Influence of the addition of chemical modified glyceraldehyde 3-phosphate dehydrogenase on the gelling properties of porcine myofibril

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Abstract—**It is well known that some sarcoplasmic proteins interact with myofibril. We have been investigating on the effect of sarcoplasmic proteins on the gelation of myofibril under low ionic strength conditions (0.2 mol/L NaCl) and found that glyceraldehyde 3-phosphate dehydrogenase (GPD) promotes the gelation of the myofibril. In this study, to elucidate the mechanism of the gelling enhancing action, GPD was chemical modified with succinic anhydrate and the effect of succinylated GPD (S-GPD) on the gelation of myofibril was studied. Two kinds of GPDs whose amino groups modified 30% and 60% were obtained as 30S-GPD and 60S-GPD, respectively. The gel strength of myofibril increased with GPD about 6 times as without GPD. The gel strength with 30S-GPD was as half as that of GPD. The addition of 60S-GPD also showed the same tendency of 30S-GPD. To clarify the mechanism of promoting of the gel formation by GPD, co-sedimentation assay was performed using SDS-PAGE analysis. Myosin heavy chain band was shifted from insoluble to soluble fraction in the presence of GPD. Soluble GPD was insolubilized and the band was shifted to the insoluble fraction co-existing of actin band. In the case of 30S-GPD and 60S-GPD, these bands were not shifted to the insoluble fraction. It is indicated that GPD had high affinity with the actin and the affinity was lost by succinylation of amino groups in GPD. Probably, GPD with positive charge and actin with negative charge would cause the electric bonds of protein-protein interaction.**

Keywords—**chemical modification, low sodium products, sarcoplasmic proteins**

I. INTRODUCTION

It is believed that myofibril, which is a salt soluble protein, is a most important ingredient among various meat proteins for the exhibition of rheological properties of meat products. The gel strength of meat product increases by the addition of salt through myosin and actomyosin released from myofibril form a gel network, increasing the water holding capacity after heating. Water soluble protein, sarcoplasmic proteins (SP) containing myoglobin and glycolytic enzymes accounts for 30% of whole meat proteins. Sarcoplasmic proteins have been advocated as no contributor of rheological properties of meat. However, some researchers recently reported that the effect of SP on the physicochemical properties of meat products. For example, it was reported on the physicail properties of sausage beef batter; great removal of SP reduced the physical properties of batter [1].

We have also investigated on the effect of SP on the rheological properties of water-washed meat and myofibril. Under low ionic strength conditions (0.15-0.2 mol/L NaCl) the higher soluble fraction of SP treated with 75% ammonium sulfate promotes the gelation of the meat emulsion [2]. Further, in the series of the studies, it was clarified that glyceraldehydes 3-phosphate dehydrogenase (GPD), which was partly isolated, played a role of the important factor to enhance the gelation of myofibril [3]. We supposed that one reason for the effect is that GPD has the binding ability to F-actin. Other researchers reported that the ability of GPD was higher in low ion strength than in high ion strength in the investigation of protein-protein interaction between various glycolytic enzymes and F-actin [4]. The affinity of GPD to myofibril is a interesting research because GPD is a basic protein with electrically positive charge at a pH<pI. So it is hypothesized that myofibrillar protein charged with negative would interact with GPD. Chemical modification technique of chemically reacting a protein with chemical reagents is used in biochemistry. Succinylation is the induced reaction of

ε-amino groups to remove the positive charge in protein by succinic anhydride. In this study, to elucidate the mechanism of the gelling enhancing action, GPD was modified with succinic anhydride to a varying degree and the effect of succinylated GPD (S-GPD) on the gel strength of myofibril was studied. Further, molecular behaviour of myofibril and S-GPD in the suspension was investigated.

II. MATERIALS AND METHODS

Preparation of myofibril and GPD

Commercial pork loin meat was purchased and kept frozen at -80°C before use. After thawing, meat was washed with 25 mM KCl-50 mM imidazole buffer (pH 6.5) and the precipitate (water-washed meat, WWM) was obtained after centrifugation (10,000×g, 30 min, 4°C). Myofibril was prepared from WWM. Briefly, WWM was rinsed with 25 M KCl-50 mM imidazole buffer (pH 7.1) twice. The obtained precipitate dispersed into the same buffer was filtrated with single layer cotton gauge. The filtrate was recovered as myofibril after centrifugal concentration.

The supernatant after the washing meat was used for the preparation of GPD. That is, the soluble fraction obtained from the supernatant with 75% saturated ammonium sulfate was treated with 20 mM EDTA and 10 mM mercaptoethanol. The treated solution was centrifuged (10,000×g, 30 min, 4°C) and then the supernatant recovered was re-treated with 90% saturated ammonium sulphate. The precipitate was lyophilized after dialysis to obtain GPD powder.

