THE EFFECT OF MYOFIBRILS ON THE RHEOLOGY OF PROTEIN GELS

B. EGELANDSDAL and J.R. MITCHELL*

Norwegian Food Research Institute, P.O.Box 50, N-1432 Ås-NLH, Norway
* University of Nottingham, Faculty of Agricultural Sciences, Sutton Bonington, Near Loughborough, Leicestershire, LE12 5RD, England

SUMMARY

Changes occuring on heating (25-80°C) myofibril suspensions at three different conditions have been examined. At pH 7 (1M NaCl) and pH 5.6 (0.3M NaCl) the proteins had precipitated before 80°C was reached. At pH 6.2 (0.6M NaCl) a weak, shrunken gel was formed. Gelation was promoted by the soluble (extracted) proteins as washed myofibrils would precipitate upon heat treatment at any of these three condition. Washed myofibrils reinforces, however, the gel formed once included in a gel network.

INTRODUCTION

Myofibrils are important structural components in muscles. They possess the ability to swell in hypertonic solutions and thus the ability to imbibe water within the myofibril (Offer and Trinick, 1982). While this property is of economical importance when meat and meat products are sold uncooked, the prevention of cooking loss is equally important when the same products are sold in the cooked state. In whole meat the endomysial sheath surrounding the myofibrils is an additional factor in determining the water binding properties (Wilding et al., 1986). When meat is comminuted, the myofibrils become shorter and the endomysium is broken so a distribution of various sized myofibrils is suspended in the aqueous phase. Thus in comminuted meat the texture and the liquid loss are to a greater extent determined by myofibrils and their proteins. Very little is, however, known about how the presence of (semi)extracted myofibrils affect the rheological properties of a homogeneous protein solution. We have studied the rheology of cold dispersions of myofibrils and this will be reported separately (Mitchell and Egelandsdal, 1987). The current paper describes the rheological changes occuring on heating myofibril suspensions. To throw further light on the role of myofibrils as fillers in protein gels, some measurements have also been made on gelatin/myofibril systems.

MATERIALS AND METHODS

A typical white (M.cutaneus trunci) bovine muscle from an electrically stimulated carcass was used. The muscle was dissected 1-2 hrs post-mortem and then stored at +11°C until the following morning (approx. 24 hrs post-mortem). Myofibrils were then isolated by the method of Harbitz et al. (1982). Three different batches were used for the work reported here. The suspensions of myofibrils were prepared by dilution with solutions of appropriate pH and ionic strengths.

Protein concentrations were determined by the biuret method (Gornall et al., 1949).

Myofibrillar swelling was estimated from sedimented volume (10 ml suspension of 7-8 mg/ml centrifuged at 9000 x g for 30 min).

The rheological changes upon heat treatment (25-80°C, 1°C/min) of the suspensions of myofibrils were monitored by using the Bohlin Rheometer System in the oscillatory mode. Other experimental details were as reported by Samejima et al. (1986).

Gelatin gels containing washed myofibrils were prepared by mixing appropriate solutions at 30°C. The mixtures were poured directly into moulds (diameter 26 mm, height 13 mm) and stored overnight at 15°C prior to measurements. A series containing heated myofibrils was prepared by heating (30-80°C, 1°C/min) the gelatin (4.8% w/v)-myofibril system prior to moulding. Before filling the moulds, the suspensions were cooled to 30°C and stirred to evenly disperse the aggregates formed. The gelatin-myofibril gels were stored overnight and measured at 15°C using parallel plate geometry (gap 12 mm).

All viscoelastic measurements were performed in the linear viscoelastic range.

Suspensions (5 g) of myofibrils were also heated (25-80°C, 1°C/min) in stoppered tubes and visual observations made at 5-10°C intervals. Cooking loss was determined by heating to specific temperatures and cooling to ambient. The water exuded from the gel was decanted and any additional water of syneresis removed by gently dabbing with tissue paper. The gel was then weighed and % cooking loss calculated.

