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SOLUBILITY OF MYOSIN AND THE BINDING QUALITY OF MEAT PRODUCTS

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Abstract

This manuscript examines how the solubility properties of myosin contribute to the binding quality of comminuted meat products. The molecular structure and physiochemical characteristics of myosin from adult chicken pectoral muscle is presented along with a review of previous studies on the mechanisms of thermally-induced myosin gel formation. It is generally accepted that the binding properties of high quality processed meats result from the denaturation and aggregation of soluble myofibrillar proteins into a protein matrix that interacts with water, dispersed fat, and other soluble and insoluble proteins in meat (Asghar et al., 1985; Foegeding, 1988; Gordon and Barbut, 1992). Recent experiments investigating the specific regions and amino acids that comprise the myosin rod and determine myosin's solubility properties are presented. The implications of these studies to meat science and the need for further research in this area to aid in the development of new processing technologies is discussed.

Introduction

Comminuted meat products rely upon the functional properties of the constituent muscle proteins. In comminuted meats, where muscle chunks must be bound together, or in meat batters where water binding, fat binding, and emulsification must be maintained during processing, the physiochemical properties of muscle proteins are central elements contributing to the ultimate quality of the final product. The solubility characteristics of proteins are particularly important since binding properties in meat products are dependent upon extracting protein from muscle tissues. Soluble muscle proteins subsequently undergo thermally induced changes in conformation and aggregation resulting in gel formation. The gelation of muscle proteins is largely responsible for binding of comminuted meats and the physical stability of various emulsified meat products (Yasui et al, 1980; Asghar et al, 1985).

Myofibrils are the principle component of muscle cells. In muscle food systems, myofibrillar proteins represent the major source of extractable protein. Myofibrils are filamentous at physiological ionic strength, and their constituent proteins cannot be solubilized without the addition of salt. Traditionally processed meat products contain 3% added salt, which is sufficient to extract and dissociate the principal contractile proteins of the myofibril, actin and myosin. Actin, the major component of the thin filament, accounts for 22% of the myofibrillar protein, while myosin, the major constituent of the thick filament, accounts for 43% of the myofibrillar protein. Purified myosin protein will form a gel when heated and such myosin gels exhibit many rheological characteristics associated with muscle food systems (Morita et al., 1987; Choe et al. 1991). However, unlike myosin, purified actin neither forms a gel nor exhibits any functional binding characteristics in model systems (Yasui et al., 1980, Sano et al., 1989), although actin has been found to exert a maximum synergistic effect on myosin gels when present at an actin:myosin molar ratio of 2.7 (Samejima et al., 1982).

The physiochemical properties of myosin have been extensively studied and numerous reviews have been published (Goldman, 1998; Baker and Titus, 1998; Cooke, 1997; Schiaffino and Reggiani, 1994). The focus of this manuscript will be to integrate the basic research performed on the solubility properties of myosin, with the applied research into the role of myosin in protein-protein interactions that determine meat binding and otherwise contribute to the textural properties of processed meat products. This manuscript will first review the basic structure of myosin proteins, followed by a discussion of myosin's participation in the functional properties of muscle food systems. Lastly, recent studies on the role of specific amino acids and regions of the myosin molecule that govern myosin solubility will be presented along with potential applications for meat processing technologies.

Myosin Structure

Skeletal muscle myosin is a large and complex structure that appears in the electron microscope as an asymmetric molecule with

two globular heads attached to a long tail. It consists of two heavy chain subunits of approximately 200 kDa and two pairs of small subunits referred to as regulatory and essential light chains. Each myosin head is associated with a regulatory and an essential light chain. The functional roles of myosin light chain subunits in muscle contractility have not been established but neither are required for actin binding, actin-activated ATPase activity, or force generation (Wagner and Giniger, 1981; Lowey and Trybus, 1995; Waller et al., 1995). The genes encoding many myosin heavy chain and light chain subunits from animals with striated muscles have been cloned and their primary amino acid sequences determined. The complete amino acid sequence of the chicken myosin heavy chain from adult pectoral muscle has recently been published (Maita et al., 1991) and a cDNA clone encoding the entire protein has been produced (Chao and Bandman, 1997). Since many of the studies carried out on muscle food systems over the past 20 years employed myosin from this species, this manuscript will focus on the structural properties and characteristics of adult chicken myosin. It should be noted however, that even within a single species there are many different myosin genes, encoding similar but not identical myosin proteins. A full discussion of the functional significance of myosin isoform diversity has been the subject of recent reviews (Bandman, 1985; Schiaffino and Reggiani, 1994)