Succinylation of GPD

GPD (0.5 g) was dissolved with 100 ml distilled water. The solution was added with succinic anhydrate (SA) as frequent small volume at below 15°C. Meanwhile, the pH of the solution was kept to 8.0-9.0 by the addition of 0.1 M NaOH during the reaction. The reaction was done at the weight ratio SA/GPD of 0.25, 0.5, 1, 2, and 4. After that, succinylated GPD (S-GPD) was obtained from the reacted solution after dialysis with distilled water. The modification rate of GPD was measured by TNBS method.

Measurement of Protein Solubility

Five percent of S-GPD (0.5 ml) was dispersed to 9.5 ml of 50 mM imidazole buffer (pH 7.0). After storing at 4°C overnight, the solution was centrifuged (3,000×g, 15 min, 4°C). The soluble protein in the supernatant was measured by the Bradford method. Protein solubility was expressed as the percent of protein concentration in the buffer against that in 0.1 M NaOH.

Measurement of gel strength

Myofibril and S-GPD were mixed in the ratio of 5.0% and 1.8% (final protein concentration) in the 50 mM imidazole buffer (pH 7.0) containing 0.2 M NaCl. The mixed suspension was put into glass vessel (20 mm h × 15 mm ϕ). After that, they were heated at 70°C for 30 min after standing on ice for 1 h. Gels were obtained after standing on ice for 3 h. The gel strength of myofibril added S-GPD was measured using texture analyzer (creep-meter rheoner 2 RE-3305, Yamaden , Tokyo Japan). The 5 mm φ plunger was inserted into the gel for 60% of the height at the penetration rate of 1 mm/sec.

Molecular behaviour of myofibril with S- GPD

To clarify the molecular behaviour of myofibril with S-GPD in the gel, co-sedimentation assay was performed. Briefly, myofibril and S-GPD were mixed in the ratio of 2.5% and 0.9% (final protein concentration) in the 50 mM imidazole buffer (pH 7.0) containing 0.2 M NaCl. The mixture was centrifuged at 15,000 × g, 15 min, 4°C and the resulting supernatant and precipitate were analyzed by SDS-PAGE.

III. RESULTS AND DISCUSSION

Effect of SA concentration on the modification rate of amino groups in GPD was shown in Fig. 1. The modification rate dramatically increased with the ratio of SA/GPD up to 1.0 and became flat from 1.0 to 4.0. At the ratio of SA/GPD of 0.25, 1 and 4, the

modification rates were 30.2, 58.9 and 60.7%, respectively.

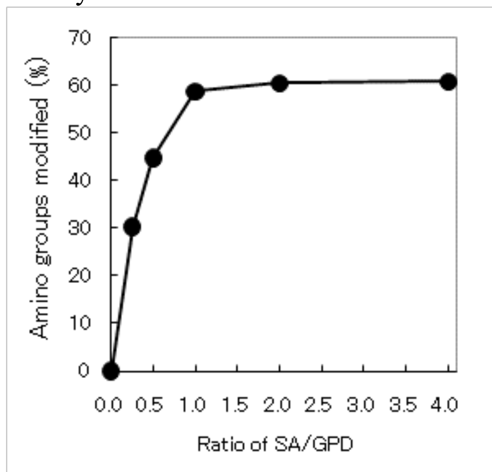

Fig. 1 Modification rate of amino groups in GPD. Succinylation of GPD was carried out by succinic anhydrate at the ratio of SA/GPD of 0.25, 0.5, 1, 2 and 4.

Effect of succinylation on the solubility of GPD was shown in Fig. 2. Succinylation at the ratio of SA/GPD of 0, 0.25 and 1 gave more than 75% of the solubility of GPD.

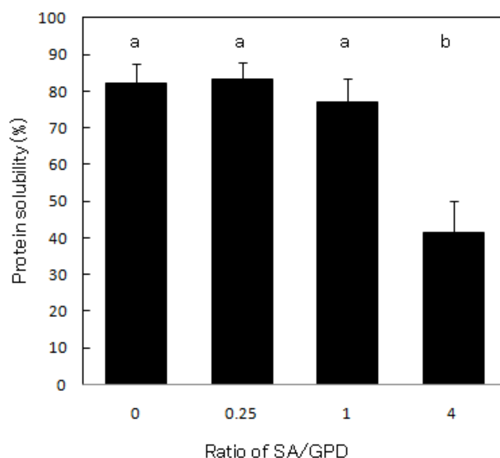

Fig. 2 Effect of succinic anhydrate on solubility of GPD. Protein solubility of GPD (0.5%) was expressed as the percent of protein concentration in the 50 mM imidazole buffer (pH 7.0) against that in 0.1 M NaOH. Different letters are significantly different ($p<0.05$).

However, the solubility at the ratio of 4 gave below 50%. So in this study, the SA/GPD ratio of 0.25 and 1 were adopted for the preparation of S-GPD. The two kinds of S-GPD with about 30% and 60% modification were obtained and hereinafter called 30S-GPD and 60S-GPD, respectively.

