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Recent Advances in Meat Science in Japan: Functionality of Muscle Proteins in Gelation Mechanism of Structured Meat Products

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Abstract

Studies on the functionality of muscle proteins in gelation mechanisms of structured meat products during the last three decades are briefly reviewed. Among muscle proteins, myosin is primarily the most important protein for gelation, whereas actin plays a complementary role in gelation when it coexists with myosin. The combined data of gelation and denaturation indicate that the gelation of myosin consists of two stages: (1) aggregation of myosin molecules through their heads at 43°C; and (2) cross-linking reaction due to helix-coil transition of the tail portion of the molecules at 55°C. In the myosin-actin system, a system consisting of 80% of free myosin 20% of actomyosin complex on heating yielded a gel with a maximum strength. The reinforced network structure of myosin by actomyosin complex is completed at 60-65°C.

Discipline: Animal industry
Additional key words: actin, cross-linking, denaturation, myosin

Introduction

During the last three decades, a great deal of works on the biochemistry of meat have been undertaken in Japan, with emphasis on post-mortem skeletal muscle which influences organoleptic quality of the meat, including color, texture relating mainly to water-holding capacity and tenderness, and flavor. The present paper reviews briefly the results of the studies dealing with functionality of muscle proteins in gelation mechanisms of structured meat products such as cured meat.

The effective contractile machinery of vertebrate striated muscle represents an elaborate framework. The motion of myosin and actin filaments is controlled by regulatory proteins and their position is supported by cytoskeletal proteins. Quantitatively, the major constituents of muscle, being functionally the most important, are a continuous array of the proteins comprising thick filaments, composed of myosin, which are interdigitated with thin filaments, composed of actin (Fig. 1)[10]. They are disposed in units of contraction (sarcomeres) by transverse binding structures (Z lines, Fig. 1). Approximately 65% of the total myofibrillar proteins is myosin and actin, both of which are contractile proteins of muscle. There are a number of regulatory and cytoskeletal proteins[10,14] listed in Table 1[14].

In this article, the authors attempt to present a discussion on the role of different proteins in the gelation mechanisms of structured meat products, which are becoming increasingly popular in consumption throughout the world, during thermal processing.

Functions of muscle proteins in gelation

The binding of chunks or ground pieces of meat in cooking is a heat-induced phenomenon which

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Table 1. Myofibrillar structural proteins of rabbit skeletal muscle[14]

Protein	Molecular weight (kDa)	Content (wt %)	Localization	Functions
Contractile proteins				
Myosin*	520	43	A band	Contracts with actin
Actin*	42	22	I band	Contracts with myosin
Regulatory proteins				
Major				
Tropomyosin*	33 × 2	5	I band	Binds to actin and locates troponin
Troponin	70	5	I band	Ca regulation
Troponin C*	18			Ca binding
Troponin I*	21			Inhibition of actin-myosin interaction
Troponin T*	31			Binding to tropomyosin
Minor				
M protein	165	2	M line	Binds to myosin
Myomesin	185	<1	M line	Binds to myosin
Creatine kinase*	42	<1	M line	Binds to myosin
C protein	135	2	A band	Binds to myosin
F protein	121	<1	A band	Binds to myosin
H protein	74	<1	Near M line	Binds to myosin
I protein	50	<1	A band	Inhibits myosin-actin interaction
α-Actinin	95 × 2	2	Z line	Gelates actin filaments
β-Actinin	37 + 34	<1	Free end of actin filament	Caps actin filaments
γ-Actinin	35	<1	?	Inhibits actin polymerization
eu-Actinin	42	<1	Z line	Binds to actin
ABP (filamin)	240 × 2	<1	Z line	Gelates actin filaments
Paratropomyosin	34 × 2	<1	A-I junction	Inhibits actin-myosin interaction
Cytoskeletal proteins				
Connectin (titin)	2800 (2100)	10	A-I	Links myosin filament to Z line
Nebulin	800	5	N_2 line	
Vinculin	130	<1	Under sarcolemma	
Desmin* (skeletin)	53	<1	Periphery of Z line	Intermediate filament
Vimentin*	55	<1	Periphery of Z line	Intermediate filament
Synemin	220	<1	Z line	
Z protein	50	<1	Z line	Forms lattice structure
Z-nin	400	<1	Z line	

* Complete amino acid sequences have been determined for those proteins marked by an asterisk.

involves protein-protein interactions, since raw meat pieces do not cohere to any significant extent. In this respect, a number of interesting findings have been reported by some meat scientists, including identification of the protein(s) which provides a binding force in cured processed meat, and the mechanism of binding in meat[2,26].

