EFFECT OF DIVALENT CATIONS ON HEAT-INDUCED GELATION OF MYOSIN

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SUMMARY

The effect of divalent cations on heat-induced gelation of myosin was studied by measuring of rigidity, turbidity, differential scanning carolimetry (DSC) and fluoresence intensity. The microstructures of the heat -set gels were observed by scanning electron microscopy (SEM).

The changes in turbidity of myosin solution thermally treated showed a maximum at about 5 - 10 mM $CaCl_2$ or $MgCl_2$ at 0.5 M KCl. The changes in the rigidity of myosin gel well consisted with those of the turbidity. The network structures of heat-set gels of myosin with or without $CaCl_2$ or $MgCl_2$ corresponded to the results of their rigidity. The results obtained from fluoresence and DSC measurements suggested that the addition of $CaCl_2$ or $MgCl_2$ induced the conformational changes in myosin molecule.

INTRODUCTION

Water-holding and binding properties are an important factors that determine the quality of comminuted meat products. These properties are closely interrelated and gelation of myosin is a decisive phenomenon which takes place in all structured meat products during thermal processing. It involves both intramolecular and intermolecular changes in proteins.

Asghar et al. (1985) has reviewed that the gel-formability of myosin was affected by several factors such as pH, ionic strength, temperature, protein concentration, myosin/actin ratio and myosin isoforms.

In the present study, we report the effect of calcium and magnesium on heat-induced gelation of myosin.

MATERIALS AND METHODS

Preparation of myosin

Myosin was prepared from rabbit skeletal muscle according to the method described by Yasui et al. (1979).

Turbidity measurements

The solutions containing 0.5 mg/ml of myosin, 0.5 M KCl, 20 mM Tris-maleate (pH 6.0) and various concentrations of $CaCl_2$ or $MgCl_2$ were incubated at 65 ^oC for 20 min. Turbidity was measured at 660 nm in 1 cm cuvetts.

Gelation

Myosin (4.5 mg/ml) in same solution described above was heated to 65 ^oC and held for 20 min. Heat-induced gelation of myosin was monitored by measuring changes in rigidity with band-type viscometer reported by Yasui et al. (1979).

Fluoresence spectra

Fluoresence spectra were measured at room temperature with a JASCO EP-770. The fluoresence intensity at 340 nm was continuously recorded. Exitation was at 290 nm.

Protein solutions containing 0.1 mg/ml of myosin in 0.5 M KCl, 20 mM Tris-maleate buffer (pH 6.0) and various concentrations of $CaCl_2$ or $MgCl_2$.

Differential scanning calorimetry (DSC)

The effect of heat and divalent cations on the denaturation of myosin was studied using a differential scanning calorimeter (Rigaku Denki DSC 8240). Ten mg/ml of myosin in the same solution described above was hermatically sealed in aluminum pans and the same buffer was used in the reference pan. DSC curved were recorded for heating of 5 ^oC/min in the temperature range from 30 to 90 ^oC.

Scanning electron microscopy (SEM)

The gels formed by thermal treatment under the same conditions for gelation were fixed, stained, and prepared for SEM using the procedure outlined by Yasui et al. (1979).

RESULTS AND DISCUSSION

The effect of divalent cations on turbidity of rabbit myosin are shown in Fig. 1 Turbidity of myosin increased with concentration of $CaCl_2$ or $MgCl_2$, and the highest values in turbidity were obtained at about 5 - 10 mM of $CaCl_2$ or $MgCl_2$. The increase in turbidity of protein solutions suggests association of protein molecule. Thus, these results indicate that changes in the mode of association of myosin molecule are different in various concentration of $CaCl_2$ or $MgCl_2$.