Most of our understanding of myosin's structural and chemical properties was derived from the study of proteolytic fragments. Treatment of filamentous myosin with papain yields two subfragment 1 (S1) protein fragments that represent the globular myosin heads and a single α-helical coiled-coil rod (Margossian and Lowey, 1982). Treatment of soluble myosin with chymotrypsin splits the molecule into 2 different fragments: heavy meromyosin (HMM) and light meromyosin (LMM) (Margossian and Lowey, 1982). The HMM fragment contains two myosin heads and approximately 1/3 of the of the N-terminal end of the α-helical coiled-coil rod, while the LMM fragment represents the remainder of the rod. The region at which trypsin cleaves the myosin rod is referred to as the myosin hinge, a more flexible part of the α-helical coiled-coil allowing the rod to bend. This flexibility allows myosin heads to project from the surface of thick filaments and facilitates actin-myosin interactions within the myofibril. Extensive physiochemical and physiological studies have been performed on S1, HMM, LMM and myosin rod fragments. These experiments have provided valuable information regarding the behavior of myosin within living muscle as well as its functionality in meat products.

The myosin head consists of the N-terminal 840 amino acid residues of the myosin heavy chain subunit and two light chain subunits. The myosin head contains all the necessary components to generate movement, including the actin-binding interface and the site for ATP hydrolysis. The myosin heavy chain fragment within the S1 can be cleaved by trypsin into three fragments (25, 50, and 20 kDa) and the location of functional amino acid residues within these peptides has been extensively studied (for review see Rayment et al., 1996). Four post-translationally methylated amino acids are found in the S1 myosin heavy chain peptide, although their functional significance is unclear. The three dimensional structure of the S1 from adult chicken pectoral muscle was determined by X-ray diffraction (Rayment et al., 1993). This revealed that 48% of the secondary structure of the myosin heavy chain within the S1 was represented by amino acids in the α-helix conformation, including a major portion of the base of the myosin head which serves as a lever arm and contains the binding sites for both light chains. The thick portion of the S1 contains a seven-stranded β-sheet motif, surrounded by α-helices, loops and turns. The actin and ATP binding sites are located on opposite sides of the protein. The three dimensional structure also revealed that the 50, 25, and 20 kDa fragments do not form individual folding domains, but that the cleavage sites represented flexible loops susceptible for proteolysis. Hydrophilicity hydropathy analyses illustrate many hydrophobic regions within the S1 (Molina et al. 1987, Chao and Bandman, 1997) which likely participate in aggregation phenomena following denaturation of the native structure.

The rod begins following a proline residue at position 840 in the adult chicken myosin heavy chain (Chao and Bandman, 1997). The amino acid sequence of the rod follows the 7 amino acid heptad motif (**a, b, c, d, e, f, g**)n characteristic of α-helical coiled-coil proteins, with **a** and **d** positions occupied predominantly by hydrophobic residues (McLachlin and Karn, 1982). Stability of the coiled-coil is provided by hydrophobic interactions at the interface of the two helices between the side chains of residues at **a** and **d** positions of opposite strands (Hodges et al., 1990). Residues at positions **e** and **g** which flank the hydrophobic core are occupied predominantly by charged amino acids which may contribute to further stabilization of the coiled-coil interface both by enhancing the hydrophobic effect and by participating in interhelical salt bridges (O'Shea et al., 1991). A putative role of these residues in myosin filament morphology has recently been proposed (Arrizubieta and Bandman, 1998). An additional repeating motif in the myosin rod that has been highly conserved includes a striking repetition of positively and negatively charged amino acid residues at positions **b**

