Rheological Properties of Salt-Soluble Protein from White and Red Skeletal Muscles†

Youling L. Xiong* and Suzanne P. Blanchard

Food Science Section, Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546

The rheological behavior of chicken pectoralis (white) and thigh (red) salt-soluble protein (SSP) was investigated. In 0.6 M NaCl, both pectoralis and thigh muscle SSP exhibited pseudoplastic flow with a maximum shear stress at pH 6.0. Shear stress and viscosity of pectoralis SSP were greater than those of thigh SSP at identical shear rates (0.23–46.1 s^{-1}), pH (5.75–8.0), and protein concentrations (1–10 mg/mL). However, relaxation time for SSP samples following shear appeared to be independent of muscle type. Shear stress steadily reduced, albeit showing a small peak around 13–19 °C, with increasing temperature from 5 to 37 °C for pectoralis and from 5 to 44 °C for thigh and then rose abruptly at higher temperatures due to gel formation. The results indicate that white and red SSP are similar in rheological patterns but differ in magnitude, which may be attributed to the various isoforms of myofibrillar proteins.

Keywords: *Myofibrillar protein; fiber types; viscosity*

INTRODUCTION

Muscles and muscle fibers are classified as fast (white, type A, or type II) and slow (red, type B, or type I) with a number of intermediates (Cassens and Cooper, 1971; Ashmore, 1974; Pette and Staron, 1990). Of the various muscle fiber types, fast-twitch glycolytic and slow-twitch oxidative fibers are two major types in white and red muscles, respectively. White fiber differs from red fiber in chemical composition as well as in histological and biochemical properties (Cassens and Cooper, 1971). More detailed studies have indicated that many of the myofibrillar components, such as myosin, troponin, and tropomyosin, exist in a variety of forms (isoforms) which exhibit variations in amino acid composition, charge, and biochemical characteristics (Pette and Staron, 1990).

Much discrepancy in functionality between myosin isoforms or salt-soluble myofibrillar proteins (SSP) from white and red fibers of skeletal muscles has been demonstrated in a number of recent investigations. In dilute solution (i.e., <4% protein), myosin, actomyosin, and SSP from white fibers generally form stronger and more elastic gels than those from red fibers (Asghar et al., 1984; Foegeding, 1987; Choe et al., 1991; Xiong, 1992; Xiong and Blanchard, 1994). However, in concentrated solution (i.e., 8% protein), this relationship is not necessarily true (Northcutt et al., 1993). The functional role of myofibrillar protein in relation to texture and physical characteristics of further processed muscle foods is fully recognized (Acton et al., 1983). In this context, fiber type-associated functionality differences could partially contribute to variations in textural attributes observed between processed white (light) and red (dark) chicken or turkey meats (Daum-Thumberg et al., 1992; Barbut and Mittal, 1993).

Proteins from white and red muscles also exhibit disparities in solution properties. Richardson and Jones (1987) showed a greater protein extractability of white turkey muscle than of red turkey muscle in salt solution under identical pH and high ionic strength conditions. Xiong (1992) and Xiong and Blanchard (1994) reported similar findings for myofibrillar proteins from white and red chicken muscles. There is evidence that viscosity of dilute myosin and SSP is influenced by the composition of fiber types from which the proteins are extracted (Asghar et al., 1984; Morita et al., 1987; Xiong and Brekke, 1989). Rheological properties of salt-extractable white and red muscle proteins are important since they can affect consistency as well as handling of comminuted meat products. For instance, the amount of energy needed for mixing and transporting meat batters and the design for material transport pipelines all entail a good knowledge of the product consistency, which can be influenced by the viscous flow of the tacky SSP extracts. The purpose of this study was to evaluate and compare the rheological (flow) behaviors of white and red muscle SSP under various shear rate, pH, and temperature conditions.

