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Comparative study of the heat-induced gelation of chicken breast and leg actomyosins affected by $FeCl_3$

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Abstract

Actomyosin were isolated from both chicken breast and leg muscles for stydying the effect of $FeCl_3$ on gelation properties of muscle proteins. The heat-induced gel strength (rigidity) and turbidity of actomyosins that included $FeCl_3$ were measured under various conditions. The maximum and minimum rigidity of thermally treated actomyosins at pH 6.0 in 0.6 M KCl occurred when $FeCl_3$ was added at the respective concentrations of 0.2 and 0.5 mM. The turbidity profiles of actomyosins that included $FeCl_3$ were quite similar to the changes of their rigidity under the same conditions between control and treatments in general. Results showed that the ferric cation accelerates intermolecular aggregating reaction, which is mainly responsible for the changes of turbidity and rigidity of actomyosins. In addition, the rigidity of leg actomyosin was higher than that of breast actomyosin. The difference in rigidity of leg and breast actomyosins lies in the characteristics of actomyosin per se from leg and breast muscles.

Introduction

Gelation of the myofibrillar proteins, myosin and actomyosin, during thermal processing is largely responsible for the physical and chemical stabilization of fat and water and for the development of the characteristic texture in comminuted and formed meat products[1,20]. Fundamental aspects of the gelling properties of muscle proteins have been extensively investigated. Previous studies have shown that the gelling properties of muscle proteins are affected markedly by thermal processing conditions such as pH, heating temperature, protein concentration, ionic strength, myosin/actin ratio and myosin isoforms[3,24,27].

Samejima *et al.*[26] investigated the effect of divalent metal salts on gelation properties of myosin and reported that the gel strength of rabbit skeletal myosin was enhaced by the addition of $CaCl_2$ and $MgCl_2$ at 10 mM. Recent studies[12,30] also reported that the same metal salts had shown a positive effect on gelation properties of chicken gizzard actomyosin and breast myofibrils. Ishioroshi *et al.*[13] further indicated that the poor quality of the breaking energy, elasticity and water holding capacity of low-salt sausage was improved by the addition of the same metal salts. On the other hand, our previous study[14] showed that the

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trivalent metal salt $FeCl_3$ enhanced the rigidity of thermal gels of chicken breast actomyosin. Myosins from breast (white) and leg (red) muscles not only differ in their biochemical characteristics but also in their gelation properties[4,7,17,18,25]. Thus, the effect of $FeCl_3$ on the heat-induced gelation of chicken breast and leg actomyosins were compared in this study.

Materials and Methods

Materials

Mature hens (White Leghorn or Rhode Island Red, approximately 16 months old) from the Chicken Farm of Rakuno Gakuen University were slaughtered. The breast and leg muscles were deboned immediately, removed of fat, tendons, large connective tissue, and then cooled in crushed ice for actomyosin preparation.

Methods

Preparation of actomyosins

Isolated breast and leg muscles were minced in a chilled grinder with plate orifices of 0.4 cm diameter. Actomyosins were extracted by the procedure of Szent-Györgyi[28] and examined by electrophoresis (SDS-PAGE) as described by Asghar *et al.*[5] for purity, and no significant variations were found between preparations. Protein concentration was determined by the Biuret method[10] using bovine serum albumin as a protein standard. The following experiments were replicated with freshly prepared actomyosin samples.

Turbidity determination

The turbidity of actomyosins that included $FeCl_3$ was determined according to the method of Samejima *et al.*[21], i. e., an actomyosin solution (0.5 mg/ml) with 0-0.50 mM $FeCl_3$ in 0.6 M KCl and 50 mM acetate or PIPES buffer was heated for 20 min at various pHs and temperatures, and then turbidity was determined at 340 nm in 1 cm cuvettes. The data presented in the figures are the mean values derived from three replications.

Measurement of gel strength (rigidity)

The rigidity of actomyosins that included $FeCl_3$ was measured as discribed by Yasui *et al.*[31] with a band-type viscometer. Three ml actomyosin solution (10 mg/ml) containing 0-0.50 mM $FeCl_3$ in 0.6 M KCl and 50mM acetate or PIPES buffer was heated at 65°C for 20 min in the glass cell of a spectrophotometer ($1 \times 1 \times 4$ cm) that was held in a thermostatically controlled water-jacket. The rigidity was measured 2 to 4 times in each set and the data given in the figures are the mean values of them. Other conditions such as pH, temperature, ferric salt and protein concentration are mentioned at appropriate places in the figures as they were varied according to the purpose of this study.