and **c** on the outer surface of the coiled-coil spaced 14 residues apart (McLachlan and Karn, 1983). These residues are believed to play a functional role in myosin rod interactions that generate the characteristic 14.3 nm axial stagger of molecules within the myosin filament (Huxley, 1974). An additional conserved feature of striated muscle myosin rods has been the insertion of 4 "skip" residues at specific positions within the α-helical coiled-coil sequence (McLachlan and Karn, 1983). The precise function for these residues remains unclear but their positions appear to correlate with bends in the myosin tail observed in the electron microscope (Offer, 1990). The rod domain can be further subdivided into 2 peptides, subfragment 2 (S2) and light meromyosin (LMM) at the highly variable and flexible hinge region (Lowey et al., 1969). The S2 represents the α-helical coiled-coil portion of the HMM, and like the HMM, the S2 fragment is soluble at low ionic strength. The amino acid sequence of the S2 shows that there are fewer hydrophobic residues and a greater number of charged residues at the **a** and **d** helical positions at the core (Maita et al., 1991; Chao and Bandman, 1997). This could render the coiled-coil less stable and account for the bend observed in this region of the rod in the electron microscope. The LMM fragment, like the full-length rod, retains the solubility characteristics of myosin. LMM aggregates in low salt usually form paracrystals with distinct axial banding patterns that exhibit 43 nm and 14.3 nm repeats and have been the focus of many studies. These features of the paracrystal are thought to be related to the 43 nm helical repeat and the 14.3 nm axial translation with which myosin heads protrude from the surface of the thick filament (Stewart and Kensler, 1986). How these structural elements of the myosin rod participate in governing myosin solubility, aggregation into myosin filaments, and the formation of myosin gels following heating is discussed in the following sections.

Myosin Solubility

In muscle tissue at physiological ionic strengths (0.15 – 0.2M) myosin is insoluble and exists as discrete thick filaments within the myofibril. Methods for the extraction and purification of myosin from skeletal muscle typically employ the use of NaCl or KCl salts at concentrations ranging from 0.3 – 0.6 M or higher, although care must be taken to prevent the salting out of protein that occurs at ionic strengths above 1.0 M. Low molecular weight polyphosphates (ie. sodium pyrophosphate) and divalent cations (ie. $MgCl_2$) are often incorporated into purification protocols in order to facilitate myosin extraction as well (Yasui et al., 1964). Since myosin's isoelectric point is close to pH 5, solubility increases as the pH is elevated above 6.0 (Malamud and Drysdale, 1978). As long as the high ionic strength conditions and appropriate pH are maintained, myosin remains soluble. Upon lowering the ionic strength below 0.3M myosin spontaneously aggregates into bipolar filaments (Huxley, 1963). Although these "synthetic" filaments generally resemble native thick filaments isolated from muscle tissue, they are more heterogeneous in size and morphology, and of course lack many of the myosin binding proteins that are structural components of the native thick filament in the myofibril. Depending upon the pH and the ionic strength, a variety of structures are observed. As a general rule, the lower the pH the longer and thicker the filament. A full discussion of the reassembly of myosin into filaments can be found in the excellent review on the morphology of synthetic myosin filaments and putative mechanisms of their assembly (Davis, 1988).

From the perspective of the meat technologist, the extraction of myosin and other salt soluble proteins from muscle is the ultimate objective in order to obtain good binding properties in restructured and comminuted meat products. The addition of 2-3% salt and 0.5% polyphosphate to meat formulations ensures adequate myosin extraction and enhanced binding at pH 6.0 or above (Siegal and Schmidt, 1979). Interestingly, not all myosin isoforms appear to be extracted equivalently. Studies of chicken breast muscle and leg muscle extraction have shown that the amount of myosin released from myofibrils as a function of time under equivalent ionic and pH conditions is greater for white muscle myosins compared to red muscle myosins (Xiong and Brekke, 1989; Xiong, 1994). Similar results have been observed by monitoring A band extraction in the phase contrast microscope of myofibrils from red and white muscles (Parsons and Knight, 1990). Porcine cardiac myofibrils have also been found to release less myosin than rabbit skeletal myofibrils when extracted under identical conditions (Samejima et al., 1992). Fast and slow/cardiac muscles do contain different myosin isoforms (reviewed in Bandman, 1985). However, it is possible that this differential myosin extraction results from significant differences in the ultrastructure of the myofibrils and the influence of specific myosin binding protein isoforms of red and white muscles rather than inherent differences in myosin isoform solubility (Parsons and Knight, 1990). Similarly, the enhanced extraction of myosin from pre-rigor meat when compared with that of post-rigor meat has been explained as resulting from the reduced number of actomyosin crosslinkages hindering myosin release from myofibrils (Offer and Trinick, 1983). However, it has been observed that myosin extractability of chicken breast (white) muscle, but not leg (red) muscle, was enhanced following rigor at pH >5.8 (Xiong and Brekke, 1991). These authors proposed that greater proteolytic activity in white muscle fibers might be

responsible for ultrastructural changes in the myofibril conducive to easier release and extraction of myosin. Other phenomena may also influence myosin extraction from skeletal muscle. Myosin solubility is markedly reduced in porcine PSE muscle compared to that of normal pigs presumably as a consequence of the protein denaturation that occurs during the early post-mortem pH decline (Camou and Sebranek, 1991).

