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Myosins from Red and White Bovine Muscles: Part 1—Gel Strength (Elasticity) and Water-Holding Capacity of Heat-Induced Gels

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ABSTRACT

Myosins were isolated from bovine M. masseter (*red*) *and* M. cutaneus trunci (*white*) *and characterized in terms of solubility, electrophoretic pattern and susceptibility to tryptic digestion. Dynamic rheological measurements showed that white myosin is generally the superior gel former: gel strength, expressed as storage modulus, is consistently higher (a) at all temperatures above the gel-inducing minimum, (b) in both 0·2*M *and 0·6*M *NaCl, (c) at all concentrations investigated (≤ 20 mg/ml) and (d) at pH $\geq 5{\cdot}8$. In 0·6*M *NaCl at pH $< 5{\cdot}8$ red myosin appears to perform better. All gels formed in 0·2*M *NaCl lost about 15% liquid on centrifugation; gels in 0·6*M *NaCl displayed an inverse relationship between storage modulus and loss of liquid. The gel strength and water-holding capacity of gels made from mixtures of red and white myosin were a linear function of mixture proportions in the case of 0·2*M *NaCl; 0·6*M *NaCl gave gels with unpredictable properties.*

INTRODUCTION

The crucial role of myosin in structured meat products is universally acknowledged (Fukazawa *et al.*, 1961; Acton, 1972; Turner *et al.*, 1979;

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Siegel & Schmidt, 1979; Asgahr *et al.*, 1985). It is also well known that several different types of muscle fibres exist (Brooke & Kaiser, 1970; Barnard *et al.*, 1971; Ashmore & Doerr, 1971; Young, 1982) and that the various fibres contain different myosin isozymes (Masaki, 1974; Gauthier & Lowey, 1979; Dalla Libera *et al.*, 1980; Silberstein & Lowey, 1981; Young, 1982; 1984).

The functional properties of myosins from different fibres have so far been studied only by Asghar *et al.* (1984) using the leg and breast muscles from broilers as sources for myosin. They found that white broiler myosin is a superior gel former compared with the red myosin, independent of pH and ionic strength.

In this paper we report on gel formation and water-binding properties of myosins from two bovine muscles, *M. cutaneus trunci* and *M. masseter*, the former having almost exclusively fast-twitch, glycolytic and oxidative fibres (white; type IIA) and the latter exclusively slow-twitch, oxidative fibres (red; type I) (Young & Davey, 1981; Young, 1982).

MATERIALS AND METHODS

Materials

Myosin

Myosin was isolated from bovine *M. cutaneus trunci* and *M. masseter*. The results presented were obtained using two batches of each myosin, isolated from two cows. The procedure used for isolation has been described elsewhere (Fretheim *et al.*, 1985). The myosin preparations were used within 2 days after isolation. Care was taken to nullify effects from storage when comparing the two myosins.

Methods

Preparation of solutions

Precipitated myosin was dissolved in a solution of 0·6M NaCl (pH 7·5) and dialyzed overnight (5°C) against the desired pH (0·04M phosphate buffer) and salt (NaCl) concentration (myosin solution volume:buffer volume = 1:100, buffer changed once). The pH of the sample after dialysis was always recorded.

Determination of protein concentration
Myosin concentrations were determined spectrophotometrically (Beckman Model 25 Spectrophotometer) using $A_{280}^{0.1\%} = 0.543$ (Kielly & Harrington, 1966). The readings were corrected for light-scattering by subtracting A_{340}.

Proteolysis by trypsin
The proteolytic sensitivity of the two myosins to trypsin was investigated at pH 7·0 (0·6M NaCl, 20°C). Myosin was incubated with trypsin at a weight ratio of 150:1. The reaction was stopped at different time intervals, ranging from 5 to 20 min, by adding trypsin inhibitor at a weight ratio of 2:1 (trypsin inhibitor:trypsin). The partially digested myosin samples were subjected to electrophoresis in polyacrylamide gels after treatment with sodium dodecyl sulfate (360 Mini vertical slab cell from Bio-Rad Laboratories, California; gels were cast and run according to the instructions given by the supplier).