Results and Discussion

Effect of $FeCl_3$ on turbidity of actomyosins

Figure 1 indicates the turbidity changes of breast and leg actomyosin solutions that included $FeCl_3$ in 0.6 M KCl heated at 65°C for 20 min at different pHs. The turibidity of actomyosin solutions increased rapidly from pH 5.5 to 6.0 and declined slightly from pH 6.0 to pH 7.0. The maximum turbidity occurred at pH 6.0.

The turbidity of actomyosin solutions that included $FeCl_3$ in 0.6 M KCl at pH 6.0 heated at different temperatures for 20 min is illustrated in Fig. 2. A small and sharp increase in turbidity occurred, respectively, between 35 to 45°C and 45 to 65°C, and then declined gradually as the temperature moved toward 75°C. The maximum tubidity occurred at 65°C. The changes of turbidity of actomyosin solutions with different $FeCl_3$ concentrations at different temperatures is similar to those changes at different pHs, i. e., the maximum turbidity occurred when the $FeCl_3$ concentration was 0.20 mM and the minimum occurred at concentrations of 0.05 or 0.50 mM. However, the turbidity of leg actomyosin solution that included or didn't included $FeCl_3$ was somewhat higher than that of breast actomyosin solution at the same pH and temperature. This suggested that leg actomyosin formed more dense aggregation than did breast actomyosin. Previous authors have reported that proteins from both poultry breast and leg muscles[8,9,16,29] had

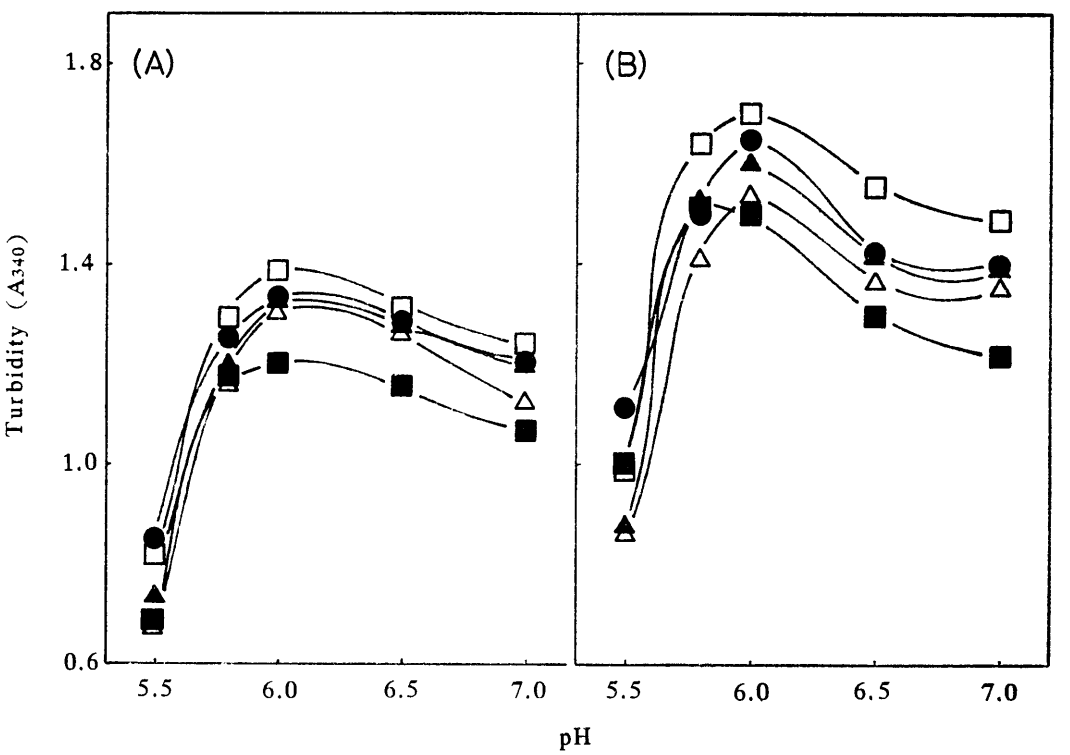

Fig. 1. Effect of $FeCl_3$ on turbidity (A_{340}) of chicken breast (A) and leg (B) actomyosin solutions at different pHs. Solution conditions: 0.5 mg/mℓ protein, 50 mM buffer, 0.6 M KCl, heating at 65°C for 20 min. pH was adjusted with acetate (5.5) or PIPES buffer (5.8-7.0).

