THE EFFECTS OF CROSSLINKING INTRODUCED BY A MICROBIAL TRANSGLUTAMINASE ON THE HEAT-INDUCED GELATION OF MYOSIN B AND ON THAT OF MUSCLE PROTEINS

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SUMMARY: This paper reports the effects of crosslinking introduced by a microbial transglutaminase on the heat-induced gelation of myosin B and muscle proteins. The microbial transglutaminase was added at a ratio of 0.01 unit/mg of myosin B or muscle protein prepared from the porcine longissimus dorsi. After incubation for 0-120 min at 40℃, the reaction mixtures were immediately heated for 30 min at 70-90℃. Analyses of myosin B (20 mg/ml) in 0.6 M KCl (pH 6.0) by SDS-PAGE showed that the heavy chain of myosin was mostly polymerized during the incubation period. The heat gel-strength of the polymerized myosin B was markedly increased as compared with that of native myosin B, and the gelation was not restrained by the presence of dithiothreitol (1 mM). The optimum pH and temperature for gelation of the polymerized myosin B were pH 6.0 and 90℃, respectively. The dependency of heat gel-strength on protein concentration was higher in polymerized myosin B than in native myosin B. Scanning electron micrography showed that the ultramicrostructure of the gels became finer as the polymerization of myosin heavy chain proceeded. These ultramicrostructral changes corresponded well to the changes in gel-strength. Similar effects of this enzyme were also observed in muscle proteins which had been treated with sodium chloride (2.0%) and phosphates (0.5%). The gel-strength of polymerized muscle proteins was increased two-fold. These results suggest the usefulness of the microbial transglutaminase for meat product processing by improving the functional properties of muscle proteins.

INTRODUCTION: Transglutaminase catalyzes a calcium-dependent acyl-transfer reaction in which the γ-carboxamide groups of peptide glutaminyl residues act as acyl donors. Primary amino groups in a variety of compounds may function as acyl acceptors. When ε-amino groups of protein lysine residues act as acceptors, cross linking of proteins via ε-(γ-glutamyl) lysine bridges occurs.

Derrick and Laki (1966) provided the first evidence for crosslinking of myofibrillar protein introduced by a transglutaminase purified from guinea pig liver. It has also been shown that a plasma transglutaminase can catalyze the formation of glutamyl-lysine bonds not only in myosin but also between myosin and actin, myosin and fibronectin, and between fibrin and actin (Cohen et al.,

1979; Kahn and Cohen, 1981).

Recently a microbial transglutaminase was found to catalyze the formation of ε-(γ-glutamyl)lysyl bonds in muscle protein (Nonaka et al., 1989). However, the functional properties of the resultant proteins as food proteins have not been clarified. To evaluate the feasibility of applying the microbial transglutaminase to meat product processing, we studied the effect of crosslinking introduced by this enzyme on the heat-induced gelation of myosin B and on that of muscle proteins.

MATERIALS AND METHODS: The muscles from the porcine longissimus dorsi (3-7 days postmortem) were minced twice through a plate with 3 mm pores. Myosin B was prepared from the muscle proteins by the method of Szent-Gyoergyi (1951).

A microbial transglutaminase (BTGase) was prepared at the Central Research Laboratories of Ajinomoto Co., Inc. The enzyme activity, determined by the method of Folk and Cole (1966), was 1.1 units/mg. The molecular weight of the enzyme was 38 k dalton and its isoelectric point was 9.0.

A myosin B solution (20 mg/ml) was prepared in 0.6 M KCl and 20 mM citrate buffer (pH 5.0) or in 0.6 M KCl and 20 mM phosphate buffer (pH 6.0-7.0). Muscle proteins were homogenized with water, sodium chloride (2.0%) and phosphates (0.5%). The enzyme was added at ratio of 0.01 unit/mg of protein. After incubation for 0-120 min at 40℃, reaction mixtures were immediately heated for 30 min at 70-90℃.

Gel strength was measured in terms of the maximum force required for the flat-ended plunger of a Rheometer (Fudokogyo Co., 5 mm in diameter) to penetrate into the sample at a constant crosshead speed of 20 cm/min.