Solubility measurements
Myosin solubility was estimated by centrifugation (Beckman, Model L5–75 Ultracentrifuge, Ti–50 rotor, 40 000 rpm for 1 h) of the suspensions (10 mg/ml) and subsequent determination of the protein concentration of the supernatant.

Turbidity measurements
The turbidity of myosin suspensions (10 mg/ml) was estimated as optical density at 600 nm (Beckman Model 25 Spectrophotometer).

Rheological investigations
The Bohlin Rheometer (Bohlin Rheology AB, Lund, Sweden) was used in the oscillatory mode. The rheological thermograms were recorded at 1 Hz, strain 0·06 and a heating rate of 1°C/min (from 25°C to 75°C). The use of this technique on myosin systems has been described in greater detail elsewhere (Egelandsdal *et al.*, 1986*a*). The temperatures given have been corrected for the difference in temperature, caused by the temperature scan, between the sample cell and the surrounding water bath. Thermogram reproducibility was $\pm 2{\cdot}5\%$ for the same batch of myosin, the variation being largely ascribed to inaccurate protein determinations. When working with myosin, we have found batch-to-batch reproducibility to be within $\pm 10\%$.

Measurements of water binding

The centrifugation method used has been described by Hermansson & Lucisano (1982). However, our weak gels were centrifuged at only 30 × *g* for 10 min.

RESULTS

Characteristics of the myosin preparations

The electrophoretogram in Fig. 1 confirms that red myosin (e) yields only two different light chains when run in SDS–PAGE (Lowey & Risby, 1971; Sarker *et al.*, 1971; Young, 1982). With white myosin (a) four bands are seen in the low molecular weight region of the electrophoretogram; from studies of rabbit and chicken white muscle myosins, light chains were expected to yield three bands (Lowey & Risby, 1971; Sarkar *et al.*, 1971), as was found by Young (1982) for

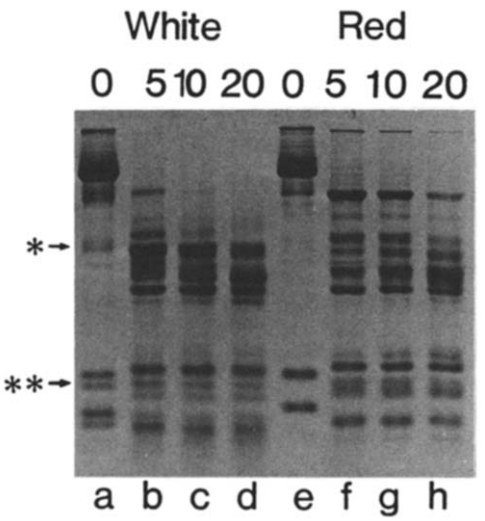

Fig. 1. Electrophoretograms (polyacrylamide gels, sodium dodecyl sulfate included) of the two myosin preparations studied and of their digests with trypsin: a, White myosin, isolated from *M. cutaneus trunci*; b, c, d, white myosin after treatment with trypsin for 5, 10 and 20 min, respectively; e, Red myosin, isolated from *M. masseter*; f, g, h, red myosin after treatment with trypsin for 5, 10 and 20 min, respectively.

* Contaminant assumed to be a proteolytic fragment of molecular weight ≈ 85 000.

** See 'Discussion' for comments on this band.

bovine white fibre myosins. This aspect of the white myosin preparation is further commented upon under the section below headed 'Discussion'.

Electrophoresis of samples treated with trypsin for various time intervals yielded results in agreement with earlier reports (Syrový, 1968): white myosin (b, c, d) is degraded at a faster rate than is red (f, g, h), i.e. the white high molecular weight fractions disappear more quickly and the low molecular weight fractions disintegrate into an electrophoretic blur.