●; control, △; 0.05 mM $FeCl_3$, ▲; 0.10 mM $FeCl_3$, □; 0.20 mM $FeCl_3$, ■; 0.50 mM $FeCl_3$.

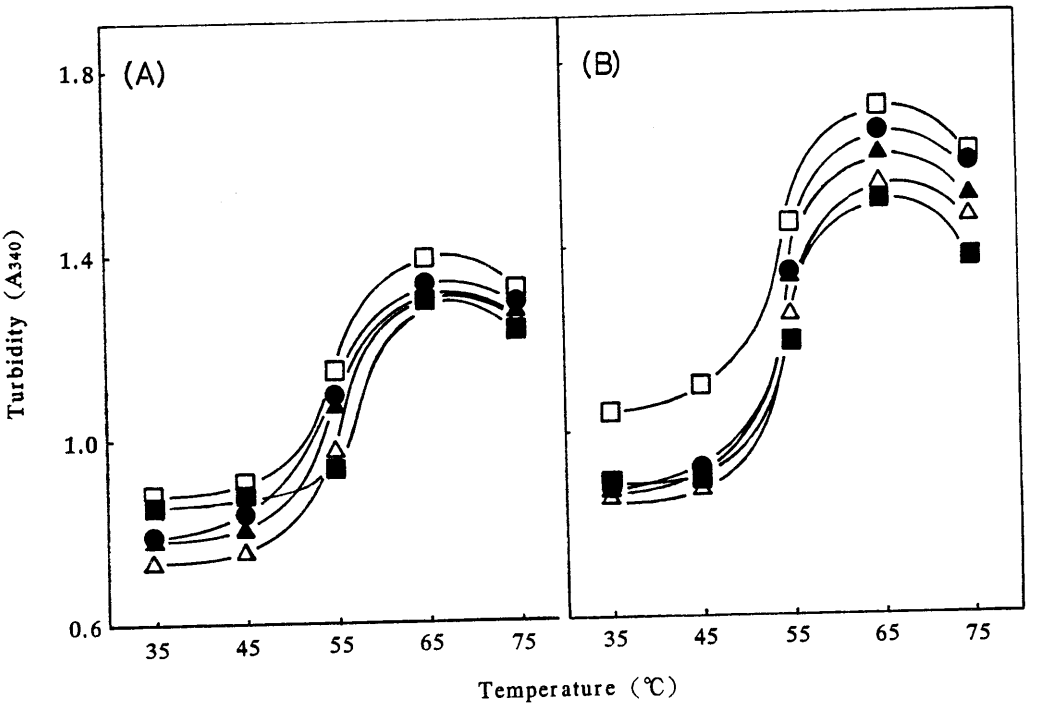

Fig. 2. Effect of $FeCl_3$ on turbidity (A_{340}) of chicken breast and leg actomyosin solutions at different temperatures. Solution condition: pH 6.0. Other conditions and symbols are the same as those described in Fig. 1.

different physicochemical characteristics and that pig cardiac myosin[23] formed much stronger aggregation than did skeletal myosin. These findings imply that actomyosins prepared from different sources and types of muscle fibrils probaly exhibit differences in turbidity properties.

However, our data shows that the turbidity characteristics of breast and leg actomyosins affected by $FeCl_3$ at the same pH temperature were similar in general. At the optimum concentration of $FeCl_3$ (i. e., 0.20 mM), the stronger three-dimensional network was formed by changing of the charge density of actomyosin as a function of polar or charged groups so as to induce the larger aggregates, and it was thought that this is why the turbidity of the actomyosin solutions increased. At low or high concentrations (e. g., 0.05 or 0.50 mM), the ferric action was weak or excessive, therefore, the turbidity of the actomyosin solutions decreased.