Fig. 2 shows the strength of heat-induced gel of myosins as a function of the pH and the concentration of $CaCl_2$. At pH 6.0 the maximum rigidity was given at 5 - 10 mM of $CaCl_2$. These changes consist with the results of Xiog and Brekke (1991). The rigidity of myosin

Fig. 1 Changes in the turbidity of rabbit myosin at various concentration of $CaCl_2$ or $MgCl_2$.
○ : $CaCl_2$, ● : $MgCl_2$

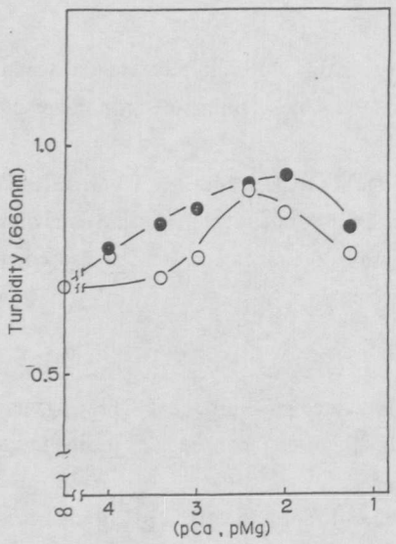


Fig. 2 Effects of $CaCl_2$ on the rigidity of heat-induced gel of rabbit myosin.
△ : pH 5.5 , ○ : pH 6.0 , □ : pH 6.5
■ : 5 mM EDTA (pH 6.0)

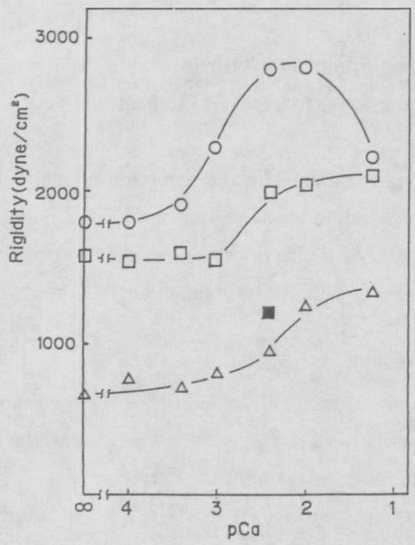
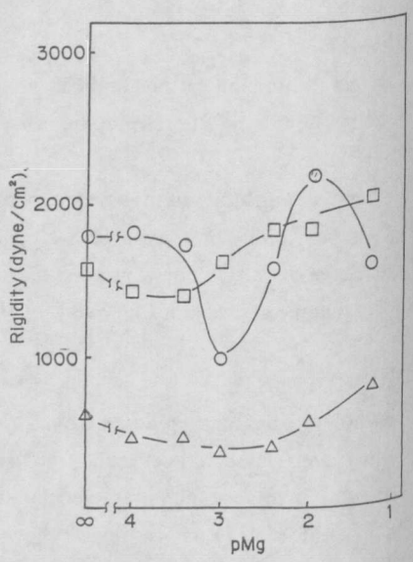


Fig. 3 Effects of $MgCl_2$ on the rigidity of heat-induced gel of rabbit myosin.
Symbols are the same as in Fig. 2.



increased with increasing of $CaCl_2$ although their values at both pH 6.5 and 5.5 were lower than those of pH6.0. The addition of 5 mM EDTA to the system at pH 6.0 before heatiung caused a considerabl reduction in the rigidity to well below that of the controls. This result suggested that calcium affected the thermal gelation of myosin.

Effects of magnesium on the rigidity of myosin gel are shown in Fig. 3. This result depicts that the magnesium did not make a distinct effect on the rigidity as well as the calcium. We have not any informations about these differences between the two divalent cations for the present.

Fig. 4 The changes in the fluoresecnce intensity of myosin induced by $CaCl_2$ or $MgCl_2$.
○ : $CaCl_2$, ● : $MgCl_2$

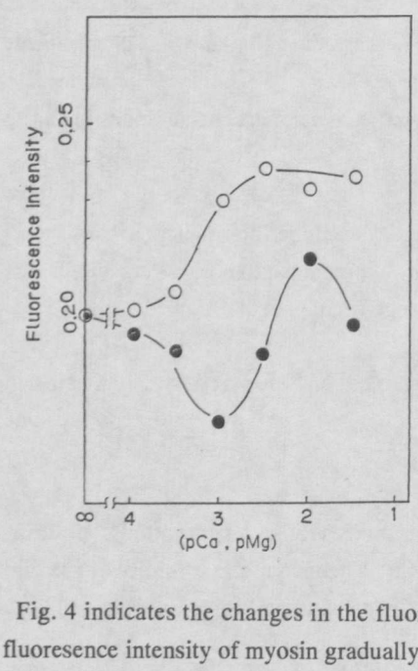


Fig. 5 Effects of $CaCl_2$(a) and $MgCl_2$ (b) on DSC profiles of myosin.

