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Influence of $FeCl_2$ and $FeCl_3$ on heat-induced gelation in porcine actomyosin

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Abstract

The influence of $FeCl_2$ and $FeCl_3$ on heat-induced gelation in porcine and myosin heavy chain (MHC) was investigated by measuring the rigidity and solubility of the actomyosin and the myosin heavy chain (MHC). The thermograms of the heat-induced gels were obtained by differential scanning calorimetry (DSC). Results showed that the maximum values for increased rigidity in the actomyosin occurred when $FeCl_2$ (1.5 mM) or $FeCl_3$ (0.3 mM) were present in the system.

The solubility of the actomyosin and MHC apparently decreased in the temperaure range of from 20 to 40 ℃. Changes in the DSC thermograms of the actomyosin were also detected by the addition of $FeCl_2$ (1.5 mM) or $FeCl_3$ (0.3 mM), suggesting that the actomyosin had been denatured by either the $FeCl_2$ or $FeCl_3$ before heating. These changes agreed well with the results of the rigidity measurements.

Introduction

It is well known that heat-induced gelation is one of the functional properties of meat proteins. Gelation during thermal processing is involved with water holding capacity, emulsifying ability and binding strength, which contribute greatly to the structural characteristics and stability of comminuted and formed meat products[5,6,8,17,18,20,21,32]. Fundamental studies have shown that gelation is a dynamic process which includes the initial aggregation of myosin heads, the unfolding of the helical tail portion and the subsequent formation of three-dimensional network structures[10,22]. The interfilamental aggregation of myosin heads is responsible for myosin gel formation, and the formation of such a gel with adequate rheological properties is affected significantly by both the physicochemical characteristics of the meat proteins and the thermal processing conditions i.e., pH, heating temperature, protein concentration, ionic strength, myosin/actin ratio and myosin isoforms[1,9,11,23,25,33,34].

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Apart from these factors, recent studies have demonstrated that some divalent metal salts ($CaCl_2$ and $MgCl_2$) and egg shell powder had a beneficial effect on the gelation properties of meat proteins such as myosin[24], actomyosin[12] and myofibril[31] and the rheological properties of meat batters[4,30] or sausages[13]. On the other hand, our previous study indicated that the heat-induced gel strength of chicken actomyosin from breast muscle was enhanced by ferric chloride ($FeCl_3$)[15]. However, how the gelation properties of porcine meat proteins are affected by iron salts ($FeCl_2$ and $FeCl_3$) remain unknown. In this study, we investigated the influence of $FeCl_2$ and $FeCl_3$ on the heat-induced gelation of porcine actomyosin.

Materials and Methods

Meat source and reagents

Fresh leg muscle of swine, purchased from local meat stores, was chosen for isolation of the actomyosin. The piperazineN, N-bis (2- ethane sulfonic acid)(PIPES) used in this study was purchased from Sigma Chemical Co. Ltd.

Isolation of actomyosin

Actomyosin was isolated according to the procedure of Szent-Gyorgyi[28] and examined for purity by 7.5% SDS-PAGE as outlined in an earlier study[2]. No significant differences were found between actomyosin samples isolated on different occasions. Protein concentration was determined by the Biuret method[7] using bovine serum albumin as a protein standard. The isolated actomyosin was stored at 4℃ prior to use.

Measurement of thermal gel strength (rigidity)

The thermal gel strength (rigidity) of the actomyosin was measured with a band-type viscometer (FUDOH RHEO METER, NRM-20002 J) as reported by Yasui et al.[32] Protein solutions containing 10 mg/ml of actomyosin, 0.6 M KCl, 50 mM acetate (pH 5.5) or PIPES (pH 5.8-7.0) and various concentrations of $FeCl_2$ or $FeCl_3$ were heated at 65℃ for 20 min. The rigidity was measured three or four times under each set of conditions and the data shown in the figures represents the mean values along with the standard deviations obtained. No standard deviation bar is shown when the standard deviation was negligible.