Effect of $FeCl_3$ on rigidity of actomyosins

Figures 3 and 4 respectively show how the $FeCl_3$ affected the rigidity of actomyosins in 0.6 M KCl heated at 65°C for 20 min at different pHs and temperatures. The results of both Fig. 3 (A) and Fig. 4 (A) had been already published recently by the authors[14]. For comparison, however, these data are shown again in this paper. On both sides of pH 6.0, the rigidity of actomyosins decreased rapidly, with the maximum and minimum occurring at pH 6.0 and 5.5, respectively. The rigidity increased initially when $FeCl_3$ was added from 0.05 to 0.02 mM,

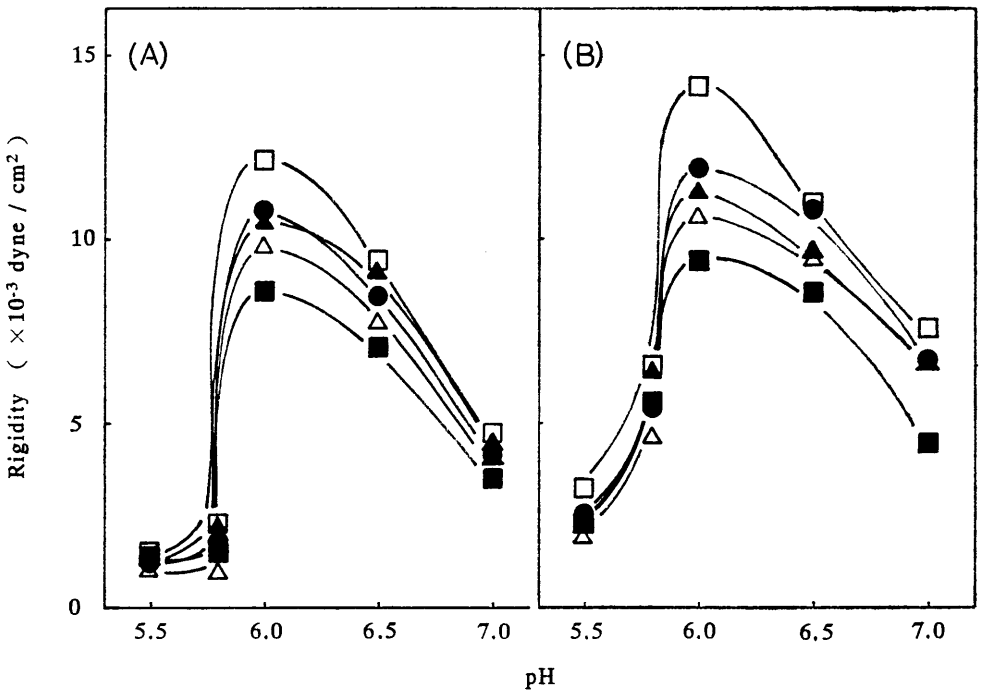

Fig. 3. Effect of $FeCl_3$ on rigidity of chicken breast and leg actomyosins at diffrent pHs. Gelation conditions: 10 mg/mℓ protein, 50 mM buffer, 0.6 M KCl, heating at 65°C for 20 min. Other conditions and symbols are the same as those described in Fig. 1.

while $FeCl_3$ concentration at 0.02 mM, actomyosins had shown the highest gel strength, then the strength had declined gradually. The minimum occurred at 0.05 and 0.50 mM when pH was, respectively, from 5.5 to 5.8 and from 6.0 to 7.0. When changing temperatures from 35 to 75°C, a sharp increase occurred from 55 to 65°C, then declined gradually as the temperature moved toward 75°C. The maximum rigidity occurred at 65°C.

These results (Fig. 3 and Fig. 4) agreed with those of Ishioroshi *et al.*[11] and Yasui *et al.*[32], who demonstrated that the optimum condition of the heat-induced gelation of skeletal muscle myosin and actomyosin was at temperatures between 60 and 70°C and at pH 6.0. Obviously, the gelation process of actomyosins was only enhanced by $FeCl_3$ through strengthening of the intermolecular aggregating reaction.