The Role of Myosin in Meat Binding

Myosin is the muscle protein responsible for texture formation and binding between meat pieces upon cooking. While myosin is not the only protein extracted with the addition of salt and polyphosphates, myosin is the only one that when purified will form a gel upon heating. Actin does not exhibit any binding or gel forming properties upon thermal denaturation, but will complement and enhance the binding characteristics of myosin. Research on experimental systems has shown that the highest gel strength is obtained when the myosin to actin ratio was maintained at 2.7, with 15-20% of the total protein present as an actomyosin complex, and the remainder being free myosin (Samejima et al., 1969). Many studies show that binding quality and binding strength of comminuted meat products as well as a plethora of model systems were correlated with the amount of extracted myosin (see review by Asghar, 1985).

Studies on the thermal gelation characteristics of myosin subfragments point to the myosin rod as being primarily responsible for the essential structure and functional properties of the gel matrix (Samejima et al., 1981; Ishioroshi et al., 1981; Sano et al., 1990; Smyth et al., 1996). Intact myosin, myosin rod, and LMM formed firm gels upon heating, while S1 exhibited poor gelling ability. Unlike its effect on intact myosin, F-actin had no effect on gel strength of any myosin rod fragments. These results indicate that the cross-linking between free myosin and actin-bound myosin was mediated through myosin rod interactions. Electron microscopy revealed the formation of globular aggregates with myosin tails radiating outward when myosin was heated (Yamamato, 1990; Sharp and Offer, 1992). This suggests that the globules represent myosin heads that aggregate when their extensive hydrophobic regions are exposed following denaturation. This arrangement of myosin molecules is remarkably similar to the way in which myosin forms a monomolecular protein film around lipid in meat batters. In raw meat batters the hydrophobic myosin head is believed to interact with the fat globule, while the radiating myosin tails interact and subsequently thicken and strengthen the protein gel matrix (Gordon and Barbut, 1992). Thus the lower gel rigidity of the denatured myosin rod fragment and the enhanced gel rigidity of actomyosin-containing systems suggests that optimum binding characteristics result from a precisely ordered array of myosin tails. Subsequent unfolding of the α-helix of the tail would then expose hydrophobic amino acids and result in cross-linking of the gel network. Non-dissociated myosin filaments also form gels when heated. However these gels tend to be more rigid than monomeric myosin gels and their properties vary with differences in filament length (Yamamoto et al., 1988). Since dissolution of myosin filaments in muscle food systems is likely incomplete, when intact filaments are subjected to heat denaturation, the myosin heads protruding from the surface may play a role in inter-filament cross-linking as has been observed in model systems with synthetic myosin filaments (Yamamoto et al., 1988).

From this discussion it is clear that myosin plays an integral part in the binding characteristics of comminuted meat products. Myosin has a similar role in meat emulsion stability. Understanding the physiochemical parameters that govern myosin solubility and aggregation is important to the meat scientist in the design, formulation, and preparation of a high quality processed meat product. These properties ultimately are derived from the amino acid sequence of the protein. The following section will present recent studies using recombinant DNA technology to investigate and identify those sequence elements within the myosin rod responsible for myosin's unique solubility properties.

Identification and characterization of regions of the myosin rod that regulate insolubility at low ionic strength

Interactions between myosin rods are responsible for myosin aggregation into native and synthetic filaments. Previous studies of myosin filament assembly and studies of the role of the rod domain in myosin solubility were carried out on proteolytically derived myosin rod fragments. Although these studies have been useful for analyzing molecular interactions between myosin rods, the use of proteases to prepare myosin fragments can result in peptide heterogeneity due to the lack of specificity of the enzymes used. For example, the non-helical conformation of the last 9-17 amino acids at the C-terminus of the myosin rod renders these amino acid residues susceptible to proteolytic activity during the preparation of the rod and LMM fragments. In recent years many myosin genes

have been isolated and the DNA sequences encoding myosin proteins have been cloned and sequenced. This has facilitated our ability to not only prepare large amounts of myosin rod and rod fragments without the use of proteases, but also permitted the introduction, removal, or substitution of specific amino acids into the myosin rod sequence.