Table 1. The sedimented volume and protein concentration in supernatants for three different batches of myofibrils.

pH	Salt concentration (M)	Batch no	Sedimented volume (ml) Initial amount of protein (mg)[a]	Protein concentration in supernatant Initial protein conc.[a]
7.0 (±0.1)	1	I	0.059	0.363
		II	0.032	0.570
		III	0.055	0.418
6.2 (±0.05)	0.6	I	0.016	0.300
		II	0.055	0.459
		III	0.044	0.291
5.85	0.3	I	0.009	0.079
5.60		II	0.007	0.057
5.60		III	0.008	0.063

[a] Before centrifugation

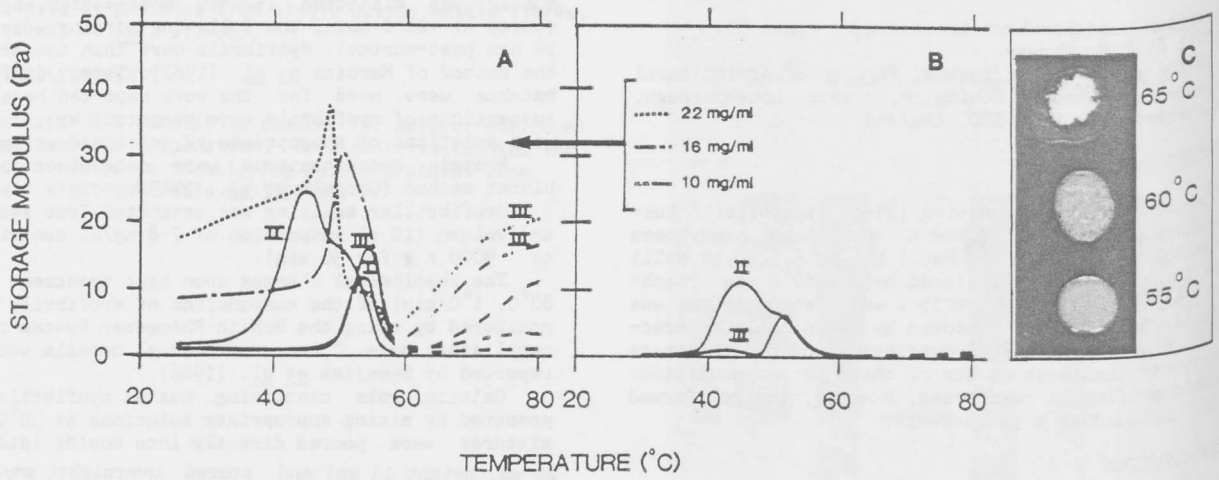

Fig. 1 The "rheological" thermograms of different batches (I-III). The thermograms of only the soluble proteins (6 mg/ml) are shown in panel B. Panel C illustrates the appearance of the protein system (22 mg/ml, batch III) after heating to the temperatures indicated with subsequent cooling to ambient. Parts of the curves in Panel A are discontinued in order to indicate less reliable results.

RESULTS

Swelling and protein extractability

The three different batches of myofibrils are characterized in Table 1.
For the low ionic strength - low pH condition both swelling and protein extractability are very low, however, for the other conditions there are no simple correlation between the two parameters. It is noteworthy that even at the highest pH and salt concentration less than half of the protein is extracted from the post-rigor myofibrils.

Heat treatment of myofibrils at pH 7 and in 1 M NaCl

Fig. 1A shows the temperature dependence of G´ for three different batches and three different myofibril concentrations. There are two or three peaks below 60°C and a minimum in G´ at about 60°C. Batches I and III behave similary while batch II is different. The supernatants also display one or two peaks below 60°C (Fig. 1B). Fig. 1C shows a photograph of the suspensions after heating to 55, 60 and 65°C and subsequent cooling. The precipitation shown in the photograph was observed to start at about 60°C. Measurement of protein concentration in the supernatant of the heated system showed that most of the protein including the soluble (=extracted) protein had precipitated by the time the final temperature of 80°C was reached. Additional results (not shown) suggest that the soluble protein in the absence of myofibrils will also precipitate but at a higher temperature.

Extensively washed myofibrils will also precipitate upon heat treatment. Dispersed, heat-treated aggregates of these myofibrils reinforce moderately the rigidity of gelatin gels, while unheated myofibrils have a much larger effect (Fig. 2).