The data in Fig. 5 reveals the changes in the gelation properties of actomyosins at different ferric concentrations under identical conditions of pH, temperature, protein and salt concentrations. The changes are consistent with the results of the turbidity (Figs. 1, 2) and the rigidity (Figs. 3, 4) at different pHs and temperatures. Breast and leg actomyosins had shown the highest gel strength (12×10^{-3} and 14×10^{-3} dyne/cm^2) at $FeCl_3$ concentration of 0.20 mM.

Liu *et al.*[15] and O'Neill *et al.*[19] investigated the gelation properties of muscle proteins treated with neutral salts and sodium dodecyl sulphate and proposed that

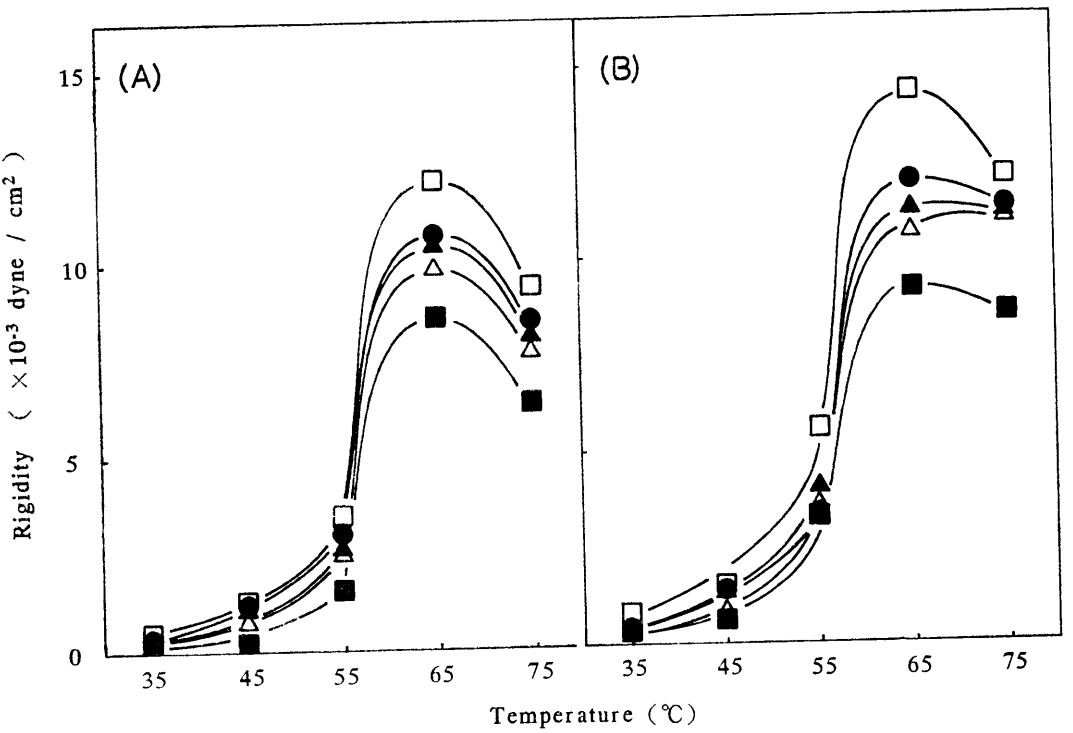

Fig. 4. Effect of $FeCl_3$ on rigidity of chicken breast and leg actomyosins at different temperatures. Gelation condition: pH 6.0. Other conditions and symbols are the same as those described in Fig. 1.

the hydrophobic protein-protein interactions play a significant role in actomyosin gel formation and stabilization. Samejima *et al.*[26] revealed that the rigidity of rabbit skeletal myosin was enhanced by $CaCl_2$ and $MgCl_2$ at the concentration of 10 mM and suggested that the cause was due to the local conformational changes in the hydrophobic environment around aromatic amino acids in myosin molecules. Xiong and Brekke[30] also found that the gel strength of myofibrils from chicken breast muscle varied with the concentration of $CaCl_2$ and $MgCl_2$, and a maximum in gel strength was seem at <5 mM. They reported that the effect of $CaCl_2$ and $MgCl_2$ on gelation properties was related to the changed protein extractability and protein-protein interaction of salt-soluble protein. However, our present study shows that the ferric cation accelerates intermolecular aggregating reaction, which is mainly responsible for the changes of turbidity and rigidity of actomyosins.