Effect of S-GPD on the gel strength of myofibril was shown in Fig. 3. Myofibril formed a strong gel by the addition of intact GPD. The gel strength was six times higher myofibril with GPD than that without GPD. Though the gel strength was also higher with 30S-GPD than myofibril alone, the strength with 30S-GPD was as half as that with GPD. The addition of 60S-GPD also showed the same tendency of 30S-GPD. Succinylation decreased the gelling enhancing action of GPD for myofibril, suggesting amino groups with positive charge in GPD play a role on the association with myofibril. Succinylation of whey protein concentrates increased retention of fat and moisture of meat patties, and decreased the shrinkage [5]. It was clarified that succinylation of GPD militated against myofibril gel.

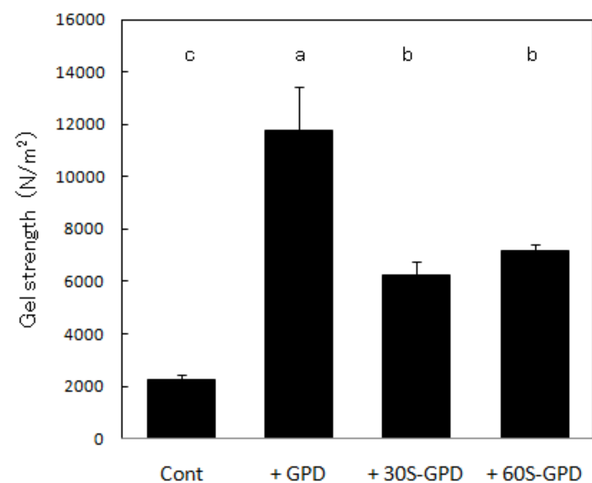

Fig.3 Gel strength of myofibril in the presence of GPD, 30S-GPD and 60S-GPD. Cont, myofibril alone; +GPD, GPD-added myofibril; +30S-GPD, 30S-GPD-added myofibril; +60S-GPD, 60S-GPD-added myofibril. The statistical significance of gel strength was evaluated by a one-way analysis of variance. Different letters are significantly different ($p<0.05$).

Co-sedimentation assay was carried out for the elucidation of molecular behaviour of myofibril and S-GPD. As shown in Fig. 4A, myosin heavy chain and actin in myofibril were insoluble at 0.2 M NaCl. However, in the presence of GPD, myosin heavy chain

was solubilized while actin was still insoluble. On the other hand, in the presence of S-GPD, myosin heavy chain band was not shown in the soluble fraction. Fig. 4B shows that molecular behaviour of GPD and S-GPD was shown in the presence of myofibril. Since GPD was soluble protein at pH 7.0, the band was observed at the soluble fraction. In the presence of myofibril, GPD band was shifted from the soluble fraction to the insoluble fraction co-existing of actin band. The results agree with a former report showing that protein-protein interaction between GPD and F-actin [6]. In the case of 30S-GPD and 60S-GPD, it was not observed that both S-GPD bands were shifted to insoluble fraction. When GPD from *Bacillus stearothermophhilus* was apo state of protein, succinylation deactivated the enzyme activity of GPD [7]. Succinylation decreased the affinity of GPD for myofibril, suggesting that GPD, whose conformation was not altered, was required for the gelling enhancing action for myofibril.

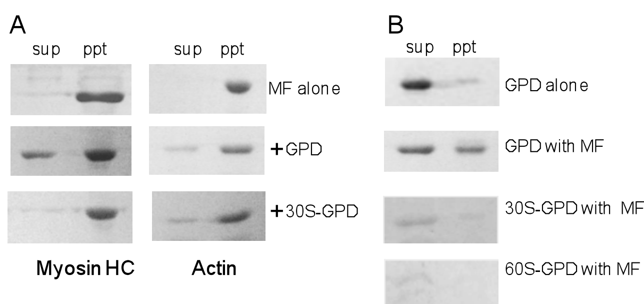

Fig. 4. Molecular behaviour of S-GPD and myofibril in the mixture. A. the behaviour of myofibril in the presence of GPD and 30S-GPD. sup., soluble fraction; ppt., insoluble fraction; Myosin heavy chain (HC) in the insoluble fraction was solubilized by the addition of GPD at 0.2 M NaCl. On the contrary, no solubilizaion of myosin HC was shown in the presence of 30S-GPD. B. Co-sedimentation assay of GPD, 30S-GPD and 60S-GPD in the presence of MF (myofibril). Insolubilization (co-sedimentation with actin) was observed in the case of GPD alone.

In particular, it was indicated that GPD had a high affinity with the actin as shown that the affinity was lost by succinylation of amino groups in GPD. Therefore, we propose that GPD with positive charge associate with actin with negative charge by electrostatic interaction, followed by depolymerisation of myosin filaments.

IV. CONCLUSIONS

Glycolytic enzyme, GPD increased the gel strength of myofibril at low ion strength (0.2 M NaCl). Succinylation decreased the effect of GPD. Further, GPD, which facilitated solubilization of myosin, was precipitated with actin, suggesting that the interaction between actin and GPD derives depolymerization of myosin filaments. On the other hand, succinylated GPD lost to associate with myofibril. This study provided important information about the mechanism of GPD strengthen myofibril gel.

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