Polyacrylamide gel electrophoresis in SDS was carried out on a slab gel (3-5 % gradient separating gel) using the SDS-tris-glycine discontinuous buffer system described by Laemmli (1970). Before being applied to the gels, samples were incubated at 100℃ for 5 min in the presence of 2% SDS, 5% 2-mercaptoethanal, 15% glycerol and 0.01% bromophenol blue.

Heat-induced gel samples were fixed in2.5%glutaraldehyde and 2% paraformaldehyde, and then dehydrated in a graded ethanol series. Using acetone as the transition fluid, the samples were dried using liquid CO_2 in a Hitachi HCP-2 critical point drier. The dried samples were mounted on aluminum stubs

and examined under a Hitachi 2300 scanning electron microscope.

RESULTS AND DISCUSSION: To detect the formation of intermolecular crosslinks, changes in the molecular sizes of myosin B after the BTGase reaction were analyzed by SDS-PAGE (Fig. 1). The fractions of the myosin heavy chain and tropomyosin diminished and ultimately disappeared as the reaction time was increased. Presumably the polymers generated by BTGase could not enter the gradient gels. No change in the fractions of actin, troponin, and myosin light chain were detected under the conditions used here. These results are in agreement with those of Nonaka et al. (1989), who treated rabbit myosin and actin with BTGase at 10℃.

When the native myosin B was incubated at 40℃ without BTGase, it formed a gel after 30 min, while the polymerized myosin B turned into a gel after 20 min (Fig. 2), These phenomena indicates that heat-induced gelation (at 40℃) of myosin B was promoted by the polymerization.

Fig. 3 shows the gel-strength of myosin B after heating at 70℃. The heat gel-strength at 70℃ markedly increased as the polymerization proceeded. This gelation was not restrained by the presence of dithiothreitol (Fig. 3). These results suggest that the heat-induced gelation of polymerized myosin B was not influenced by intermolecular disulfide bond formation.

The effects of protein concentration and pH on heat-induced gelation are shown in Fig. 4 and Fig. 5, respectively. The dependency of heat gel-strength of polymerized myosin B on protein concentration was higher than that of the native myosin B, and the optimum pH was shifted to the alkaline side. A marked difference in the dependency of heat-induced gelation on the heaing temperature was seen (Fig. 6). The heat gel-strength of polymerized myosin B increased greatly, while that of native myosin B decreased gradually with increasing temperature.

Scanning electron micrographic observation of gels heated at 60℃ and 90℃ showed that the ultramicrostructure of polymerized myosin B gel differs markedly from that of native myosin B. In the case of native myosin B, the three-dimensional network structure of the gel became looser upon heating at 90℃. On the other hand, the polymerized myosin B gel became more stable, probably due to the formation of a tight three-dimensional network structure, upon heating at 90℃. These structural changes upon heat treatment corresponded well to the changes in gel-strength.

These results suggest that the mechanism of the heat-induce of polymerized myosin B is different from that of native myosin B.

The heat gel-strength of muscle protein was also increased by the action of BTGase (Fig. 8). However, it was shown that the heat-induced gelation of muscle proteins crosslinked by BTGase has a different dependency on heating temperature and protein concentration from that of BTGase-treated myosin B. Although the reason for this is not clear, the results suggest that BTGase may be useful in meat product processing .

CONCLUSION: The heat-induced gel of myosin B crosslinked by BTGase showed markedly increased gel-strength as compared with that of native myosin B. Muscle proteins treated with BTGase also showed the same effect. This enzyme is expected to have applications in the meat processing industry.