In Fig. 2 the solubility and turbidity of the respective myosin preparations are seen to depend in different ways on pH. It is shown by the solubility curves that, at pH values $<5{\cdot}7$ (0·6M NaCl), more white myosin than red can be brought into solution and that about

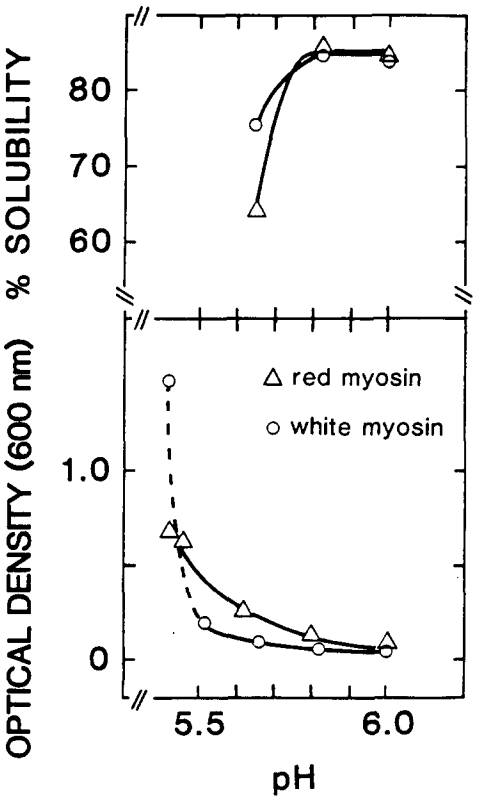

Fig. 2. Solubility and turbidity (optical density) of the two myosin preparations (10 mg/ml; not heat treated) as a function of pH in 0·6M NaCl. The turbidity of white myosin at pH $<5{\cdot}5$ is commented on in the 'Discussion'.

85% of both myosin preparations (10 mg/ml) goes into solution at pH 6·0. The turbidity measurements reveal a sharp increase in the turbidity of white myosin suspensions at pH < 5·5, presumably due to protein denaturation (see 'Discussion'). At lower salt concentrations, for example in 0·2M NaCl, both myosins were virtually insoluble (results not shown).

Rheological thermograms of red and white myosin at pH 6·0

Figure 3 shows rheological thermograms of the two myosins at two different ionic strengths at pH 6·0. The figure reveals the superior gel formability of white myosin compared with red at both ionic strengths and within the entire temperature range investigated. The fact that the thermograms are not simple, sigmoid curves has been reported on earlier (Egelandsdal *et al.*, 1986*b*; Ishioroshi *et al.*, 1979); it is related to the step-wise denaturation and gelation of myosin.

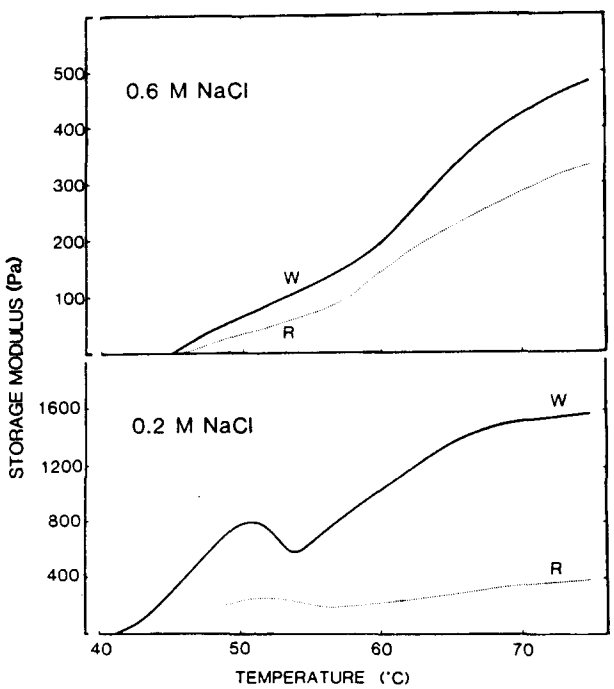

Fig. 3. Rheological thermograms of red (R) and white (W) myosin (10 mg/ml) at pH 6·0.