Following the preferential extraction methods developed by Hanson and Huxley[4], Huxley and Hanson[5] and Perry's group[15,16], myofibrils simplified from beef *M. semitendinosus* (Fig. 2(a)) were prepared to compare the binding quality of sausage manufactured from them in connection with their physicochemical properties. The results indicated

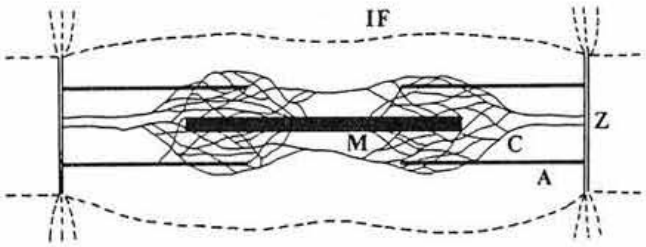

Fig. 1. Diagrammatic illustration of a longitudinal section of a sarcomere of vertebrate skeletal muscle[10]
M: Myosin filaments, A: Actin filaments,
C: Connectin filaments, IF: Intermediate filaments,
Z: Z lines.

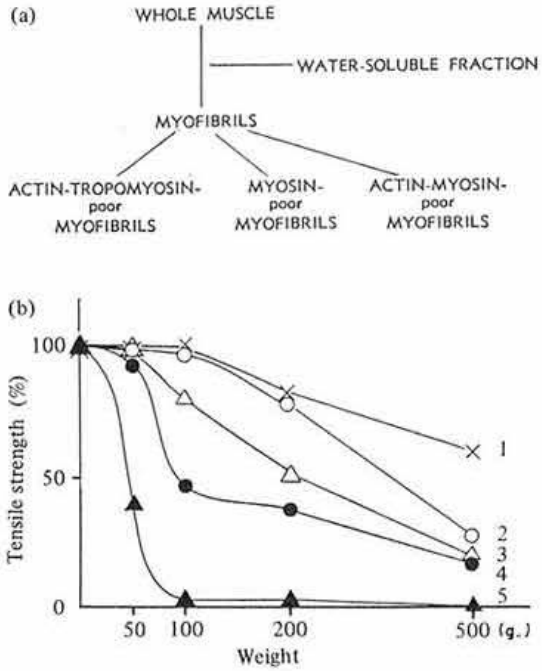

Fig. 2. Relationships between binding quality of meat and meat proteins
(a) Classification of various muscle models from fresh beef muscle.
(b) Tensile strength of sausage from various muscle models.
1: Whole muscle, 2: Intact myofibrils, 3: Actin and tropomyosin-poor fibrils, 4: Myosin-poor fibrils, 5: Actin-myosin-poor ("ghost") fibrils.

that myosin played a decisive role in affecting the binding quality of sausage, because a poorer binding took place in the sausage prepared from fibrils

Table 2. Comparison of physicochemical properties in preparations of various muscle molecule

	Remaining after extraction (%)		Super precipitation	Binding quality
	Protein	ATPase		
Whole muscle	100	100	+	+
Isolated myofibrils	71.4	97.4	+	+
Actin- and tropomyosin-poor myofibrils	45.4	92.6	+	+
Myosin-poor myofibrils	55.2	25.9	+	±
"Ghost" myofibrils	24.8	9.3	+	−

of low ATPase activity than that of high ATPase activity (Table 2). As shown by tensile strength (Fig. 2(b)), the binding property varied primarily according to the quantities of native myosin ATPase in the myofibrils. These results imply that adequate quantities of native myosin in fibrils are indispensable to compact binding, and that water soluble proteins, actin and other regulatory proteins have no direct effect on that quality.