MATERIALS AND METHODS

Preparation of Salt-Soluble Proteins (SSP). Pectoralis major and thigh muscles were excised from 36–48-h postmortem carcasses of chicken broilers slaughtered at a local poultry-processing plant. Pectoralis and thigh muscles were used since they are relatively homogeneous in their white and red fibers, respectively (Suzuki et al., 1985; Rosser and George, 1986). All carcasses were kept on ice after slaughter. Myofibrils were isolated using a 0.1 M NaCl/50 mM sodium phosphate (Na_2HPO_4/NaH_2PO_4) buffer (pH 7.0), and SSP was extracted from the purified myofibrils in 0.6 M NaCl/50 mM sodium phosphate at pH 6.0 (Xiong, 1992). Three myofibril isolations and SSP preparations (replications) were conducted on different days. SSP samples were kept on ice and utilized within 24 h. Protein concentration was determined by the biuret method (Gornall et al., 1949) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as standard.

Sample Treatments. Proteins are large electrolytes; as such, their interactions with each other and with solvents can be altered by changing the solid concentration and pH. To establish a shear stress–shear rate relationship as a function of protein concentration, SSP was suspended and diluted to 1, 4, 6, 8, and 10 mg/mL in 0.6 M NaCl/50 mM sodium phosphate (pH 6.0). To determine the pH effect, a suspension

* Author to whom correspondence should be addressed.

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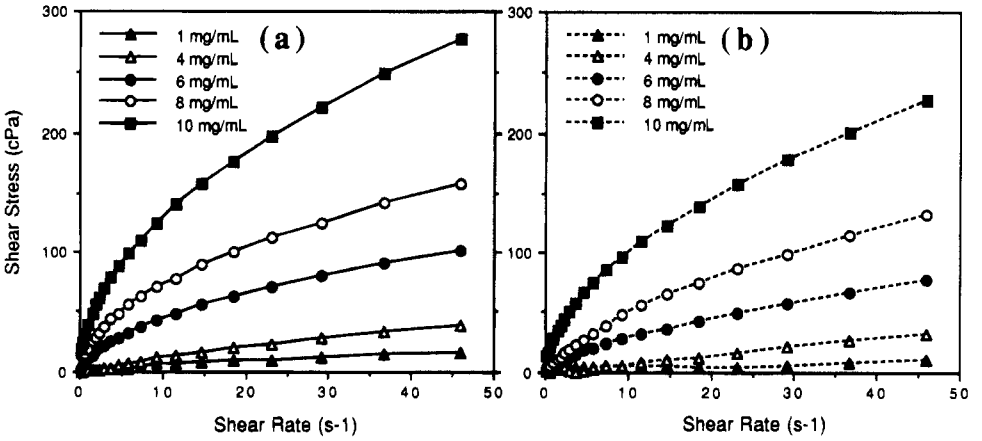

Figure 1. Relationships between shear stress and shear rate for chicken P. major (a) and thigh (b) salt-soluble protein suspensions (0.6 M NaCl/50 mM sodium phosphate, pH 6.0) at 5 °C.

Table 1. Rheological Parameters of Salt-Soluble Protein Suspensions Calculated Using the Power Law Equation[a]

rheological parameter	pectoralis protein					thigh protein					P value
	1 mg/mL	4 mg/mL	6 mg/mL	8 mg/mL	10 mg/mL	1 mg/mL	4 mg/mL	6 mg/mL	8 mg/mL	10 mg/mL	
k (N s^n m^{-2})	0.6 f	3.2 ef	8.2 de	18.8 c	53.9 a	2.0 ef	2.6 ef	5.4 de	11.9 d	34.4 b	0.001
n	0.89 a	0.69 bc	0.62 bcd	0.53 cde	0.43 e	0.48 de	0.80 ab	0.69 abc	0.63 bcd	0.49 de	0.007

[a] Means in the same row with no common letter differ significantly ($P < 0.05$).

of SSP (10 mg/mL) in the same phosphate buffer was adjusted to pH 5.75, 6.00, 6.50, 7.00, and 8.00 (±0.01) with 0.1 or 1 N NaOH or HCl. In our preliminary study, we observed significant changes in rheological properties for SSP suspensions during storage at 0 °C. For instance, after 24 and 48 h of storage, shear stress of SSP suspensions (10 mg/mL) decreased by about 14 and 44% for P. major and by 4 and 42% for thigh, respectively. Therefore, all SSP suspensions were used immediately after preparation.

