-holding in muscle, although we agree that the surface interactions created by the three-dimensional network and their effects on water-structure, as well as electrostatic and osmotic forces, keep the bulk water in the system. The effects of pH, salts, phosphates, denaturation etc. can be explained using these interaction hypotheses. All the discussed hypotheses on water-holding in muscle seem to include solution/protein interactions and structural aspects.

This review summarises the surface interactions of the previous hypotheses and stresses the hydrophobic/hydrophilic interactions of proteins. The new aspects, not dealt with in meat science using modern terminology much before, are the properties of water, like low density/high density water or chaotropic/kosmotropic effects on water and proteins. The concept formulated by Collins and Washabaugh (1985) provides a relatively simple model that can be linked with the common hypotheses used in meat science. Later research has further deepened and quantified the foundations by presenting surface charges, free energy of solution and surface free energy for ions, ion pairs and non-polar substances. These aspects have also been discussed earlier by Hamm (1972), but using different terminologies and developing from ideas originally suggested by Ling (1965). As Hamm (1972) claimed that the hydrophobic pushing and polar effects affect the whole bulk water in muscle protein system, Collins and Washabaugh (1985) suggested that the effect reaches to two to five layers of bulk water. Collins, Neilson, and Enderby (2007), however, recently stated that the electrostatic effects of ions on water molecules are short-ranged to one or two molecules only. The interaction of water and protein surfaces, as well as the capillary hypothesis (as presented by Offer & Trinick, 1983), can also be linked with the models presented by Collins and Washabaugh (1985). Also, the protein–solution interaction term (Flory–Huggins interaction constant) in the osmosis hypothesis of Offer and Knight (1988) can be related to the chaotropic/kosmotropic phenomena and surface tension.

We stress the fact that the total amount of heavy meromyosin is larger than that of light meromyosin, and, in addition, the water-accessible surface is approximately three times larger than that of the myosin shaft. All three dimensions of S1 units are of colloidal size, and of S2 units of molecular size. Therefore their interaction with water phase could be even more important than claimed above. Increased attention should be paid to transverse structural elements and not only the backbones of the filaments. The cross-bridges also have varying lengths due to different circumstances, but the longitudinal elements are rather stable (Offer & Knight, 1988).

Still, even though the models presented above explain rather well the water/protein interactions close to the inter-phase, these models

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do not give a good explanation for the holding of bulk water. Therefore, in addition to the hypotheses presented above, models like Hamm's (1972) hydrophobic pushing/polar pulling in three-dimensional network or Offer's osmotic effect caused by the uneven distribution of ions (Offer & Knight, 1988), are needed. On the contrary, with capillary forces, the differences in the amount of bulk water at high moisture contents are not easy to explain. Consequently, the classical hypotheses of bulk water-holding are still applicable.

It should also be noted that myosin is an extraordinary molecule. It is able to form a gel when solubilised and heated in optimal circumstances, in increasing concentrations from 0.2% (Ishioroshi et al., 1979). The gel strength increases by a logarithmic factor of 1.8, meaning that the gel-forming is a second-order reaction. This exceptionally high gel-forming ability shows that myosin itself is well able to create conditions in which it holds huge amounts of water. Therefore a high water-holding can be expected also when myosin is still in the filamental form, with S1 and S2 units sticking out of the filament shaft.

Myosin filaments are more or less disrupted when salts are added. All this indicates that there is enough capacity for water-holding in meat, but the covalent bonds and other structural aspects that determine the longitudinal and/or transverse myofibril dimensions, seem to dominate, thus controlling the amount of bulk water-holding.

Especially, the effect of ions and salts on the water-holding of actin and myosin filament system should be considered. The order of effectiveness of ions to structure water can be derived from the Hofmeister/lyotropic series and expanded to the Law of matching water affinities. There is an interplay between ions, filament surfaces and water. Hofmeister ions modify the filament/water inter-phase, which further affects the hydrofobicity, charge distribution and consequently water-holding of the system.

As Pollack (2003) mentioned, muscle is considered [one of] the most important and best achievements of Mother Nature. She seems to closely guard her secrets!

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