Denaturation of myosin

Myosin is a double-headed enzyme protein which possesses two pear-shaped heads (S-1, Fig. 3) and a characteristic coiled-coil tail (rod, 100% α-helical) which is connected by a hinge region. It has three important biological functions: i.e., (1) ATPase activity, (2) actin binding ability, and (3) thick filament formability under the physiological ionic

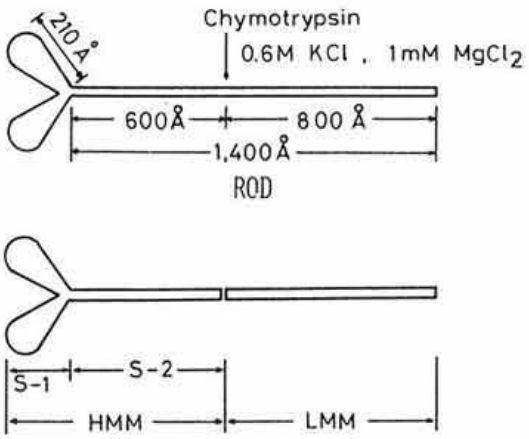

Fig. 3. Schematic diagram of the myosin molecule

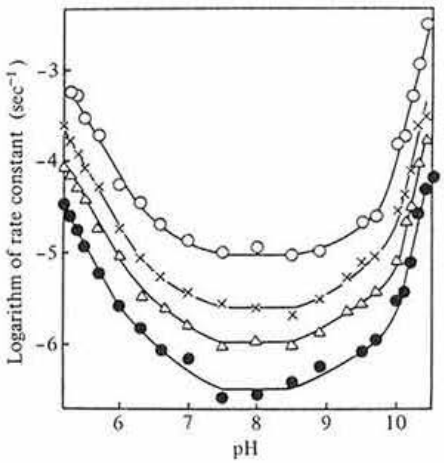

Fig. 4. Effects of temperature and pH on the inactivation rates of myosin ATPase in 0.5 M KCl
○: 25°C, ×: 20°C, △: 15°C, ●: 10°C.

environment. The former two functions reside exclusively in the S-1 region and the latter one is localized in the rod. It is possible to fragment the molecule into heads and a tail by limited proteolysis and to investigate their functions separately.

When isolated and dissolved in high salt solution such as 0.6 M KCl or NaCl, myosin is rather labile and denatures its functions easily even at the body temperature of animals used. At 35°C and neutral pH, its enzymic activity decreases exponentially and viscosity concomitantly increases[7], suggesting local as well as overall conformational changes of the molecule. Fig. 4 summarizes the inactivation behavior of myosin ATPase under varying denaturation conditions. It is evident from Fig. 4 that the inactivation consists of two independent components: i.e., pH-independent, but temperature-dependent inactivation which is seen between pH 7.5 and 8.5; and temperature-independent, but pH-dependent inactivation taking place in either acidic or alkaline region.

All data using electronmicroscopy[7], ultracentrifugation[7] and gel-filtration[7] indicated that the S-1 region of the molecule fused into large aggregates during the course of inactivation. This implies that the apparent increase in viscosity is solely due to the head-head aggregation of the myosin molecules. Then, what happens with the tail portion of the molecule? Studies on the thermal denaturation of the chymotryptic rod subfragment showed typical helix-coil transition between 35 and 70°C with an apparent Tm at about 53°C (Fig. 5). The reaction was found to be partially irreversible. The secondary structure once melt at high temperatures never restored to its initial level at pH 6. Changes in the difference spectra of the rod at 285 nm[18] and fluorescence emission spectra at 339 nm[17] measured under the same condition indicates that local conformation changes in the polypeptide chain residing proximal to aromatic amino acid residues are gradually transferred from a moderately hydrophobic environment inside the protein molecule to an exposed polar environment, and that those changes probably never return to their original positions[18].

Heat-induced gelation of myosin

Binding of meat pieces by curing and subsequent cooking implicates that the meat system is converted irreversively from dispersed sol-type to solidified gel-type. Regarding such transformation as that generally observed on high polymers, changes in rigidity (shear modulus) of the myosin system were investigated under the same conditions for the denaturation studies of the protein. As shown in Fig. 6, it was found that the heat-induced gelation of myosin on stepwise heating ranging over 10 to 70°C showed an optimum level of its temperature and pH at 65°C and 6, respectively (Fig. 6(a), (b)). The derivative plot revealed the change with two stages at approximately 43 and 55°C (Fig. 6(a) insert).