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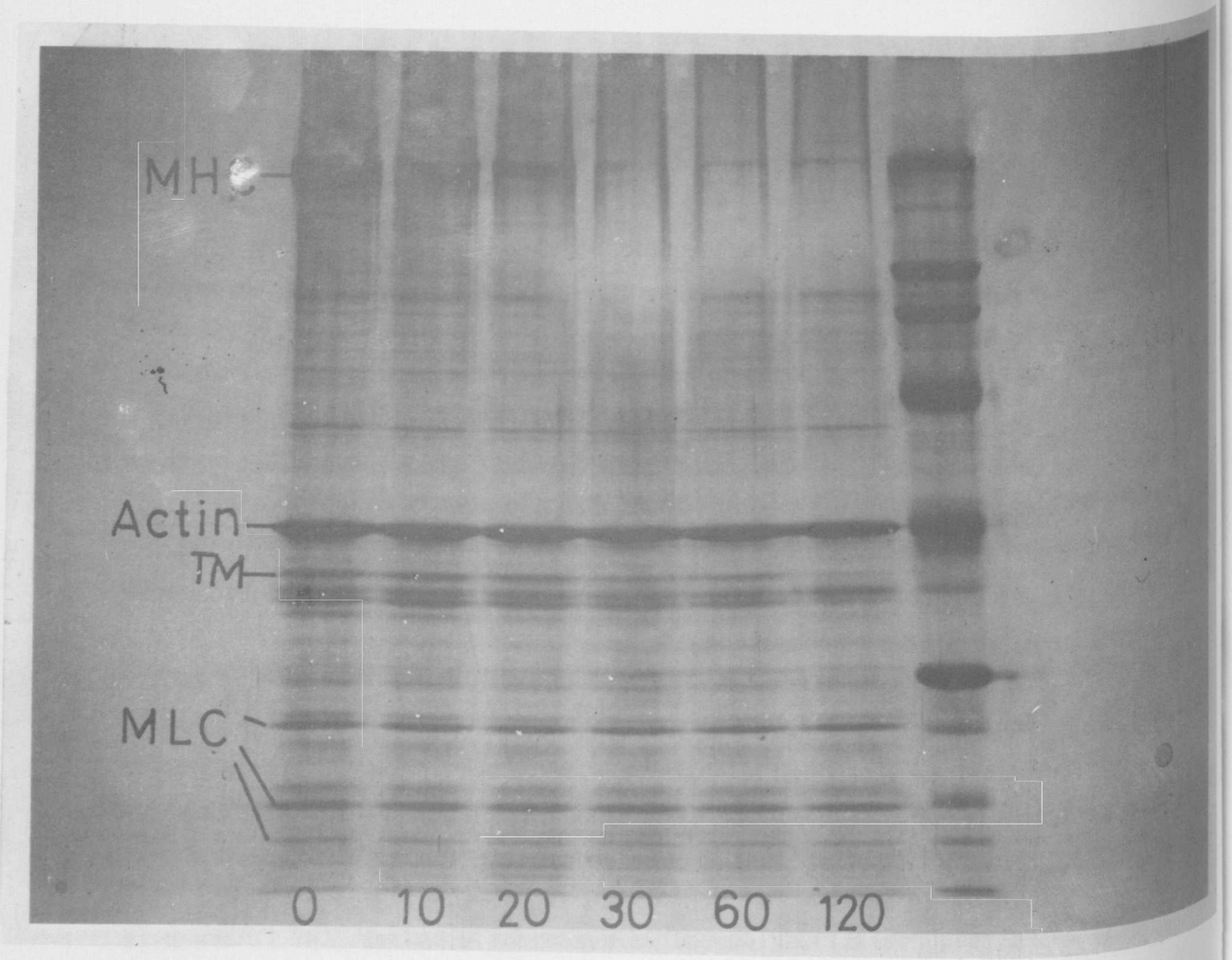
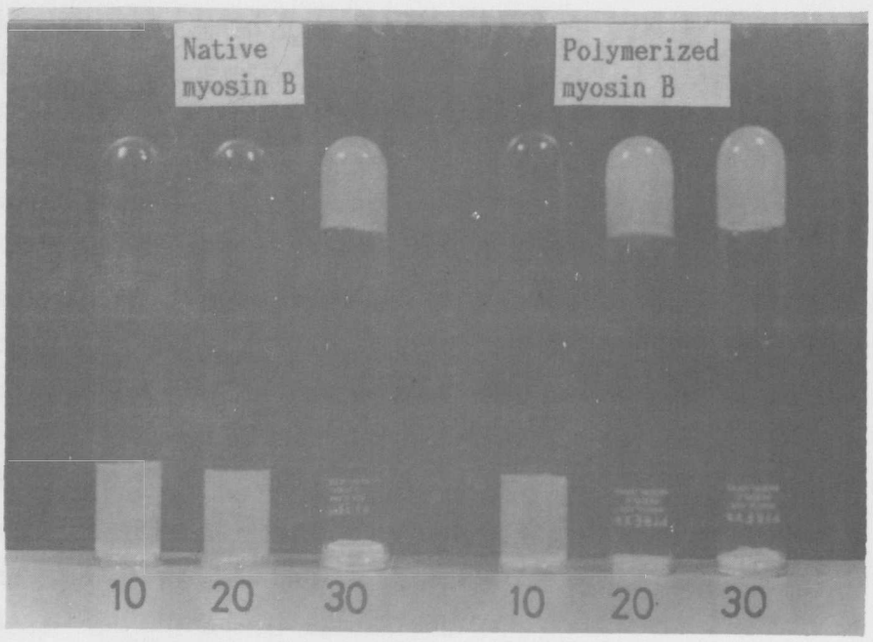


