# THE ROLE OF DISULPHIDE BONDS IN CHICKEN BREAST MUSCLE MYOSIN GELATION

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**Abstract**

The role of disulphide bonds in the gelation of chicken breast muscle myosin in 0.6 M NaCl, 50 mM sodium phosphate buffer, pH 6.5 was investigated. Myosin heated in the presence of 4 mM DTT aggregated from 49 to 70 °C, while myosin heated in the absence of DTT aggregated between 46 and 70 °C. Furthermore, the control myosin was more aggregated at 85 °C than myosin heated in the presence of DTT. Onset of gel formation was delayed by 5 °C in myosin heated in the presence of DTT and overall gel strength was lower, when compared to myosin heated in the absence of DTT. At 25 °C, the microstructure of myosin in the presence and absence of DTT were identical. At 55 and 65 °C myosin heated in the absence of DTT had a uniform three dimensional aggregated network structure, while the microstructure of myosin heated in the presence of DTT appeared melted, flat and lacked uniformity. These results indicate that disulphide bonds are formed between 48 and 54 ° C and their formation influences the degree of structural organisation observed in myosin gels.

## Introduction.

Gelation of muscle proteins during thermal processing is largely responsible for the characteristic texture, cohesion and stability of comminuted meat products. Myosin and/or actomyosin are the principle proteins responsible for gelation in meat systems (Gordon and Barbut, 1992). Myosin contains approximately 42 thiol groups, of which 12 to 13 are contained in each globular head region, one in each alkali light chain (LC) and two in each dithionitrobenzoate LC (Hofmann and Hamm, 1978). However, the role of the SH groups in myosin and actomyosin gelation is unclear.

The objective of this study was to determine the role played by disulphide bonds in chicken breast muscle myosin gelation, by selectively inhibiting SS bond formation using the reducing agent, dithiothreitol (DTT). This was achieved by heating myosin in the presence and absence of 4 mM DTT and assessing differences in protein-protein interactions, dynamic viscoelastic properties and gel microstructure. These results may further the understanding of the overall myosin gelation process.

## Materials and Methods

**Protein purification**. Breast muscle was extracted from 6 week old commercial meat type broilers as described by Wang and Smith (1994) and stored in 40% $(NH_4)_2SO_4$ at -20 °C. Prior to use myosin was suspended in 0.6 M NaCl, pH 6.5 or 0.6 M NaCl, 4 mM DTT, pH 6.5, and dialysed against three changes of the same buffer at 4 °C for 48 h. Protein concentration was determined by measuring absorption at 280 nm using an extension coefficient of $E^{1\%} = 5.5$ for myosin (Swenson and Ritchie, 1980). Protein concentration was adjusted to 10 mg/ml using the appropriate buffer.

**Thermal Aggregation**. Thermal aggregation was monitored by measuring the increase in absorbance at 340 nm in a Cary 3E UV/Vis spectrophotometer (Varian Analytical Inst., Sunnyvale, CA.) as described by Smyth et al. (1996a) . Samples were heated (in triplicate) from 25 to 85 °C at a rate of 1 °C/min.

**Oscillatory Rheology**. Storage (G') modulus was measured using a Carri-Med CSL 100, controlled stress rheometer (Carri-Med Ltd., Dorking, UK) as described by Lynch and Mulvihill (1995). Myosin solutions were equilibrated at 25 °C for 3 min, and heated from 25 to 85 °C at a rate of 1 °C/min. Data reported are the mean of six replicates.

**Gel Preparation for Environmental Scanning Electron Microscope (ESEM)**. Myosin gels were prepared for the ESEM by heating solutions at a rate of 1 °C/min in disposable culture tubes using a polystat circulating bath connected to a Bioblock Scientific Polystat Programmateur (Cole Parmer Inst. Co., Chicago, IL). All samples were prepared in triplicate and covered with teflon tape to avoid evaporation during heating. Once each sample reached its predetermined temperature it was removed from the water bath and cooled to 10 °C for 10 min, then stored at 0 °C until viewed within 24 h in the ESEM.

**ESEM**. Samples were imaged as described by Smyth et al. (1996b).

## Results and Discussion

**Gel Formation.** When myosin was heated in the absence of DTT, turbidity increased rapidly from 46 to 51 °C, remained relatively constant from 51 to 55 °C and increased slowly from 55 to 70 °C, where it reached a plateau (Fig. 1). In contrast when myosin was heated in the presence of DTT, turbidity increased rapidly from 49 to 50 °C and then increased more slowly from 51 to 70 °C. Turbidity values remained constant between 70 and 85 °C (Fig. 1). At temperatures greater than 46 °C, turbidity of myosin solutions heated in the absence of DTT was greater than that of myosin in the presence of DTT.