Rheological Measurements. Rheological tests were replicated two or three times using freshly prepared SSP samples. Within each replication, measurements were made at least in duplicate. The rheological tests were performed at 5 °C (unless otherwise noted) using a Model VOR Bohlin rheometer (Bohlin Instruments, Inc., Cranberry, NJ) equipped with a bob and cup (cylinder) apparatus and a torque element of 91.6 g·cm. The bob and cup diameters were 25 and 27 mm, respectively. The following tests were conducted: shear rate sweep (0.23 → 46.1 s^{-1}); time sweep (0 → 300 s) at 0.23-, 2.3-, and 23-s^{-1} shear rates; temperature scan (5 → 60 °C at 1 °C/min) at a 14.6-s^{-1} shear rate; and shear–relaxation in which the SSP suspension was sheared at 23.2 s^{-1} for 5 s and stress decay immediately following shear was monitored during sample relaxation. For shear stress measurements, 13-mL aliquots of protein samples (0 °C) were loaded in the prechilled (5 °C) cup and allowed to equilibrate for 5 min before shear was initiated. Except for the relaxation test, shear stress and viscosity of the samples were measured, integrated, and averaged over 15-s intervals.

Data obtained from shear tests were treated with the power law equation, which gives the relationship between shear stress and shear rate as

$$\tau = k\dot{\gamma}^n$$

where τ (N m^{-2} or pascal) is shear stress, k (N s^n m^{-2}) is the consistency index, $\dot{\gamma}$ (s^{-1}) is shear rate, and n (dimensionless) is the power law index. By taking logarithms of the equation and plotting log τ against log $\dot{\gamma}$, k and n values can be easily obtained from the graph. Alternatively, the term "apparent viscosity", defined as the ratio of shear stress to shear rate, was used to differentiate from "dynamic viscosity", which applies to Newtonian flow only.

Rheological data were analyzed using general linear model procedures of the Statistix 3.5 program (Analytical Software Inc., St. Paul, MN). AOV was performed to determine treatment effects (protein concentration, fiber type). Differences ($P < 0.05$) between individual means within the same rheological parameter groups were identified by the least significant difference test (Snedecor and Cochran, 1989).

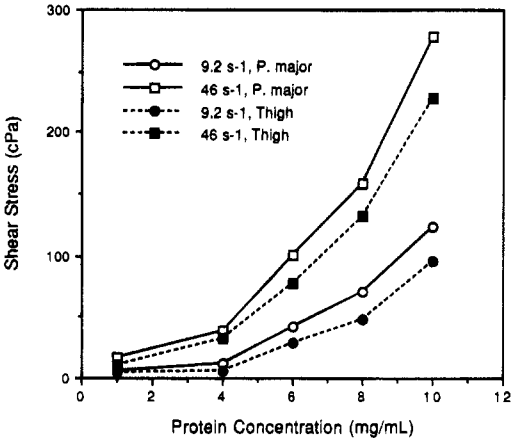

Figure 2. Shear stress at two shear rates for chicken P. major and thigh salt-soluble protein suspensions (0.6 M NaCl/50 mM sodium phosphate, pH 6.0) as a function of protein concentration at 5 °C.

RESULTS AND DISCUSSION

The hydrocolloidal nature of muscle proteins in aqueous dispersions is manifested by their ability to resist flow under the influence of an external force. SSP of both white and red muscles exhibited pseudoplastic flow (Figure 1); i.e., shear stress increased rapidly at low shear rates but slowly at high shear rates. This flow type was particularly evident at protein concentrations greater than 6 mg/mL. The magnitude and relationships between shear stress and shear rate for all protein suspensions were highly reproducible among replications. Suspensions of P. major SSP also consistently produced greater ($P < 0.05$) stress values than did thigh SSP at similar concentrations. Evaluating the empirical power law equation with experimental data and then plotting log τ *vs* log $\dot{\gamma}$ yielded a series of k and n values (Table 1). The n values were less than 1, thus verifying