Effect of protein concentration on gel strength at pH 6·0, 0·2M NaCl

Figure 4 gives values for the storage modulus (G') of heat-induced gels as a function of protein concentration (pH 6·0, 0·2M NaCl). The inset shows the corresponding log/log relationships, and the slopes of the two lines reveal that the storage moduli had practically the same concentration dependence: with white myosin $G' \propto C^{2.0}$ and with red

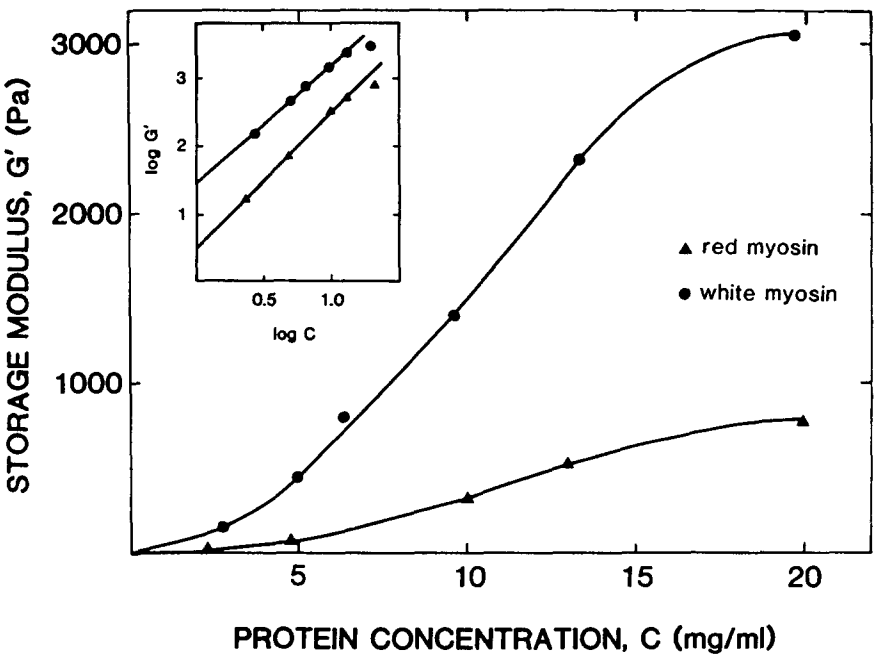

Fig. 4. Storage modulus at 75°C as a function of protein concentration for red and white myosin gels; pH 6·0, 0·2M NaCl. Inset: The corresponding log/log plot.

$G' \propto C^{2.1}$ ($C \leq 8$ mg/ml). From a series of rheological thermograms (*cf.* Fig. 3), recorded at different protein concentrations, we also calculated the dependence of the storage modulus on concentration at various temperatures throughout the gelation process (*cf.* Egelandsdal *et al.*, 1986*b*). G' was found to undergo most of its change in concentration dependence, from $C^{\approx 3.8}$ (for both myosins) to the above values, in the temperature range 54–56°C.

Effect of pH and ionic strength on the strength and water-holding capacity of the myosin gels

Figure 5 confirms the general superiority of white myosin at forming gels but reveals one exception: in 0·6M NaCl, pH < 5·8, red myosin performs better than white. The figure also agrees with earlier reports (Ishioroshi *et al.*, 1979; Hermansson *et al.*, 1986) that heat-induced myosin gels of low ionic strength are generally stronger than heat-induced gels of high ionic strength.

There was no significant difference in liquid loss from gels made in 0·2M NaCl, regardless of myosin source or pH, i.e. about 15% was lost (Fig. 6). On the other hand, the loss of liquid from gels made in 0·6M

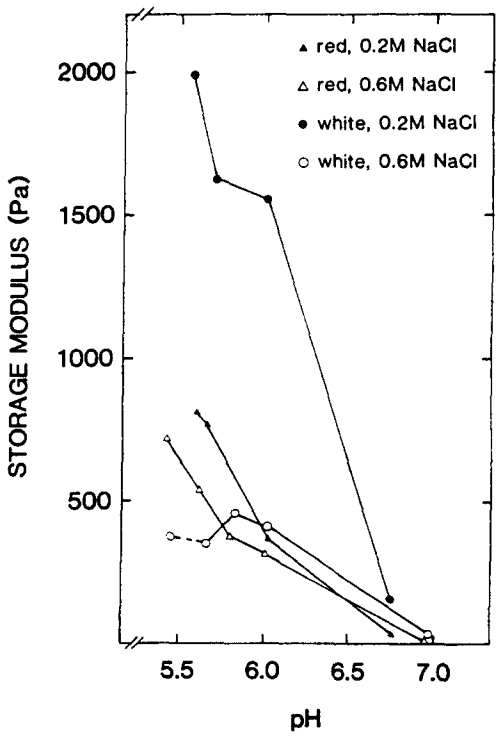

Fig. 5. Storage modulus at 75°C as a function of pH for red and white myosin gels; 10 mg/ml. The measurement of white myosin in 0·6M NaCl at the lowest pH value is uncertain since the sample had attained some 'structure' during preparatory pH salt equilibration.