Determination of solubility

Protein solutions containing 1.5 mg/ml of actomyosin, 0.5 M NaCl, 50 mM PIPES (pH 6.0) and various concentrations of $FeCl_2$ or $FeCl_3$ were heated at various temperatures for 20 min. The absorbances at 280 nm of the supernatant (A) of the unheated samples and the absorbances at the same wave length of the supernatant (B)

of the heated samples after centrifugation at 10,000 rpm for 5 min were used to make an estimate of the solubility of the actomyosin. The equation used for calculation was expressed as: [Solubility (%) = (B) × 100/(A)]. The data presented in the figures were the mean values derived from the three replicates. The standard deviation bar is omitted because its value was negligible.

Solubility of myosin heavy chain (MHC)

Protein solutions, adjusted in the same way as those described above, were heated at various temperatures for 20 min, and then centrifuged at 10,000 rpm for 5 min. The supernatants (0.3 ml) containing uncoagulated protein were analysed by SDS-PAGE. The relative changes in the unaggregating behaviour of the MHC were manifested from the SDS-PAGE profiles by densitometer (Model 1650, Bio-Rad) recordings.

Differential scanning calorimetry (DSC)

DSC analysis was performed on a Rigaku Denki DSC-8240 to study the effect of the $FeCl_2$ and $FeCl_3$ on the denaturation of the actomyosin. Protein solutions containing 25 mg/ml of actomyosin, 0.5 M KCl, 20 mM PIPES buffer (pH 6.0) and various concentrations of $FeCl_2$ or $FeCl_3$ were transferred to aluminum hermatically sealed pans and weighed to 20 mg. Distilled water was sealed in another pan to be used as a reference. The heating rate was kept at 5℃/min. Data analysis program was applied to the thermal curves in order to provide the temperatures for the maximum transition (Tmax).

Results and Discussion

Effects on rigidity

The changes in the rigidity of the heat-induced gels of porcine actomyosin in 0.6 M KCl at pH 6.0 at various concentrations of $FeCl_2$ are shown in Fig. 1 (a). An increase in rigidity could be observed of up to 1.5 mM for $FeCl_2$ which then decreased gradually to 8.0 mM $FeCl_2$. Fig. 1 (b) exhibited a maximum increment in rigidity when 0.3 mM $FeCl_3$ was present under the same conditions. We have reported that the rigidity of the heat-induced gelation of chicken actomyosin from breast muscle was enhanced by $FeCl_3$[15]. As for changes in rigidity against various $FeCl_3$ concentrations, however, these results showed no significant differences, which might be a reflection of the approximate physicochemical characteristics and gelation properties of the samples (chicken and porcine actomyosin) used in the two experiments. Figure 2 shows that the rigidity of actomyosin increased with pH from 5.5, reached a maximum at pH 6.0 and declined toward pH 7.0. With the addition of 1.5 mM $FeCl_2$ (a) and 0.3 mM $FeCl_3$ (b), the maximum rigidity of the actomyosin was