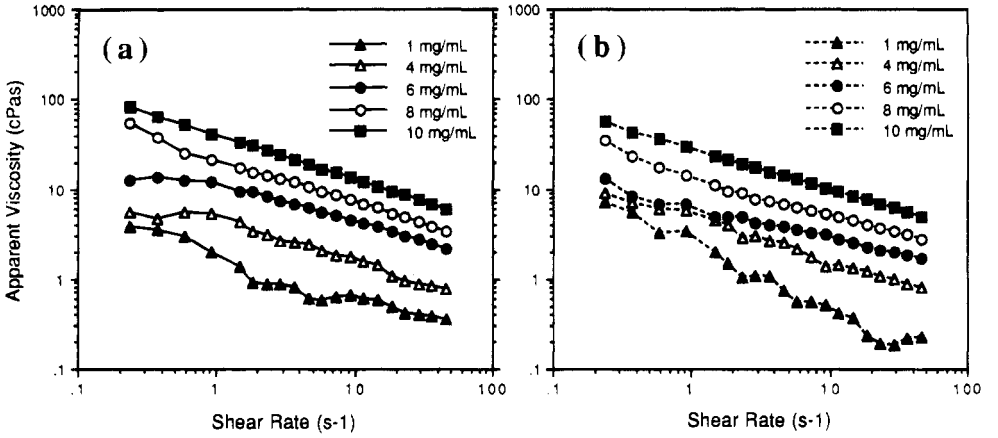

Figure 3. Viscosity *vs* shear rate plots for chicken P. major (a) and thigh (b) salt-soluble proteins (in 0.6 M NaCl/50 mM sodium phosphate, pH 6.0) at 5 °C.

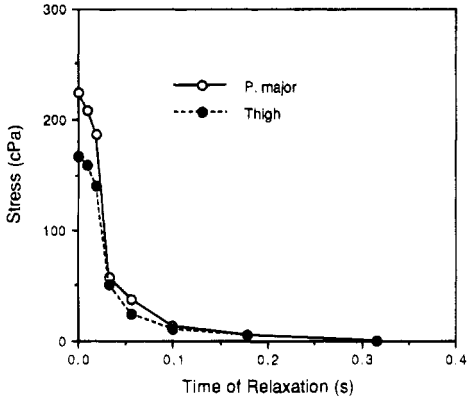

Figure 4. Changes in stress values for P. major and thigh salt-soluble protein suspensions (10 mg/mL protein in 0.6 M NaCl/50 mM sodium phosphate, pH 6.0) after a 5-s shearing (23.2 s^{-1}) at 5 °C.

that the SSP suspensions were of pseudoplastic flow characteristics. As protein concentration increased, the n values decreased ($P < 0.05$) overall, while the k value increased ($P < 0.05$), thus indicating deviation from Newtonian flow and an increase in viscosity. The protein concentration effect is further illustrated in Figure 2, where an exponential response of shear stress to protein concentration is manifested. The rheological behavior of SSP is also presented in terms of apparent viscosity, i.e., the ratio of shear stress to shear rate (Figure 3). On a log–log plot, there was a linear relationship between apparent viscosity and shear rate. Some fluctuations existed at low protein concentrations, which was probably due to limitations in the analytical sensitivity of the instrument.

The deviation from Newtonian flow, particularly for high concentrations of SSP, was probably caused by reduction in protein–protein and protein–water interactions. In essence, SSP is a mixture of the actomyosin complex, various other myofibrillar components, possibly some myosin and actin molecules or filaments, and nonsedimentable myofibrils. Hence, factors such as dissociation of protein aggregates and deformation and realignment of polymers would all contribute to the reduced stress response at high shear rates. Indeed, SSP suspensions did not relax instantaneously when shearing was stopped; instead, stress decay took about 0.3 s for both P. major and thigh samples (Figure 4). The results were consistent with the pseudoplastic flow behavior and suggest that SSP suspensions possessed some kind of "structural" characteristic.