The storage modulus of myosin solutions heated in the absence of DTT increased sharply between 48 and 75 °C and remained constant between 75 and 85 °C, where a rigidity modulus of 120 Pa was recorded (Fig. 2). Wang and Smith (1994) observed a similar trend in the development of G' of chicken breast muscle myosin. Increases in G' of protein solutions during heating reflect the formation of an elastic gel network by partially denatured interacting protein molecules (Egelandsdal et al., 1986; Young et al. 1992; Wang and Smith, 1994). When myosin solutions were heated in the presence of 4 mM DTT a 5 °C delay in the onset of gelation was observed (Fig. 2). G' increased from 53 to 75 °C and remained constant between 75 and 85 °C, where a rigidity modulus of 95 Pa was obtained. These results are consistent with the turbidity data presented in figure 2. The results indicate that while disulphide bond formation is not a prerequisite for chicken breast myosin gelation, intermolecular disulphide bonds contribute significantly to gel network formation. This finding was emphasised further when myosin gel microstructure was examined.

**Gel Microstructure**. The microstructure of myosin at 25 °C in the presence and absence of 4 mM DTT were identical (Fig. 3a and 3b). At 55 °C, myosin in the absence of DTT formed an aggregated three dimensional network, consisting of uniform triangular and star shaped aggregates (Fig 4a). In contrast, myosin heated in the presence of DTT appeared as if it was melted and lacked the structural organisation observed in the control myosin (Fig. 4b). The structure was flat, with large star and triangular shaped interlinked aggregates. Myosin in the absence of DTT at 65 °C formed a continuous dense uniform network, with a small pore size distribution (Fig. 5a). In comparison myosin heated in the presence of DTT consisted of unevenly distributed star shaped aggregates and contained several large voids (Fig. 5b). The difference in microstructure formed by myosin when heated in the presence and absence of DTT illustrates the contribution of disulphide bonds in gel matrix formation.

## Conclusion.

Disulphide bonds are formed between 48 and 54 °C in chicken breast muscle myosin. By inhibiting disulphide bond formation a weaker gel structure was formed, which lacked the structural organisation observed in the control myosin at 55 or 65 °C.

## References

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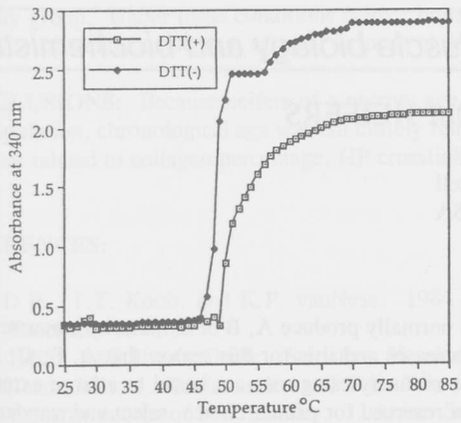



Figure 1. Turbidity profile of (1% (w/v) chicken breast myosin in 0.6 M NaCl, pH 6.5), heated from 25 to 85 °C at 1 °C/min in the presence and absence of 4 mM DTT.

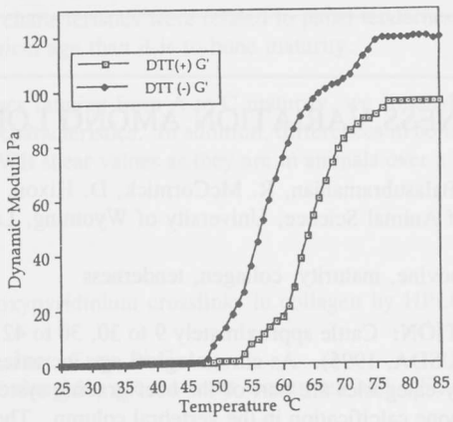



Figure 2. Storage (G') moduli of chicken breast muscle myosin (1% (w/v) in 0.6 M NaCl, pH 6.5) heated from 25 to 85 °C at 1 °C/min in the presence and absence of 4 mM DTT.