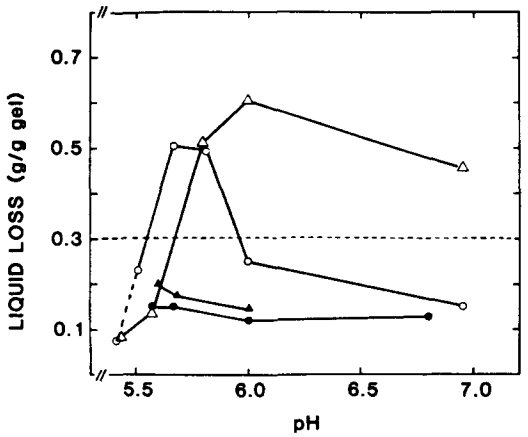

Fig. 6. Liquid loss, measured at 20°C, from red and white myosin gels (10 mg/ml; heated to 75°C) as a function of pH. Symbols as in Fig. 5. Values higher than 0·3 g per gram of gel are uncertain due to more or less extensive breakdown of the gels; lower values were reproducible within ±15%. See legend for Fig. 5 re uncertainty of value for white myosin in 0·6M NaCl at pH 5·4.

NaCl was found to depend both on type of myosin and on pH. It should be noted that, in the case of 0·6M NaCl, the gel with the highest storage modulus at any given pH (Fig. 5) also displayed the lowest loss of liquid in the centrifugation test.

Effects of mixing red and white myosin in different proportions

Figure 7 reveals that the relationship between storage modulus and protein composition is highly variable for mixed myosin gels: while at low ionic strength (0·2M; Fig. 7B) direct proportionality appears to determine the storage modulus, the relationship in 0·6M NaCl (Fig. 7A) is non-linear in a manner depending on pH. The gel-strengthening effect of mixing the two myosins at pH 5·65 is particularly noteworthy. The broken curve (Fig. 7A, both panels) represents the relationship which individual, independent (red and white) protein networks would produce if each were governed by $G' \propto C^2$ and the gel strengths were additive. Clearly, this is not the case. The implications are discussed below.

Made in 0·2M NaCl, mixed gels loose the same amount of water upon centrifugation as the pure gels, i.e. about 15% (results not shown).

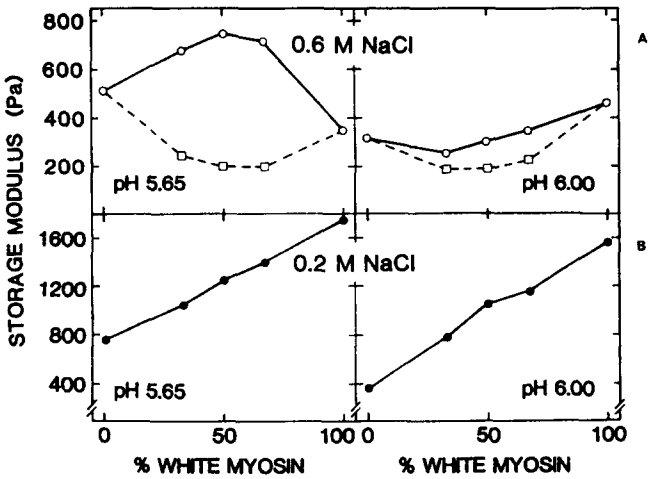

Fig. 7. Storage modulus at 75°C as a function of the proportion of white to red myosin; total protein concentration, 10 mg/ml. The broken curves in the upper panels were calculated from the storage moduli of the respective non-mixed gels assuming that the red and white myosin networks were independent, that both have concentration dependences of C^2, and that their storage moduli were additive.