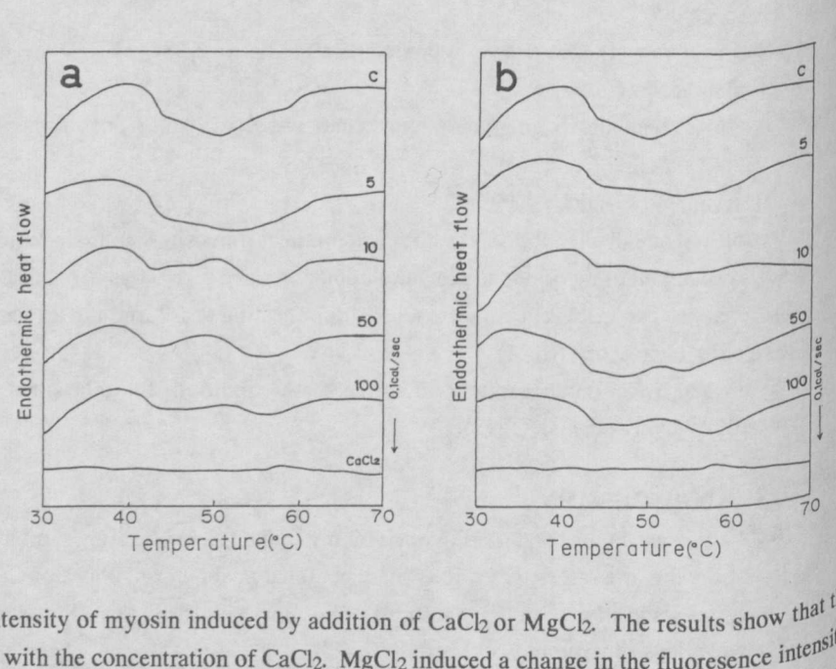


Fig. 4 indicates the changes in the fluoresence intensity of myosin induced by addition of $CaCl_2$ or $MgCl_2$. The results show that the fluoresence intensity of myosin gradually increases with the concentration of $CaCl_2$. $MgCl_2$ induced a change in the fluoresence intensity of myosin at about 10 mM. However, the effect of $MgCl_2$ on the fluoresence intensity of myosin differed from those of $CaCl_2$. These results imply that aromatic amino acid residues (especially tryptophan residues) are gradually transferred by the divalent cation from a moderately hydrophobic environment inside the myosin molecule to an exposed polar environment.

Fig. 6 Effects of $CaCl_2$ (○) and $MgCl_2$ (△) on changes in the enthalpy of myosin