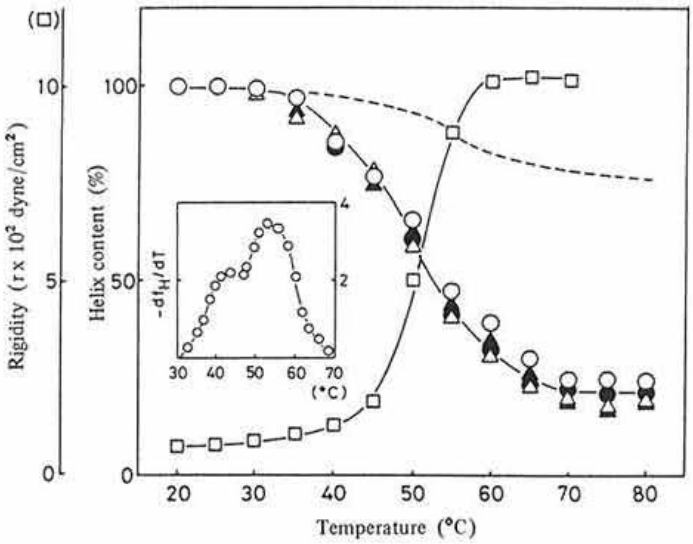

Fig. 5. Temperature profile of the rod obtained from ORD and CD measurements
Changes in helical content of the rod were derived from the measured ORD and CD parameters during thermal treatment[18]. The protein (0.5 mg/ml) in 0.6 M KCl and 20 mM phosphate buffer (pH6) was thermally treated for 20 min at various temperatures specified on the abscissa.
□: Rigidity, ○: Helical content of the rod obtained from ORD, △: from CD measurements in the absence (○, △) and presence (●, ▲) of 1 mM DTT. Dotted line: Reversibility of the helix content after overnight storage at 0°C of samples.
(Insert): Derivative plot of fraction of helix (fH) of the rod as a function of temperature (ΔfH/ΔT).

The combined data of gelation and denaturation demonstrate the relationship between these two functions of myosin (Fig. 7). The figure presents the changes in ATPase actively and turbidity of myosin as well as in helical content of the rod together with that in rigidity of the myosin solution (4.5 mg/ml in 0.6 M KCl) equilibrated for 20–25 min at different temperatures varing from 10 to 70°C under the constant value of pH 6. The inactivation of myosin ATPase took place exponentially in the temperature range over 20 to 40°C, and the activity was lost almost completely at 40°C. The turbidity of the solution increased linearly but slightly in the above temperature range. Thereafter, the rise was drastic and reached a maximum value at 60°C. Then, it leveled off. The helix-coil transition in the rod, as revealed by an optical rotatory dispersion (ORD) and circular dichroism (CD) studies, commenced at about 40°C and completed at 65°C with an apparent Tm at around 50°C. On the other hand, the rigidity of the system also increased linearly with elevation of temperature from 20 to 30°C. Beyond this level of temperature, the increase of rigidity was exponential and attained a maximum value at 60 to 65°C. It is to be mentioned that among those parameters, the presence of 1 mM dithiothreitol (DTT), SH-protecting reagent, has no concerns with the inactivation of myosin ATPase and the helix-coil transition of the rod, but it does affect the turbidity and thermogelling reaction.

Thus, a series of incidences occurring during the stepwise heating of the myosin solution may be described as follows: First, thermal treatment of the solution first induces local conformation change around the enzymic active site domain in the head region of the molecule without oxidation of SH-group(s). Second, as a result of this change, the inactivated myosin molecules tend to aggregate through their heads. The reaction is more or less SH-oxidation-dependent and it triggers the incipient