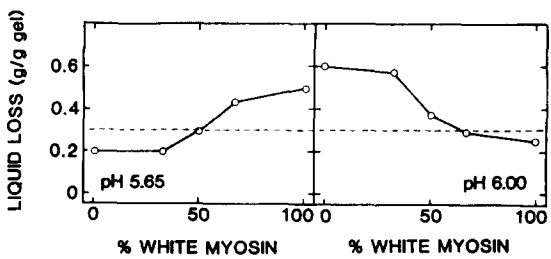

Fig. 8. Liquid loss, measured at 20°C, from mixed gels as a function of the proportion of white to red myosin; total protein concentration, 10 mg/ml, 0·6M NaCl, heated to 75°C. Reproducibility of results: See legend for Fig. 6.

In the 0·6M NaCl case it is seen (Fig. 8) that liquid loss depends in a complex manner on the proportion of white myosin. The curves are not analogous to the ones for the storage moduli (Fig. 7A) but they corroborate the observation that mixed gels at 0·6M NaCl constitute complex systems.

DISCUSSION

The fourth band in the low molecular weight region of the electrophoretogram (Fig. 1) of white myosin corresponds to troponin-I (Syrový, 1984). It seems unlikely, however, that the isolation procedure would allow so much contamination by troponin, *cf.* the purity of the red myosin preparation. The white myosin band at molecular weight $\approx 85\,000$ is, according to our experience, a proteolytic fragment and, conceivably, the 'troponin' band could also arise through proteolytic breakdown of myosin. On the other hand, earlier work has not excluded the possibility that myosin from bovine *M. cutaneus trunci* may include a fourth light chain with properties re SDS–PAGE nearly identical to those of troponin-I. It should be pointed out that many of our various preparations of bovine myosin display a band in this position, the intensity varying from barely discernible to about the intensity seen here.

Our bovine white myosin, isolated from *M. cutaneus trunci*, was found to have properties similar to those described by Asghar *et al.* (1984) for chicken white myosin, i.e. white myosin yields heat-induced gels of superior strength (G'), pH 5·8 (0·6M NaCl) gives maximum gel strength and the concentration dependence is $G' \propto C^{\approx 2}$. It appears likely, therefore, that the slight contamination of the preparation (Fig. 1) has not appreciably affected gel formation. We have, on other occasions, observed the presence of a proteolytic fragment to have a negative effect on gel formation (unpublished results); troponin-I has been reported not to affect gel formation by actomyosin (Samejima *et al.*, 1982).

The red myosin preparation, isolated from bovine *M. masseter*, appeared to be very pure (Fig. 1), taking into account that these gels were, for clarity, heavily loaded with sample. Nonetheless, our bovine red myosin yielded results which were, in part, quite different from those obtained by Asghar *et al.* (1984) on chicken red myosin: no maximum in gel strength at pH 5·9 (Fig. 5), the red myosin formed better gels than white myosin at pH $< 5{\cdot}7$ in 0·6M NaCl (Figs 5 and 6), and gel strength (G') had a concentration dependence of about C^2 (Fig. 4, inset). The apparent discrepancy may, at least in part, stem from the fact that the two red myosin preparations in question were prepared from different muscles of different species. Bovine *M. masseter* is known to be homogeneous in fibre type (type I) (Young & Davey, 1981; Young, 1982), which is rather unusual. *M. gastronemius* of chicken is a red muscle but

we are not aware to what extent it is homogeneous in fibre type. Fast-twitch fibres would contribute white myosin which, in turn, would contribute comparatively little towards the total gel strength (G') at low pH values and high ionic strength.