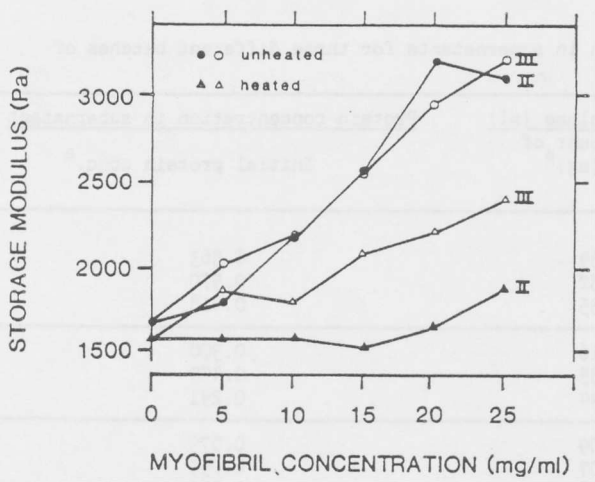

Fig. 2 The effect of increasing concentration (initial concentration as defined in Tab. 1) of washed myofibrils (batches II and III) on the storage modulus of 4.8% w/v gelatin of pH 7 (1M NaCl) gels.

Heat treatment of myofibrils at pH 6.2 in 0.6 M NaCl

Similar measurements to those performed at pH 7 were made at pH 6.2. The major difference between these two conditions was that the myofibrils did not precipitate upon heat treatment at pH 6.2, but formed weak gels. Fig. 3A shows the storage modulus as a function of temperature. The thermogram looks surprisingly similar to those reported in Fig. 1A except that the magnitudes for G´ are much larger. The thermograms of supernatants (not shown) gave peaks at similar temperatures to the total system seen in Fig. 3A. The cooking loss is the largest at 60°C and above 70°C. Fig. 3B shows a photograph of the system and three different temperatures. An obvious weakening of the system is seen at 60°C, i.e. the gel appears soft and releases more water.

Washed myofibrils will precipitate at this condition, but when included in gelatin gels they make G´ increase, both in the cold, dispersed state and in the heat-treated, dispersed state (Fig. 4).

Heat treatment of myofibrils at pH 5.6 and in 0.3 M NaCl.

In 0.3M NaCl (pH 5.6) only precipitates (from about 50°C) or very loose, shrunken gel lumps will form both from suspended myofibrils and for the small amount of extracted proteins. However, washed myofi-

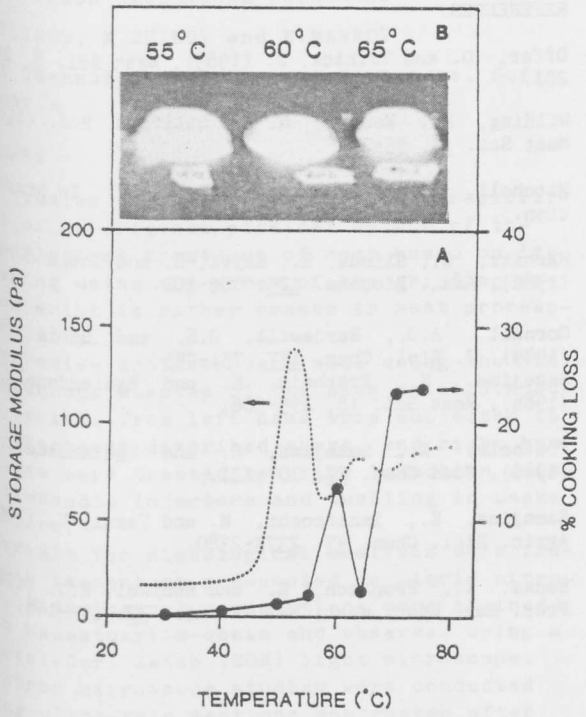

Fig. 3 The effect of heat treatment on storage modulus (possible erratic values for G´ are indicated by a discontinuous curve) cooking loss (panel A) of a myofibril suspension (Batch III, 22 mg/ml, pH 6.2, 0.3M NaCl). Photographs of the gels at three different temperatures are shown in panel B.