Extensive studies of non-muscle myosins indicated that the region near the C-terminus of the rod is important for filament formation (Sinard et al., 1990; Hodge et al., 1992). Initial studies using proteolytically prepared or bacterially expressed recombinant muscle myosin rod fragments also show that an intact C-terminus is essential for ordered aggregation in vitro. It was shown that removal of the C-terminal 100 amino acids drastically altered LMM solubility (Atkinson and Stewart, 1991) and that removal of as few as 17 amino acids from the C-terminus of the rod could effect solubility (Maeda et al., 1991). NMR spectroscopy revealed that these latter C-terminal 17 residues are not in a α-helix but in an unfolded and more freely mobile conformation (Kalbitzer et al., 1991). Recently a 29 amino acid sequence beginning 65 residues from the C-terminus of the rod was shown to regulate insolubility and ordered aggregation of a shortened LMM fragment into paracrystalline arrays at low ionic strength (Sohn et al., 1997). In these experiments it was observed that removal of the 65 C-terminal amino acids from a 369 amino acid LMM fragment produced a low salt soluble protein. Furthermore, the addition of the 29 amino acid sequence to a low salt soluble 389 amino acid rod fragment derived from the N-terminus of the rod, produced a rod fragment with similar solubility and aggregation properties as the LMM. This 29 amino acid sequence was termed the assembly competence domain (ACD) and appears to be conserved in all myosin rod sequences that have been determined (Sohn et al., 1997).

Experiments in our own laboratory however suggest that myosin solubility and assembly involve more than the 17 amino acids from the C-terminus and the ACD. We have confirmed the importance of the C-terminal region of the myosin rod in the molecular interactions that lead to the formation of myosin filaments using monoclonal antibodies whose binding sites in the myosin rod have been mapped (Moore et al., 1992). 5C3 is a monoclonal antibody that binds to the myosin rod within 12 amino acid residues of the C-terminus. NA4 is a second monoclonal antibody that binds near the end of the myosin rod, approximately 100 amino acid residues from the C-terminus. Both of these antibodies will bind to soluble myosin in 0.5 M NaCl and prevent myosin from aggregating into higher order structures in low ionic strength buffers. Thus these antibodies are capable of rendering myosin soluble at physiological salt concentrations. Antibodies that bind to the middle or N-terminal end of the LMM domain (EB165 and AB8) had no effect on myosin solubility or the morphology of filaments formed (Wick et al., 1996). Thus, it appears that the C-terminus of the muscle myosin rod participates in molecular interactions that are required for fibrillogenesis

Further studies to map the precise location of amino acid residues that participate in these interactions have suggested that multiple regions within the C-terminal 100 amino acids contribute to the overall solubility of the LMM. When the 16 amino acids from the C-terminus of a 650 amino acid chicken LMM were deleted (the equivalent region to the 17 residues in the rabbit LMM which were removed in the experiments by Maeda et al., 1991), a LMM protein that required slightly lower ionic strength conditions to aggregate was produced. However, unlike previously published results (Maeda et al., 1991), the chicken LMM lacking the non-helical tailpiece still remained insoluble at 50 mM KCl. This discrepancy between the two studies may be due to differences between chicken and rabbit myosins, but is more likely due to the different sizes of the LMM proteins employed in the studies. The former experiment used only a 262 amino acid C-terminal LMM fragment, while our studies employed the full length 655 amino acid peptide. In other published studies, removal of 92 C-terminal amino acid residues from a 567 amino acid LMM fragment was required to produce a LMM peptide soluble at ionic strengths down to 0.05 M (Atkinson and Stewart, 1991). This suggests that additional C-terminal deletions were likely required in order to alter the solubility of the chicken LMM peptide.