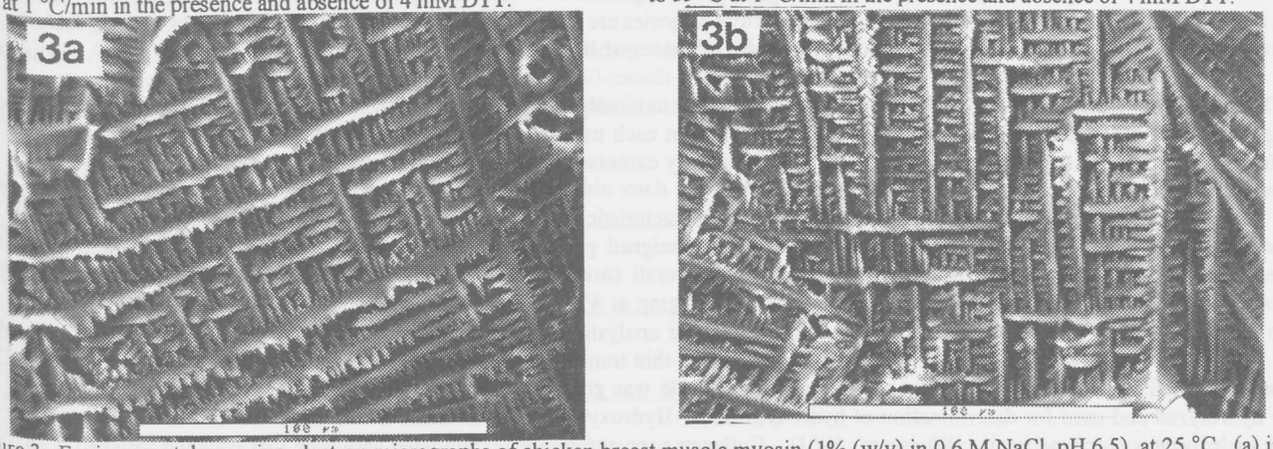
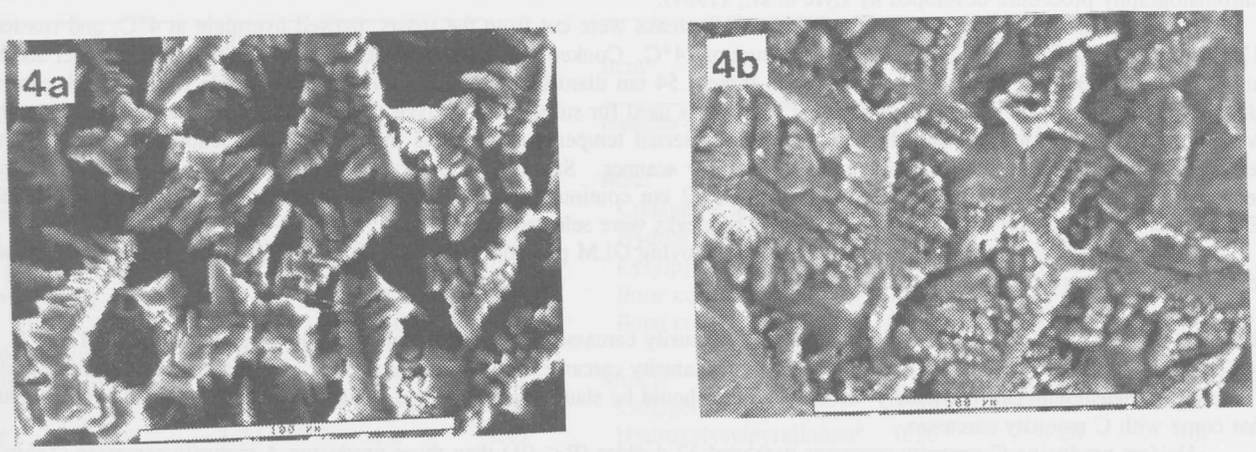



Figure 3. Environmental scanning electron micrographs of chicken breast muscle myosin (1% (w/v) in 0.6 M NaCl, pH 6.5), at 25 °C, (a) in the absence of 4 mM DTT; (b) in the presence of 4 mM DTT.



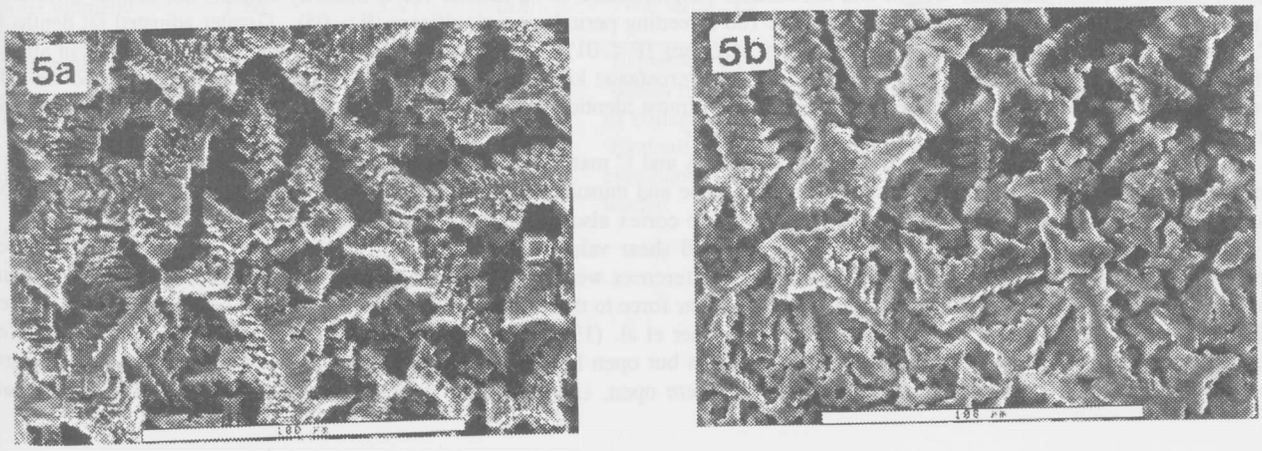




Figure 4-5. Environmental scanning electron micrographs of chicken breast muscle myosin (1% (w/v) in 0.6 M NaCl, pH 6.5) at 55 °C (figure 4) and 65 °C (figure 5), (a) in the absence of 4 mM DTT; (b) in the presence of 4 mM DTT.