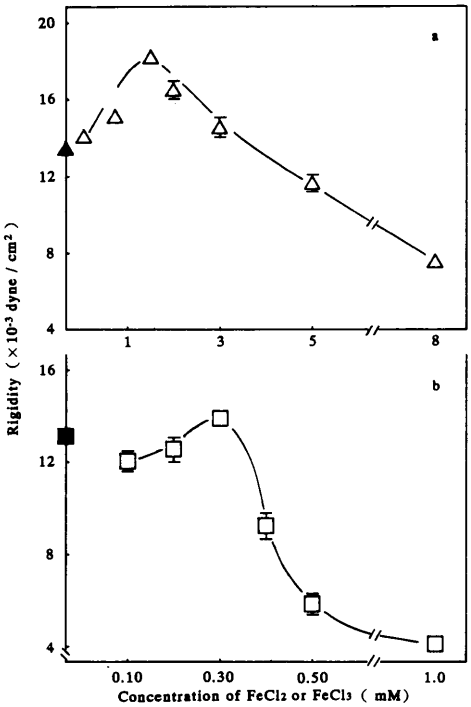

Fig. 1 Effect of concentrations of $FeCl_2$ (a) and $FeCl_3$ (b) on rigidity of heat-induced gels in actomyosin. Solution conditions: 10mg/ml protein, 50mM PIPES, 0.6MKCl, pH 6.0. Solutions were heated at 65℃ for 20min. Bars represent standard deviation.
▲, ■ : control ; △ : with $FeCl_2$ (a) ;
□ : with $FeCl_3$ (b)

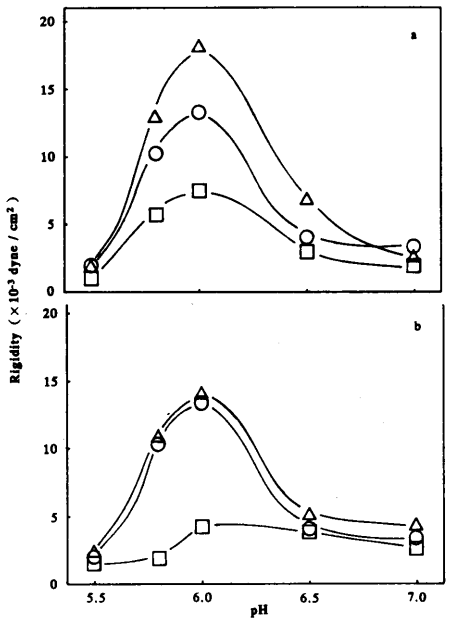

Fig.2 Effect of $FeCl_2$ (a) and $FeCl_3$ (b) on rigidity of heat - induced gels in actomyosin at different pHs.
pH was adjusted with acetate (5.5) or PIPES (5.8-7.0). Other conditions were the same as those described in Fig. 1.
(a), ○ : control ; △ : with 1.5 mM $FeCl_2$;
□ : with 8.0 mM $FeCl_2$
(b), ○ : control ; △ : with 0.3mM $FeCl_3$;
□ : with 1.0 mM $FeCl_3$

again observed to be at pH 6.0. This suggests that the gelation process of the actomyosin was influenced by both $FeCl_2$ and $FeCl_3$. In addition, it appears that $FeCl_2$ (1.5 mM) had a greater effect in enhancing the rigidity of the actomyosin than did $FeCl_3$ (0.3 mM).

Changes in solubility of actomyosin

The effect of $FeCl_2$ or $FeCl_3$ on the solubility of actomyosin together with 0.5M NaCl at pH 6.0 was temperaturs dependent which is shown in Fig. 3. The solubility of the actomyosin decreased along with increasing temperatures of from 30 to 40℃, and then changed slightly with a further increase in temperature of up to 80℃. However, appreciable differences were found when $FeCl_2$ (1.5 mM) and $FeCl_3$ (0.3 mM) were added. The solubility of the actomyosin decreased markedly with the addition of $FeCl_2$ in the temperature range of 20 to 40℃. Thereafter, there were no distinct changes found among the controls and the Fe-treatments. The optimum temperature for the heat-induced gelation of skeletal muscle myosin and actomyosin is

between 60 to 70℃[8,33]. However, our data indicate that the heat-induced aggregation of actomyosin was accelerated by $FeCl_2$ and $FeCl_3$ even at low temperatures (from 20 to 40℃), though with no obvious changes at high temperatures (from 40 to 80℃).

Liu et al.[16] and O'Neill et al.[19] investigated the effect of neutral salts and sodium dodecyl sulphate on the gelation properties of meat proteins and suggested that hydrophobic protein-protein interaction plays a significant role in actomyosin gel formation and stabilization. A study by Samejima et al.[24] showed that the enhancement of rigidity in rabbit skeletal myosin by $CaCl_2$ and $MgCl_2$ was due to local conformational changes in the hydrophobic environment around the aromatic amino acid residues in the myosin molecules. Xiong and Brekke[31] also reported that the effect of $CaCl_2$ and $MgCl_2$ on the gelation properties of myofibrils is related to the change in protein extractability and protein-protein interaction of the salt soluble protein.