Some rigidity values of actomyosins that included $FeCl_3$ in 0.6 M KCl at pH 6.0 heated to 65°C for 20 min at diferent protein concentrations are depicted in Fig. 6. The changes of rigidity at different $FeCl_3$ concentrations were similar to those changes at different pHs and temperatures, i. e., the maximum and minimum rigidity occurred at 0.20 and 0.50 mM, respectively. The rigidity of actomyosins that included $FeCl_3$ increased with increasing protein concentration in an almost linear manner. Protein concentration is considered an important determinant of the strength of muscle protein gels[2,11,22]. Thus the positive effect of increasing

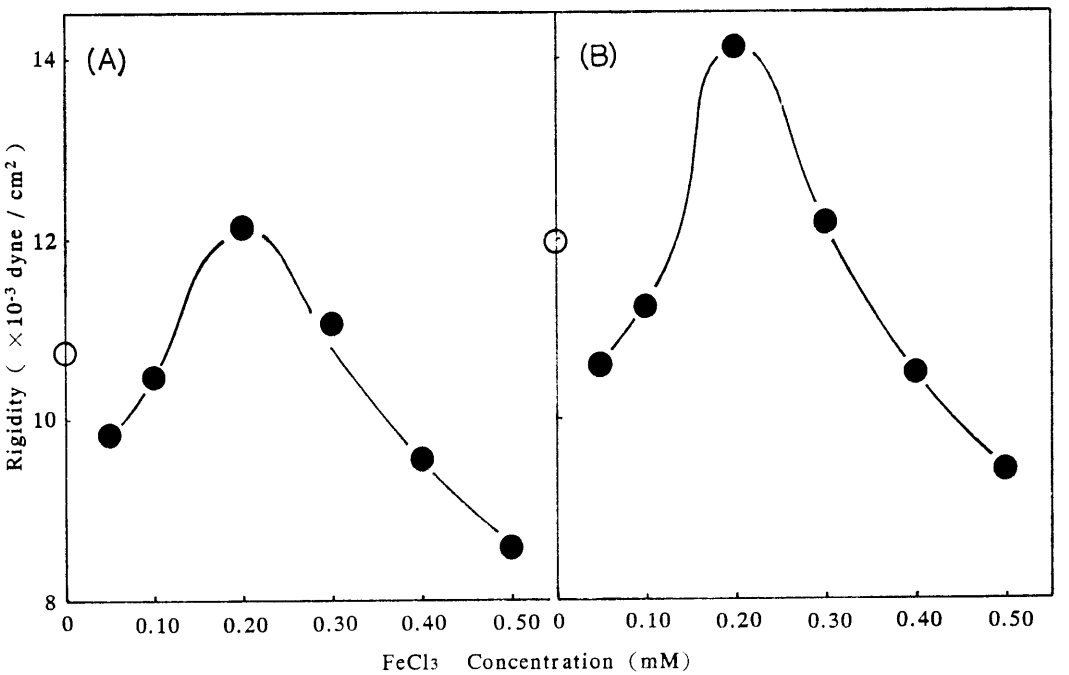

Fig. 5. Effect of $FeCl_3$ on rigidity of chicken breast and leg actomyosins at different ferric concentrations. Other conditions are the same as those described in Fig. 1.

○ : control, ● : with $FeCl_3$.

protein concentration with 0.20 mM $FeCl_3$ on the rigidity perhaps relates to an increased number of interaction sites and cross-links of actomyosin on heating.

Even though the changes of the gelation properties of breast and leg actomyosins affected by $FeCl_3$ under various conditions were similar, the rigidity of leg actomyosin was greater that of breast actomyosin. In the study of the relationship between the gel strength and the biochemical properties of muscle proteins, Samejima *et al.*[25] pointed out that the strength of thermal gels of long myosin filaments formed by dialysis was much higher than those of short filaments formed by dilution. Morita *et al.*[17] showed that the strength of heat-induced gels of both red and white muscle myosins is closely related to their morphological properties. Choe *et al.*[6] also reported that the gel structure of leg myosin rods revealed a finer network structure than that of breast myosin rods, which was coarse and more aggregated than the former. Based on those results, it seems that the difference in gel strength lies in the characteristics of actomyosin per se from leg and breast muscles, although there was no detailed investigation of this in this work.