We have now generated many LMM fragments with C-terminal mutations in order to more precisely map those regions required for insolubility at low ionic strength. Consistent with previous studies we have found deleting a total of 100 amino acids from the C-terminus is necessary in order to generate a completely soluble LMM fragment (Atkinson and Stewart, 1991). LMM fragments lacking either 44 or 72 amino acids of the C-terminus had similar solubility properties as LMM fragments lacking 16 amino acids. The aggregates formed from the mutated LMM proteins were also examined in the electron microscope. It was observed that all the LMM fragments formed paracrystalline structures. The intact LMM, the LMM lacking 16 residues, and the LMM lacking 44 residues all formed paracrystals that had a similar staining pattern. The LMM lacking 72 residues also formed a paracrystal, but it had a

unique staining pattern compared to the other aggregates, suggesting a different arrangement of the mutant LMM peptide within the paracrystalline lattice. The significance of these observations lies in the fact that an LMM protein lacking the ACD (Sohn et al., 1997) was still insoluble at low ionic strength, and competent to form ordered structures. We conclude from our observations that there are multiple domains near the myosin tail that contribute to myosin aggregation in low salt. Removal of any one may alter the ability to aggregate, but is not absolutely essential for aggregation. Future mutations will be required to precisely define the borders of these regions and to determine whether sequences other than the ACD within the C-terminal 100 amino acids can also restore the ability to aggregate in low salt conditions. Based on our studies it also appears that the total size of the LMM peptide contributes to aggregation. Thus the effect of removing any single solubility domain may cause one fragment to become fully soluble while the same deletion of a larger LMM peptide may have little or no effect. Thus it cannot be ruled out that different results may be obtained in studies using the full-length myosin rod as opposed to the full-length LMM fragment. Clearly future analyses of myosin rod mutations are necessary to provide further insights into the precise roles of the rod sequence in determining myosin's solubility characteristics.

Implications and future research needs

Binding quality is a functional characteristic of comminuted and restructured processed meat products, which is dependent upon the physical state of myosin and its interactions with other muscle proteins. Extensive studies of myosin solubility and heat denaturation and aggregation have resulted in an understanding at the molecular level of the thermally induced formation of myosin containing gels. Current hypotheses suggest that the addition of NaCl to 2-3% dissociates myosin filaments and generates a soluble pool of myosin. The addition of 0.5% polyphosphates can further augment myosin release from myofibrils by disrupting actin-myosin linkages in post-rigor muscle. Upon initial heating, the denaturation of the S1 domain results in aggregation, mediated through the newly exposed hydrophobic domains. Upon further heating, the myosin rod unfolds with the resultant formation of a protein gel network. Formulations lacking salt and polyphosphate lack binding properties. Although intact myosin filaments when heated will also form a gel-like protein network, without soluble myosin molecules in the matrix, there appears to be insufficient binding to maintain structural integrity of comminuted products.

Meat processors have attempted to address the issue of making products with less salt in order to satisfy consumer's and health official's concerns regarding the potential long-term impact of excessive salt in the diet. Clearly a goal of future studies should be to further characterize means of dissociating myosin from myofibrils without employing high ionic strength conditions. Current studies aimed at clarifying the mechanism(s) of myosin assembly in the myofibril should provide further information on which regions of the myosin molecule should be the focus of future studies of the meat scientist. An understanding of the role of specific amino acids and specific regions of the myosin rod that are involved in maintaining intermolecular myosin interactions should help meat scientists design new technologies for generating binding properties based upon intrinsic meat proteins and without employing exogenous binding agents.

Current studies have clearly identified the C-terminal 100 amino acids of the myosin rod as the crucial domain that initiates the aggregation of myosin into macromolecular aggregates. A crucial question that has yet to be answered is whether myosin can be dissociated from myofibrils by disrupting the interaction of the C-terminal amino acids of myosin rods within the myosin filament? If the answer to this question is yes, research strategies employing engineered proteases that specifically cleave the myosin rod near the C-terminus may be employed to determine whether sufficient free myosin to generate binding activity can be achieved? Additional methods for intervening in the specific interactions within this solubility domain may also lead to the development of processed meats with little or no added salt.

Further research into the contribution of various regions of the myosin molecule in thermally induced gel formation may also facilitate the design of new meat processing technologies. Extensive studies with proteolytically-derived myosin fragments have proven useful in increasing our understanding about how soluble myosin participates and interacts with other meat proteins to form the network that binds muscle chunks during heating. These studies have enabled meat scientists to modulate heating parameters to achieve maximum bind without the loss of excessive water holding capacity and other quality characteristics. With the use of recombinant DNA technology it is now possible to produce specific myosin fragments in sufficient quantity for rheological studies to

investigate how any region of the molecule mediates the formation of a thermally induced gel network. Ultimately, the results of such studies may lead to the discovery of new alternative binding agents that can functionally substitute for soluble myosin protein and thus permit the production of salt-free processed meats in the future.

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