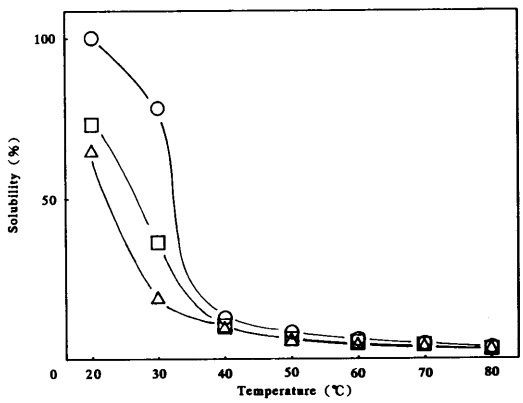

Fig. 3 Effect of $FeCl_2$ and $FeCl_3$ on solubility of actomyosin.
Solutio nconditions:1.5mg/ml protein,50mM PIPES, 0.5M NaCl, pH6.0. Solutions were heated at 20, 30, 40, 50, 60, 70, and 80℃ for 20 min and then cenrifuged at 10,000 rpm for 5min. ○: control; △: with 1.5 mM $FeCl_2$; □: with 0.3 mM $FeCl_3$

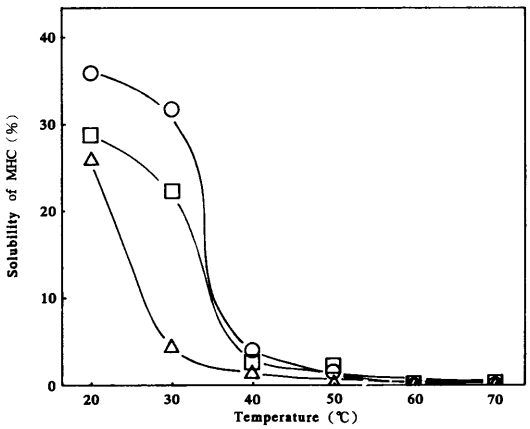

Fig. 4 Effect of $FeCl_2$ and $FeCl_3$ on solubility of MHC. Soluutions were heated at 20, 30, 40, 50, 60 and 70℃ for 20 min and centrifuged at 10,000 rpm for 5min. Other conditions and symbols were the same as those described in Fig. 3.

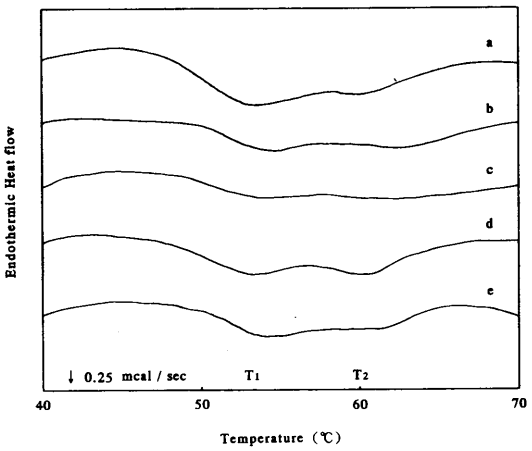

Fig. 5 Effect of $FeCl_2$ and $FeCl_3$ on DSC thremograms of actomyosin.
Solution conditions: 25mg/ml protein, 20mM PIPES, 0.5M KCl, pH6.0. pH was adjusted with PIPES(6.0).
a:control; b:with 1.5 mM $FeCl_2$; c:with 8.0 mM $FeCl_2$; d:with 0.3mM $FeCl_3$; e:with 1.0 mM $FeCl_3$

However, our results on solubility show that the ferric or ferrous cations changed the charge density of the protein through polar or charged groups, thus accelerating in ferric-protein, ferrous-protein and protein-protein aggregating reactions, which seemed to be mainly responsible forthe changes in rigidity.