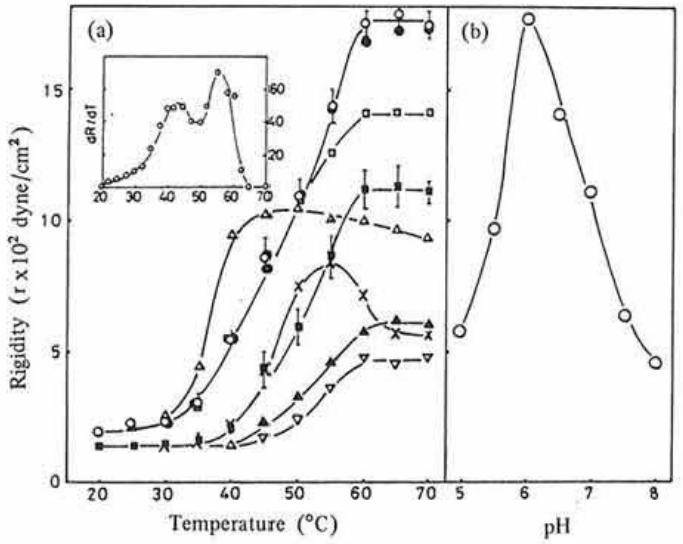

Fig. 6. Changes in the rigidity of thermally treated myosin
(a): Myosin (rabbit) dissolved in 0.6 M KCl or NaCl and 20 mM citrate (pH 5–5.5), phosphate (pH 6–7) or tris-HCl (pH 7.5–8) buffer was incubated for 25 min at temperatures from 20–70°C. Rigidity of each system was measured at the temperature indicated. × : KCl at pH 5, △: KCl at pH 5.5, ○: KCl at pH 6, ●: NaCl at pH 6, □: KCl at pH 6.5, ■: KCl at pH 7, ▲: KCl at pH 7.5, ▽: KCl at pH 8.
Bars for pH 6 and 7 indicate standard errors of 10 experiments on 6 myosin from different animals.
(Insert): Derivative plot as a function of temperature. Differential rigidity ($\Delta R / \Delta T$) of the system at pH 6.0 (KCl) was plotted against temperature.
(b): pH-dependence of heat-induced gelation of myosin in 0.6 M KCl at 65°C.

gelation of the system. Third, above 40°C, the secondary structure of the rod unfolds. Fourth, exposed hydrophobic residues produce intermolecular cross-linkages among molecules and complete their network through out the system. The molecular mechanism of the heat-induced gelation of the myosin system is schematically illustrated in Fig. 8. The figure indicates that the gelation of myosin consists of two stages, i.e., aggregation of myosin molecules through their heads at 43°C and another cross-linking reaction due to helix-coil transition of the tail portion of the molecules at 55°C.

Effect of actin

Unlike myosin, actin is a multifunctional protein and can bind itself with not a few other proteins[11]. In high salt solution, it binds to myosin and forms actomyosin complex, whose structure looks like arrowheads under the electronmicroscope. An extract of meat with high salt solution contains both components of thick and thin filaments in the myofibril, myosin and actin, and they exist as actomyosin complex[22]. Nakayama and Sato[12,13], who examined the rheological properties of heat-set meat protein gel, reported that the binding strength of reconstituted as well as natural actomyosin was greater than that of myosin alone. In contrast, Macfarlane et al.[8] found a stronger binding force in myosin than an actomyosin gels. Siegel and Schmidt[21] observed that a high proportion of myosin to actin within the ratio of 3 to 8 by weight resulted in high binding ability.

To resolve this disparity, the effect of actin on

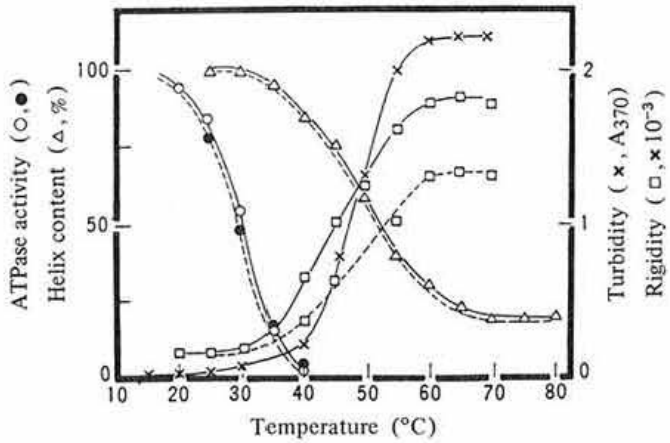

Fig. 7. Heat-induced changes in physicochemical properties of myosin and its subfragments (0.6 M KCl, pH6)
○ and ●: EDTA-ATPase activity of myosin and S-1, respectively, □: Rigidity of myosin, ×: Turbidity of myosin, △: α-helix content of myosin rod in the absence (solid lines) and presence (dotted lines) of 1 mM DTT.