The flow patterns of P. major and thigh SSP demonstrated in Figures 1–4 indicate a close resemblance to one another. Nevertheless, at all protein concentrations, shear stress and consistency index k for P. major SSP were greater ($P < 0.05$) than for thigh SSP. This was in agreement with a previous observation on chicken whole breast and leg myofibrils (Xiong and Brekke, 1989). The discrepancy between P. major and thigh may be explained in light of histological variations among fiber types and associated differences in physicochemical properties of proteins. Compared with chicken thigh, which has a preponderance of type I red fibers (Suzuki et al., 1985), chicken P. major is comprised of homogeneous type II white fibers (Rosser and George, 1986) and has different isoforms of myosin, troponin, and tropomyosin (Pette and Staron, 1990). The molecular weights of type I fiber myosin heavy chain and light chains are known to differ from those of type II fiber counterparts (Sarkar et al., 1971; Robbins et al., 1986). In addition, the percent myosin heavy chain and light chains also differs between white and red SSP, with light chain 3 being relatively deficient in red SSP (Hay et al., 1973; Xiong and Brekke, 1989). It is possible that these fiber type-dependent variations in molecular size and composition had contributed to the observed rheological differences between P. major and thigh proteins. Although the exact compositions, e.g., the amount of myosin and actin relative to the actomyosin complex, of white *vs* red SSP were not clear, there was evidence that in 0.6 M KCl chicken breast myosin formed long filaments, whereas chicken leg myosin produced relatively short aggregates (Morita et al., 1987). Thus, it seems plausible that some different protein–protein interactions existed in white and red SSP suspensions, and this would influence the stress properties of the aqueous protein dispersion.

Proteins are large zwitterions whose charges are pH-determined. Hence, alterations in protein–protein and protein–solvent interactions can be expected when the pH is changed. Figure 5 illustrates the relationships between shear stress and shear rate for various pH values (5.75–8.00). Despite the magnitude difference, both P. major and thigh SSP showed maximal stress at pH 6.00. This result differed from the finding of Morita et al. (1987), who reported a constant viscosity value for chicken leg myosin within pH 5.8–7.6 but a maximal viscosity for chicken breast myosin at pH around 5.8. The discrepancy between the two studies could be attributed to the fact that different proteins (myosin *vs* SSP) were used. It is possible that at pH 6.00 protein–

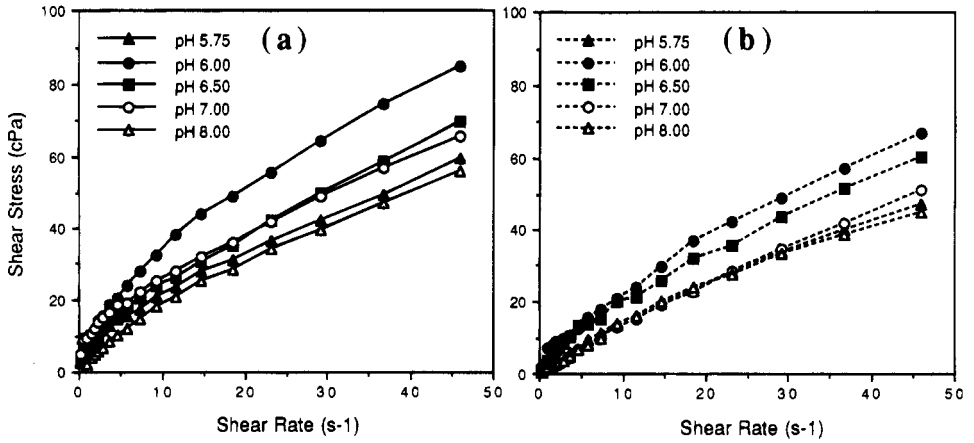

Figure 5. pH dependence of shear stress at various shear rates for P. major (a) and thigh (b) salt-soluble protein suspensions (10 mg/mL protein in 0.6 M NaCl/50 mM sodium phosphate, pH 6.0) at 5 °C.

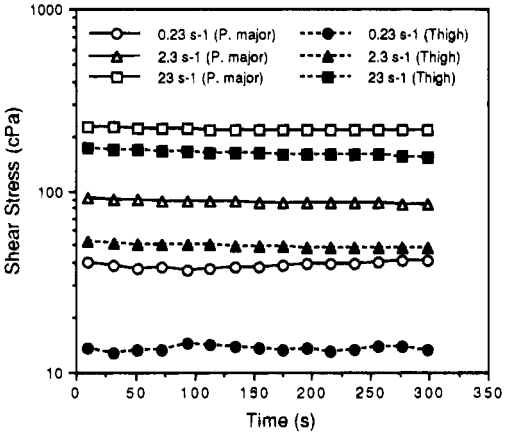

Figure 6. Monitoring shear stress changes at constant shear rates for P. major and thigh salt-soluble protein suspensions (10 mg/mL protein in 0.6 M NaCl/50 mM sodium phosphate, pH 6.0) at 5 °C.