Solubility features of MHC

The thermal gels were centrifuged at 10,000 rpm for 5 min and analyzed by SDS-PAGE, the changes in the remaining amounts (relative) of MHC in the supernatants are shown in Fig.4. A significant reduction of MHC occurred under the presence of 1.5 mM $FeCl_2$ and 0.3 mM $FeCl_3$ within a narrow range of temperatures (20 to 40℃). Myosin has a greater gel forming ability than most other myofibrillar proteins[1,20,25]. Therefore, it can perhaps be explained as $FeCl_2$ and $FeCl_3$ affecting the actomyosin gelation by changing the association or aggregation reaction of the myosin. The results of Fig.4 were similar to those of Fig.3.

DSC thermograms of thermal gel

Two major transitions (T_1 and T_2) were observed (Fig.5) at temperatures of about 54 and 60 ℃ in the typical DSC thermogram of the actomyosin control (a). According to the results of some researchers[14,27,29], T_1 and T_2 corresponded to the transitions of myosin and actin, respectively. As the concentrations of added $FeCl_2$ increased from 1.5 to 8.0 mM (b and c), the peak heights in the DSC profiles decreased markedly at the denaturation temperatures of myosin and actin. This implies a significant effect for $FeCl_2$ on the denaturation of actomyosin. Unlike the case of $FeCl_2$, the peaks at the two major transitions showed no change when the concentrations of $FeCl_3$ were increased from 0.3 to 1.0 mM (d and e), although their peak heights were somewhat lower than those of the controls. The changes in the thermograms suggest the occurrence of denaturation prior to thermal treatment. The actomyosin was denatured more severely by $FeCl_2$ than by $FeCl_3$.

Samejima et al.[24] studied the effects of divalent cation son the rigidity of myosin and reported that enthalpy originating in rabbit myosin was reduced by increasing the concentrations of $CaCl_2$ or $MgCl_2$ at 5-10 mM. Similar effects were also observed by Barbut and Findlay[3], and Stabursvik and Martens[26] in their DSC studies. Our data suggest that the actomyosin had been more or less denatured by both the $FeCl_2$ and $FeCl_3$ prior to heating. $FeCl_2$ and $FeCl_3$ seem to act in a similar manner on myosin and actin as denaturants in the case of $CaCl_2$ or $MgCl_2$. Fig. 5 also indicates that $FeCl_3$ was a weaker denaturant for actomyosin than $FeCl_2$.

Therefore, it can be concluded from the results of the present investigation that gelation and/or aggregation of porcine actomyosin was enhanced by both $FeCl_2$ and $FeCl_3$ but more so by the former. Ferrous or ferric cations may change the charge density of actomyosin through polar or charged groups, thus accelerating the ferrous-protein, ferric-protein and protein-protein aggregating reactions, which are primarily involved in changes in porcine actomyosin gelation.

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要　約

豚のアクトミオシンの加熱ゲル化反応に及ぼす $FeCl_2$ および $FeCl_3$ の影響をゲル強度とアクトミオシンおよびミオシン重鎖の（MHC）溶解度を測定することによって検討した。また，これらの金属塩のアクトミオシンにおよぼす効果を示差走査熱量分析計によって測定した。$FeCl_2$ と $FeCl_3$ をそれぞれ 1.5 mM および 0.3 mM 添加した時アクトミオシンの剛性率は最大となった。$FeCl_2$ や $FeCl_3$ が存在すると，アクトミオシンと MHC の溶解度は比較的低温度（20 — 40℃）で低下した。アクトミオシンの示差熱量曲線の変化が $FeCl_2$ や $FeCl_3$ 添加によって観察された。この結果は，豚のアクトミオシンが $FeCl_2$ や $FeCl_3$ により加熱前に変性することを示している。これらの変化は，ゲル強度測定結果を強く支持するものである。