Reaction time (min)

Fig. 1. Changes in the SDS polyacrylamide gel electrophoresis of myosin B during the course of BTGase-reaction at 40 ℃
Myosin B were incubated with BTGase for 0, 10, 20, 30, 60, and 120 minutes. MHC, myosin heavy chains; TM, tropomyosin; MLC, myosin light chains.



incubation time (min)

Fig. 2. Gelation of native myosin B and polymerized myosin B incubated at 40℃

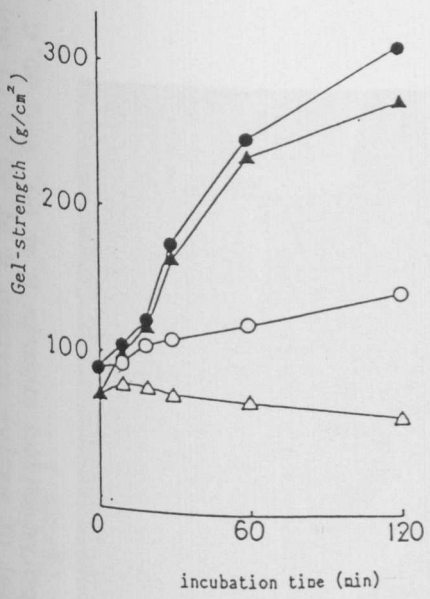


Fig. 3. Effect of incubation time at 40℃ and dithiothreitol (DTT) on heat gel-strength of native and polymerized myosin B
Heating condition, 70℃-30 min; ●, polymerized myosin B; ▲, polymerized myosin B with DTT; ○, native myosin B; △, native myosin B with DTT

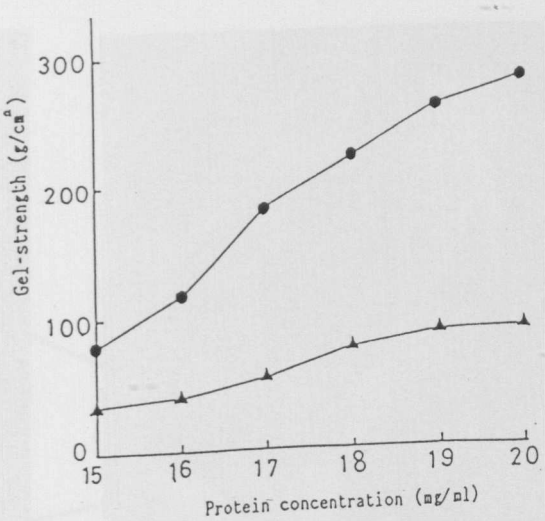


Fig. 4. Effect of protein concentration on heat-induced gelation of native and polymerized myosin B
Reaction of BTGase, 40℃-60 min; heating condition, 70℃-30min; ●, polymerized myosin B; ▲, native myosin B

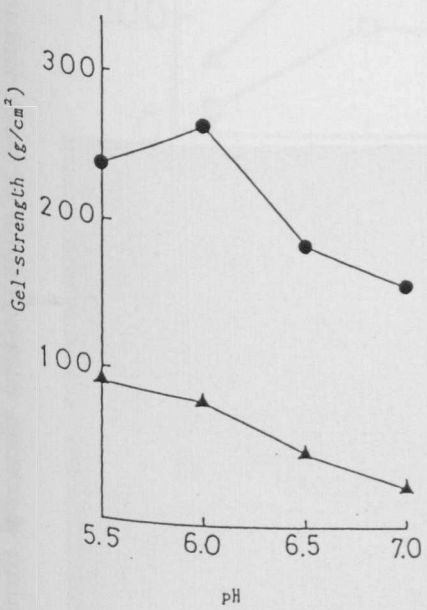


Fig. 5. Effect of pH on heat-induced gelation of native and polymerized myosin B
Reaction of BTGase, 40℃-60 min; heating condition, 70℃-30min; ● and ▲, same as those in Fig. 4.