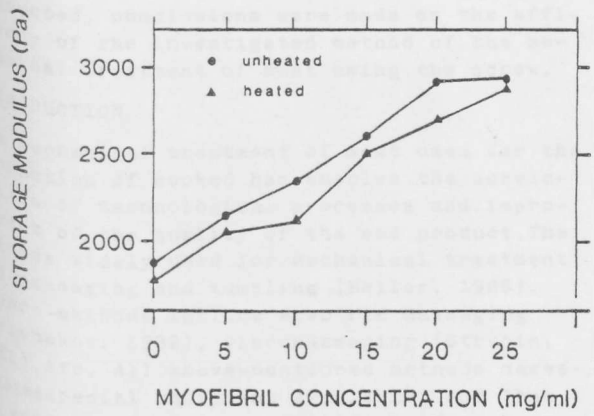

Fig. 4 The effect of increasing concentration (initial concentration as defined in Tab. 1) of washed myofibrils (batch II) on the storage modulus of 4.8% w/v gelatin gels of pH 6.2 (0.6M NaCl).

brils dispersed in a gelatin gel will still reinforce the gel for both the heated and unheated systems (results not shown).

DISCUSSION

The rheological thermograms (Figs. 1 and 3) are complex. Before any basic interpretations are made, it has to be considered to what extent genuine rheological parameters are being measured. We would take the view that both precipitation and shrinkage of a gel can result in artifacts.

For pH 7 precipitation (Fig. 1C), and for pH 6.2 shrinkage are severe a bove 65°C (Fig. 3) and the data above 60-65°C should therefore be treated with considerable caution. The interesting peak in G´ at 50°C is, we believe, a genuine phenomenon since it is readily apparent also on examining systems that have been heated to around this temperature and then cooled. In passing, it is worth noting that low phase angles and reproducible G´ values can be obtained even for the precipitates and we therefore consider that it is important to combine thermorheological measurements with visual observations of the systems.

The next point to consider is what changes are responsible for the peak in G´ at around 50°C. The increase in G´ is accompanied by an increase in phase angle (result not shown). This suggests that we are not initially monitoring a gelation phenomena, but protein denaturation perhaps followed by aggregation which is not sufficiently extensive to form a continous network. Limited aggregation leads to a drop in G´ while extensive aggregation leads to precipitation (Figs. 1A and 1C) causing the present technique to fail.

Visible precipitation is also observed for the supernatants of pH 7 at about 65°C, i.e. some time after G´ has started to drop (Fig. 1B). Therefore an interpretation in terms of solubilized myosin, the major soluble protein at pH 6.2 and 7, seems a reasonable starting point.

At pH 6.2 (in 0.6M NaCl) heat-induced aggregation is limited and precipitation does not take place (Fig. 3B). Myosin is a far better gelling agent at pH 6.2 compared to pH 7 (Fretheim et al., 1986). This is probably caused by the different temperature differences between rod and head denaturation at the two pH´s (Samejima et al., 1983) in addition to the larger ionic strength used at pH 7. The weak gel/suspension of dense aggregates seen at 60°C (Fig. 3B) is eventually reinforced by further (part of the rod) denaturation of myosin.

The final point to consider is the relative importance of the myofibrils as opposed to the proteins extracted into solution. For unheated myofibrils G´ can be related to the degree of swelling (Mitchell and Egelandsdal, 1987). Upon heating G´ should decrease when the myofibrils aggregate and precipitate. This drop in G´ between 50°C and 60°C (Figs. 1A and 3A) should therefore not only be interpreted in terms of souble protein aggregation but also myofibril aggregation.

As myofibrils dispersed in an aqueous solution devoid of proteins can only precipitate upon heat treatment at the protein concentration discussed here, the myofibrils have practically no water binding ability. Thus at pH 7 (1M NaCl) where the gelling ability of the soluble proteins is poor, the presence of myofibrils will only make things worse. At pH 6.2 (0.6M NaCl) the gelling ability of the soluble proteins is sufficient to prevent precipitation of myofibrils, however, the presence of myofibrils impair the water binding as shrinkage/precipitation is not observed for the heat-treated supernatant except at low (<4 mg/ml) protein concentrations. However, the gel in Fig. 3A is stronger (higher G´) than could be deduced from measurements on the soluble proteins only. This is perhaps due to the reinforcing effect seen for myofibrils incorporated into gelatin gels (Figs. 2 and 4). It is interesting that after heating myofibrils at pH 7, myofibrils reinforce the gelatin gel much less than at pH 6.2. This suggests that both the size, the mechanical strength and the protein content of the myofibril could be of importance.

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