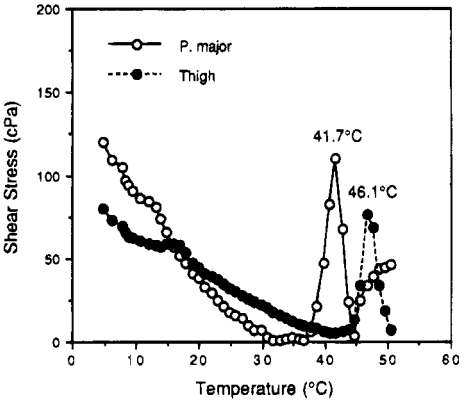

Figure 7. Changes in shear stress values of P. major and thigh salt-soluble protein suspensions (10 mg/mL protein in 0.6 M NaCl/50 mM sodium phosphate, pH 6.0) heated to 50 °C; heating rate, 1 °C/min; shear rate, 14.6 s^{-1}.

protein and protein–solvent interactions were balanced so as to produce a strong friction when sheared. However, at lower pH, protein charges decreased, resulting in the formation of more tightly bound aggregates and reduced solute–solvent interactions. Alternatively, as the pH increased to ≥6.5, increased charge repulsion led to decreased association of protein molecules, thereby decreasing the shear stress and apparent viscosity.

Time-dependent flow behavior (e.g., thixotropic) was observed in fish actomyosin solution incubated at mild temperatures (Wu et al., 1985). For chicken SSP suspensions stored at 5 °C, the stress value did not change with a continuous shear (Figure 6), thus indicating that chicken SSP suspensions were not susceptible to "thinning" when subjected to shear. This confirms the observation of pseudoplastic flow and suggests that the external force did not cause a permanent deformation of some possible "structural" components in SSP.

Figure 7 illustrates shear stress of SSP as a function of temperature. For P. major SSP, shear stress decreased from 123 cPa to almost 0 as the temperature was increased from 5 to 30 °C; for thigh SSP, shear stress also rapidly declined from its original 80 cPa. The great sensitivity of SSP to temperature was expected, and the inverse relationship between shear stress and temperature probably resulted from reduced protein–protein and protein–water interactions due to weakening of the hydrogen bonds. The abrupt shear stress increase at >37 °C for P. major and at >44 °C for thigh apparently resulted from protein coagulation or gelation, and the plunge immediately after reaching a peak was likely due to the collapse of the gel. There was a rate change in shear stress reduction as evidenced by a small peak (transition) at 13.2 ±0.7 °C for P. major and at 18.8 ± 1.7 °C for thigh. The origin of the peak was not clear, but it was also noted by Wu et al. (1985) for fish actomyosin. The peak temperature due to gelation was also lower for P. major (41.1 ± 0.5 °C) than for thigh (49.3 ± 1.7 °C), thus further substantiating that at pH ~6.0 gelation of white muscle SSP occurs more readily than gelation of red muscle SSP (Foegeding et al., 1991; Xiong and Blanchard, 1994). The similar responses to protein concentration (i.e., exponential) and fiber type (i.e., white > red) for shear stress of protein suspension (see Figures 3 and 4) and "strength" of the gel (Foegeding et al., 1991) indicated that the strength of thermally induced gels is related to the viscosity of the unheated protein suspension. Morita et al. (1987) reported that conditions favoring filamentogenesis and hence, increased viscosity, of unheated myosin dispersion allowed the formation of strong thermal gels.

Overall, salt-soluble proteins of chicken muscle exhibited time-independent, pseudoplastic flow when subjected to shear. However, under identical conditions (pH, protein concentration, shear rate), P. major SSP suspensions showed greater stresses and viscosities than thigh SSP, which could be ascribed to the isoforms in myofibrillar proteins. Viscosity of the protein suspension was inversely related to the onset temperature for SSP gelation and, therefore, might be indicative of

protein−protein interactions conducive to formation of gel networks. The study has produced further evidence of disparities in myofibrillar protein functionality between white and red muscles, which may help explain quality variations in processed muscle foods made from white and red meat.

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