In conclusion, the gel strength of chicken leg actomyosin was higher than that of breast actomyosin, the difference lying in the characteristics of actomyosin per se. The gel strength of actomyosins was enhanced by the trivalent salt $FeCl_3$ at 0.20 mM. The mechanism was attributed to the intermolecular aggregating

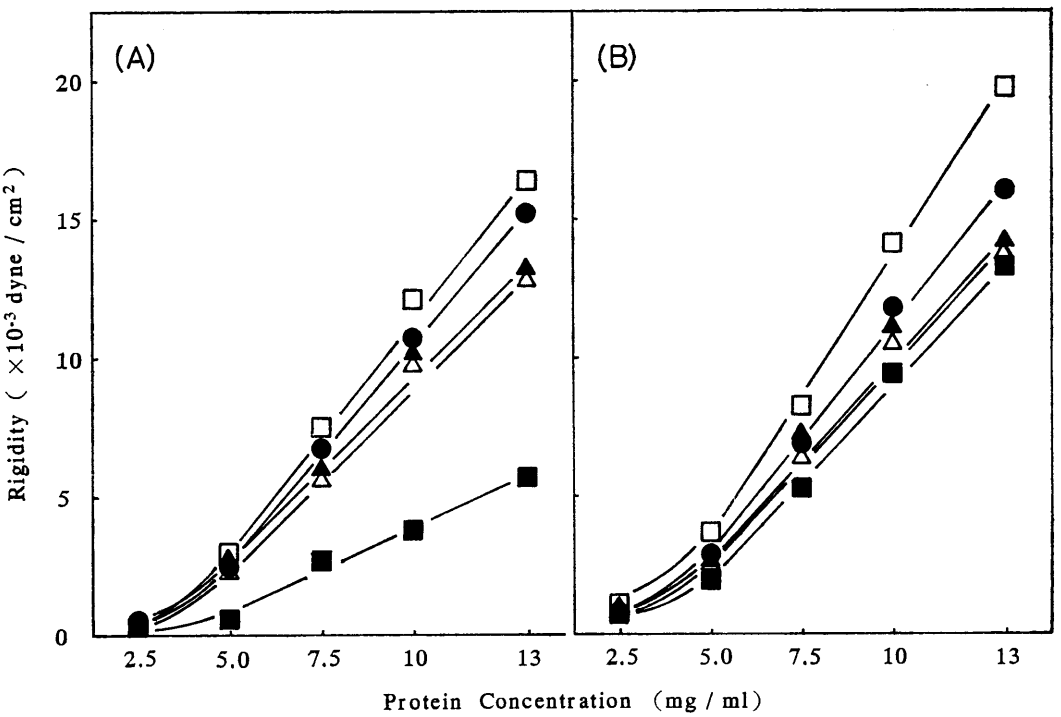

Fig. 6. Effect of $FeCl_3$ on rigidity of chicken breast and leg actomyosins at different protein concentrations. Gelation condition: pH 6.0. Other conditions and symbols are the same as those described in Fig. 1.

reaction of actomyosins as a function of the ferric substance.

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要　　約

鶏胸及び脚筋アクトミオシンの加熱ゲル化反応に及ぼす三価の陽イオン ($FeCl_3$) の効果を加熱にともなう濁度変化とゲル強度を測定することによって調査した。0.6 M KCl, pH 6.0 でのアクトミオシンの加熱ゲル強度は、$FeCl_3$ を 0.20 mM 添加した時に最大値となり、0.50 mM の時に最小値になった。アクトミオシンの濁度へ及ぼす $FeCl_3$ の影響は、加熱ゲル強度の結果と一致していた。結果は鉄イオンによる分子間凝集反応促進がアクトミオシンの濁度と剛性率の変化に影響することを明らかにしている。また、脚筋のアクトミオシンの剛性率は、胸筋のそれよりも高いことを示していた。脚筋と胸筋アクトミオシンの剛性率の差異は、それぞれのアクトミオシンの特性の違いによるものと考えられる。