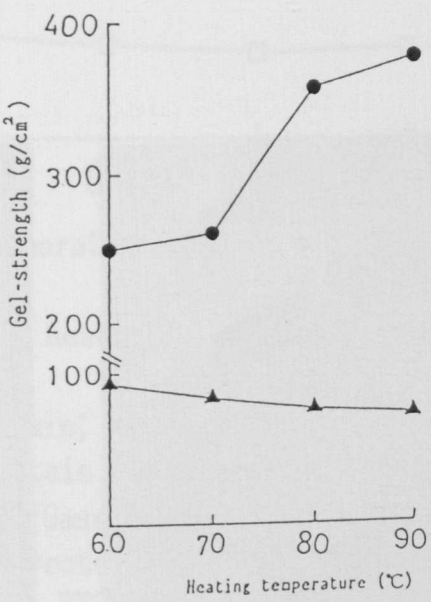
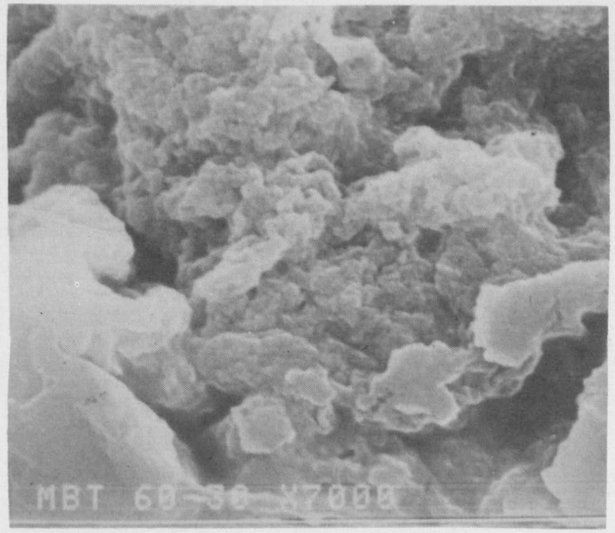
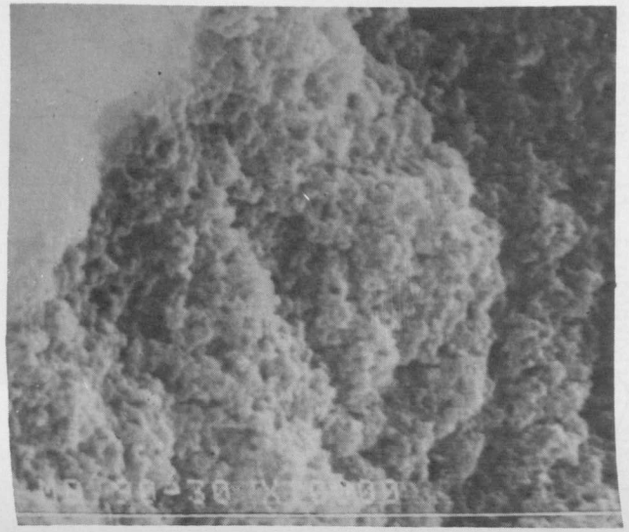


Fig. 6. Effect of heating temperature on heat-induced gelation of native and polymerized myosin B
Reaction of BTGase, 40℃-60 min; heating time, 30min; ● and ▲, same as those in Fig. 4.





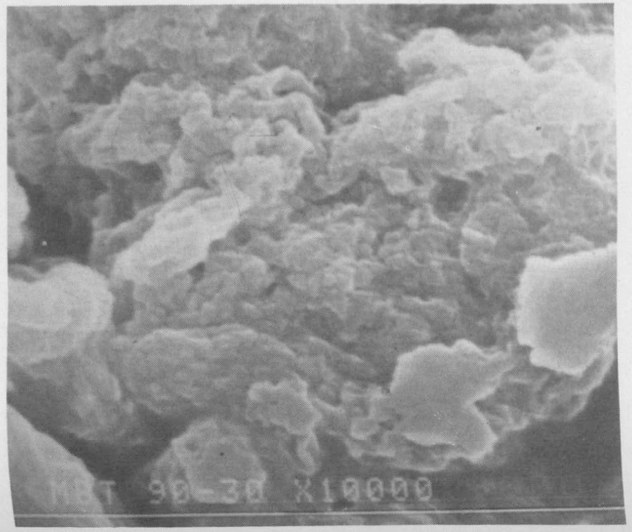


Fig. 7.- Scanning electron micrographs of heat-induced gels of native and polymerized myosin B at 60° and 90°C. Reaction of BTGase, 40°C-60 min.; a) gel of native myosin B at 60°C; b) gel of native myosin B at 90°C; c) gel polymerized myosin B at 60°C; d) gel of polymerized myosin B at 60°C.