Our observation that there is a pronounced difference in gel strength pH dependence for red and white myosin in 0·6M NaCl (Fig. 5) can be tentatively understood in terms of different stabilities towards denaturation. It has been reported from this laboratory (Stabursvik & Martens, 1979) that white myosin (in samples of rabbit muscle) is markedly destabilized (lower denaturation temperature) upon lowering of pH from 6·0 to 5·35 (0·15M NaCl) while red myosin is little affected. Furthermore, we have shown (Fretheim *et al.*, 1985) that at pH 5·5 (0·6M KCl) a large proportion of myosin from bovine *M. longissimus dorsi* (a mixed muscle) would denature in the course of 16 h at 5°C. There is reason to believe, therefore, that protein denaturation and ensuing, irreversible aggregation at the lower pH values, leave less white than red myosin in solution (0·6M NaCl) for the formation of heat-induced gels. Scattered aggregates of pre-denatured molecules contribute little towards a strong network, i.e. relatively weak white myosin gels are obtained. Nonetheless, it is at present unclear to what extent the drop in storage modulus for white myosin below pH 5·8 (0·6M NaCl) can be attributed to partial denaturation.

From the above it may appear contradictory that measurements of protein solubility (Fig. 2, upper panel) indicate red myosin to be less soluble than white at $pH < 5{\cdot}7$. The observed, relative decrease in red myosin solubility is, however, ascribed to the formation of filaments which contribute towards the relatively high strength of red myosin gels at the lower pH values (Fig. 5). It is seen from the lower panel in Fig. 2 that the white myosin turbidity curve strongly supports the concept of white myosin destabilization, implying denaturation and aggregate formation, at $pH < 5{\cdot}5$.

It should be noted that myosin molecules in filaments are stabilized against denaturation (Wright & Wilding, 1984). Thus, the mechanism of filament formation would lend more 'protection' to red myosin than to white at low pH values in 0·6M NaCl, concurring with the above. Similarly, at low ionic strength (0·2M NaCl) filaments prevail, little or no denaturation occurs prior to heating, and lowering of pH results in consistently increasing gel strength (Fig. 5) in the pH range investigated.

The observed change in the dependence of the storage modulus

on concentration in the course of heat treatment, from $C^{\approx 3\cdot 8}$ to $C^{\approx 2}$ between 50 and 60°C for both myosins, sheds further light on the filamentous systems. The former value reflects the concentration dependence of an essentially native (non heat-denatured) gel (Egelandsdal *et al.*, 1986*b*). More importantly, the close analogy in gelation behaviour suggests that the two myosins yield filamentous gels having similar junctions. In agreement, Fig. 7B revealed an essentially linear relationship between storage modulus and the proportion of red and white myosin in mixed gels of low ionic strength.

At high salt concentrations (0·6M NaCl) no linear relationship between gel characteristics and the proportion of red and white myosin was found (Figs 7A and 8).

Asghar *et al.* (1984) reported a similar finding for chicken myosin mixtures. We are at present unable to delineate the mechanisms behind these results. Obviously, the non-linearity of the curves precludes the possibility of a single, coupled network as appears to be the case for gels formed at low ionic strengths (Fig. 7B). Furthermore, the deviations from the broken curves in Fig. 7A preclude the possibility of two interpenetrating gel networks, one for each myosin. A phase separated system, having multiple regions of one or the other individual network, is conceivable but our results do not provide evidence for it.

An understanding of the observed phenomena will probably need to accommodate three aspects of these myosin systems: (1) ionic charges and their distribution on the two myosins as a function of pH; (2) stability towards thermal and acid denaturation for the two myosins; (3) gel-forming interactions of the monomeric (or dimeric), the denatured (i.e. as various aggregates) and the filamentous states of myosin. Work aimed at elucidating some of these aspects is under way.

Caution should be exercised when interpreting water-binding measurements on weak gels. Nonetheless, it was much as expected that filamentous gels (low pH, low ionic strength), being more ordered and presumably of smaller average pore size, generally had better water-binding properties than gels formed from essentially monomeric myosin molecules (Fig. 6). It is less obvious why the weak (Fig. 5) white myosin gel at pH 7 in 0·6M NaCl retained water so well. Again, the explanation probably rests with the extent of order and resulting pore size within the gel. Order generally increases with increasing distance from pI for monomeric protein systems at high (0·6M) ionic strengths (Hegg, 1982) and this usually implies smaller pore size for the gel.

Obviously, gel formation and water binding are not the only physico-chemical differences between red and white myosins. Work is under way in our laboratory to further characterize myosins from bovine muscles.

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