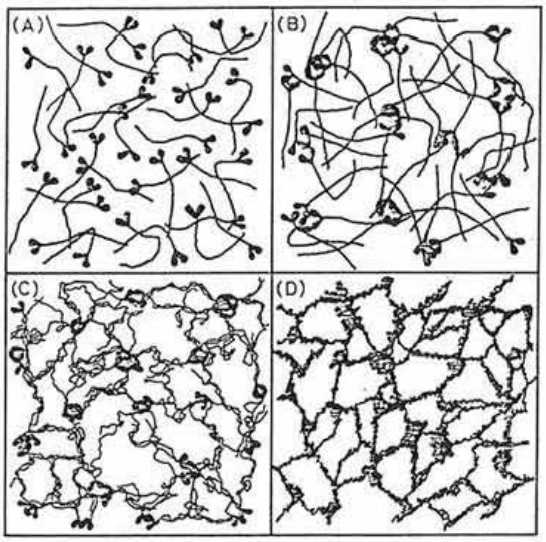

Fig. 8. Schematic diagrams of the heat-induced gelation of myosin
(A): Before heating, myosin monomers are dispersed.
(B): At −43°C, head-head interactions among molecules.
(C): At −55°C, cross-linking among tail portions of molecules.
(D): At 60–65°C, completion of the network system.

the heat-induced gelation of myosin was studied. Under the standard conditions, employed by the study, i.e., pH 6 and temperature of 65°C with a holding time of 20 min, it was found that a specific ratio of myosin to actin was essential in developing a stronger gel than the case of gelation by myosin alone. The maximum strength of gel was observed at a myosin to actin mole ratio of 2.7, which corresponds to the weight ratio of myosin to actin, or about 15 (Fig. 9). The increase in the weight ratio of myosin beyond this limit causes a decrease in gel strength.

Although under the physiological ionic environment conditions in living muscle, the stoichiometry of myosin-actin interaction was reported to be at a molar ratio of 0.5[9], it has been found to be about 0.26 molar ratio, under the present experimental conditions, which corresponds to a 2.96 weight ratio. This means that before heating, the system from which the maximum gel strength emerges is composed of fully decorated F-actomyosin and surplus free myosin. These data suggest that heat-induced gel strength of the myosin-actin system depend more on the free myosin to actomyosin weight ratio rather than in the myosin to actin ratio.

The above idea is verified by the experimental result presented in Fig. 10, where myosin and

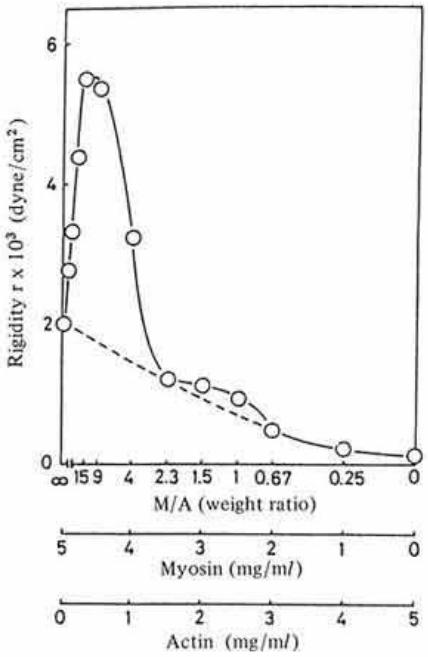

Fig. 9. Changes in the rigidity of the heat-induced actomyosin gels as a function of the weight ratio of myosin to actin
Dotted line: Myosin alone, Solid line: Actomyoshin.
The protein samples (5 mg/ml) dissolved in 0.6 M KCl and 20 mM phosphate buffer (pH 6) were incubated for 20 min at 65°C. Rigidity of each system was measured at the temperature indicated. In the case of myosin alone, only buffer was added in place of the actin containing solution, so that concentration of myosin varied as indicated in the scale for the myosin concentration.