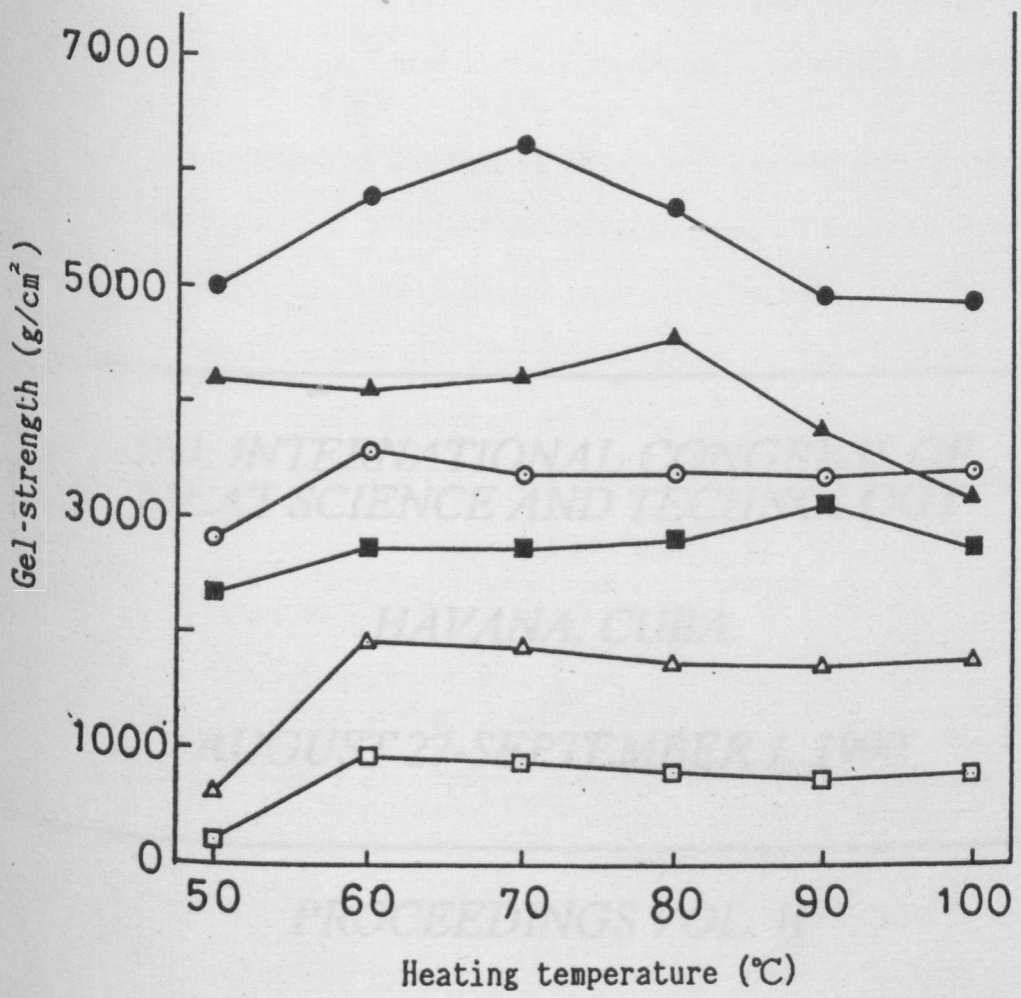


Fig. 8. Effect of BTGase on heat-induced gelation of muscle proteins
Reaction of BTGase, 40℃-60min; ●, muscle protein (16%) with BTGase; ○, muscle protein (16%) without BTGase; ▲, muscle protein (13%) with BTGase; △, muscle protein (13%) without BTGase; ■, muscle protein (10%) with BTGase; □, muscle protein (10%) without BTGase.