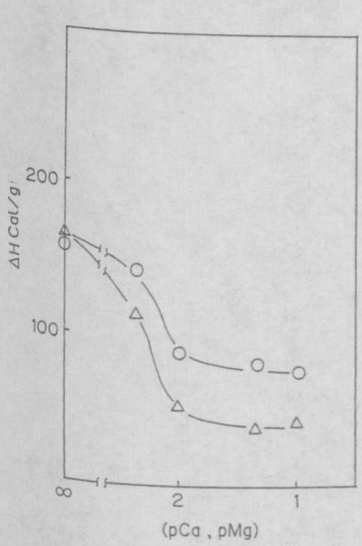
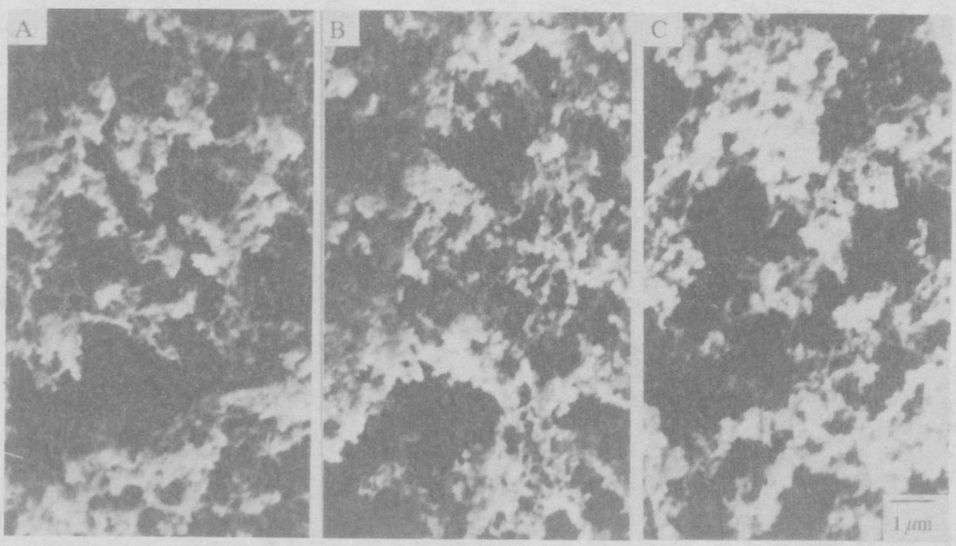


Fig. 7 Scanning electron micrographs for heat-set gels of mysoin..
A : myosin , B : with 10 mM $CaCl_2$
C : with 10 mM $MgCl_2$



The effects of $CaCl_2$ and $MgCl_2$ on the DSC-thermograms of myosin are shown in Fig. 5 (a) and (b), respectively. A major transition is observed with a Tm of about 50 ^oC, with two minor transitions at temperatures of 45 and 57 ^oC in the typical DSC profiles for control myosin. The results show that the major transition of about 50 ^oC is decreased by the addition of 5 - 10 mM $CaCl_2$ or $MgCl_2$. However, the two minor transitions were remained at the higher concentration of divalent cations (100 mM $CaCl_2$ and $MgCl_2$), although their peak hights were lower than those of control. According to the study of Samejima et al. (1983), the three endotherms are thought to correlate with the Tms of the principal domains in the myosin molecule, i.e. the major (50 ^oC) and the two minor (45, 57 ^oC) transitions correspond to the myosin head and tail portions, respectively.

The data on the changes in enthalpy derived from DSC thermograms as a function of $CaCl_2$ and $MgCl_2$ concentrations are depicted in Fig. 6. With increasing of both $CaCl_2$ and $MgCl_2$ concentrations, the magnitude of enthalpy for myosin decreased. Barbut and Findlay reported that the enthalpy originated in myosin was reduced by increasing the $MgCl_2$ concentrations in their DSC measurements. The results indicated that myosin molecules become very susceptible to denaturation with addition of divalent cation. The result also suggests that $MgCl_2$ has relatively stronger destabilization effects than $CaCl_2$ for myosin molecule.

Fig. 7 shows SEM pictures of myosin gels taken from the heat-set gels. The micrographs indicate a changes in morphology of the gels arising with addition of 10 mM $CaCl_2$ or $MgCl_2$. The microstructures of the heat-induced gels vary in pore size distribution, and in network strand sharp and thickness. SEM pictures for the samples prepared with 10 mM $CaCl_2$ (B) and $MgCl_2$ (c) indicate the thiner and smoother microstructure than that of control myosin gel (A). The results obtained from these morphological observations consist with the changes in the turbidity (Fig. 1) and the rigidity (Fig. 2 and 3).

In the study of gelation properties of chicken myofibrils treated with calcium and magnesium chlorides, Xiong and Brekke (1991) reported that the extractability of salt-soluble protein and myofibril gel strength of chicken leg muscle were increased by $CaCl_2$ and $MgCl_2$. They concluded that the divalent cations affected chicken myofibril gelation by changing the extraction and protein-protein interaction of salt soluble protein.

We conclude from the results of the present investigation that the enhancement on the rigidity of myosin heat-set gel was due to the changes in the tertiary structure of myosin molecules induced by $CaCl_2$ or $MgCl_2$.

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