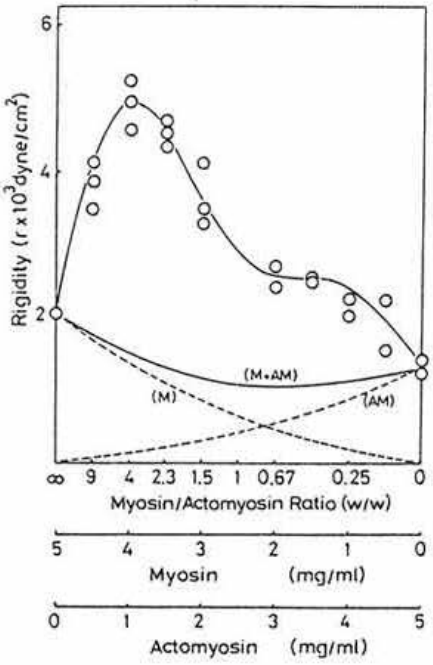

Fig. 10. Changes in the rigidity of the heat-induced gels of myosin-actomyosin mixtures as a function of myosin/F-actomyosin weight ratios
Conditions were the same as in Fig. 9.
(M): Myosin alone, (AM): F-actomyosin alone, (M + AM): Sum of (M) plus (AM).

actomyosin were mixed at different weight ratios. It is obvious that a system consisting of 80% free myosin and 20% of actomyosin on heating yielded a gel with a maximum strength. The gelation mechanisms of this system is schematically illustrated in Fig. 11. The figure includes: (A) before heating, free myosin molecules and actomyosin complexes are dispersed; (B) at about 43°C, like in the case of Fig. 9, head-head interactions of free myosin molecules commence; (C) at about 55°C, cross-linking between tail portions of free myosin molecules and those of bound myosin molecules with actin becomes apparent due to helix-coil transition; and (D) the reinforced network structure by huge actomyosin complexes is completed at 60–65°C.

Research needs

With regard to the functionality of myosin in the gelation process, recent studies[6,24] have indicated that at 0.1 to 0.2 M ionic medium (NaCl or KCl), myosin always produced a gel with a much greater strength than the case at high molarity. Whether this is the result of low ionic strength *per se*, or the formation of myosin filaments at low molarity is not clear. It is also little known as to how the presence of minor regulatory proteins as well as cytoskeletal proteins would modify the gel behavior[23] of myosin in the model systems. The answers to those questions are important not only in academic implications but also from practical considerations.

The authors' latest studies[1,19,20] have revealed a marked difference in the thermogel behavior of myosin derived from avian red and white muscles

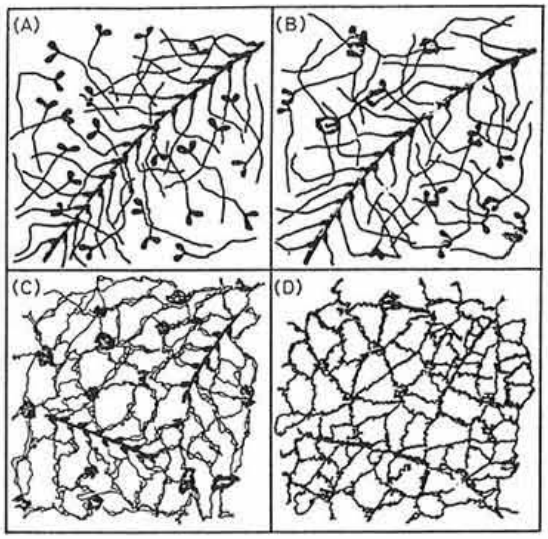

Fig. 11. Schematic diagrams of the heat-induced gelation of actomyosin in the presence of surplus free myosin
(A): Before heating, myosin and actomyosin are dispersed.
(B): At −43°C, head-head interactions among free myosin molecules.
(C): At −55°C, cross-linking among tail portions of free myosin and among those of free myosin and actomyosin.
(D): At 60–65°C, completion of the myosin-actomyosin network system.

and porcine cardiac muscle. It would be of interest to investigate the heat-and/or acid-induced[3] gelling characteristics of myosin from smooth muscles as well as from nonmuscle sources. Perhaps such an endeavor would help reveal how different isoforms of myosin influence the stability and quality characteristics of restructured meat and meat products.

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