Effect of gluconate salt on mechanical and structural properties of heat-induced myosin gel

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INTRODUCTION

Gluconate salt is a potential food additive whose chemical structure is analogous to sorbitol. Recently, Takeshita *et al.*¹⁾ reported that sodium gluconate not only protected myofibrillar protein against thermal inactivation but also solubilized it as a salt. Since these properties are closely related to the ability of gel forming of flesh paste, gluconate salt makes possible to improve consistency of minced fish product, *e.g.* kamaboko²⁾. In this work, we elucidated the effect of gluconate salt for thermal gelation of fish myosin, which is major component of salt soluble proteins governing elasticity of kamaboko. The mechanical and structural properties of heat-induced myosin gel prepared with sodium- or potassium-gluconate were studied. Furthermore, in order to obtain some information about molecular structure of myosin in a solution of gluconate salt, we studied partial specific volume and adiabatic compressibility of myosin.

MATERIALS AND METHODS

Myosin was extracted from red sea bream ordinary muscle and purified according to the method of Reinach *et al.*³⁾. At the final stage of purification each myosin was dissolved in a solution containing of 0.6M of various salts (KCl, NaCl, K-gluconate, Na-gluconate) and stored on ice. Stocked myosin was used within 3days after purification. Protein concentrations were determined by biuret method using bovine serum albumin as the standard. Stocked myosin was diluted to appropriate protein concentration with a buffer solution (pH6.5) of 20mM potassium phosphate, 1mM EDTA, 1mM 2-mercaptoethanol and 0.6M various salts to make myosin gel at appropriate protein concentration. Thermal gelation of myosin was performed in the

1.5ml cuvette whose temperature was precisely controlled by thermobath. Mechanical strength of the gel was tested with Rheoner RE-3305 (Yamaden Co.) using a cylindrical plunger ($\phi=5.0\text{mm}$).

Specimens for scanning electron microscopic observation were prepared according to the method of Yasui *et al.*⁴⁾ with slight modifications. Before the critical point drying, ethanol used for dehydration was replaced by isopentyl acetate. The dried specimens were coated with platinum palladium and observed with Hitachi JSM-T200 at an accelerating voltage of 10kV.

Specific volume of myosin was determined from the density of myosin solution measured with a precision density meter, DMA-02C (Anton Paar, Gratz, Austria). Adiabatic compressibility was obtained from the velocity of sound in the myosin solution. The measurement of the sound velocity of a solution was performed by a 'sing-around pulse method'⁵⁾.

RESULTS

Fig.1 shows the typical patterns of the force development in the puncture test of the heat-induced myosin gels. It is clear that the breaking strength of the myosin gel prepared with gluconate salt is extremely larger than that of the gel with potassium or sodium chloride. The averaged breaking strengths were about 7.7- and 4.0-fold enhanced in case of potassium and sodium salt, respectively. The enhancement of breaking strength is not only reinforced effect of the heat-induced myosin gel by gluconate salt. It is well known that long heating time in the processing of kamaboko causes a degradation of 'ashi' (elasticity of kamaboko). The mechanical strength of the myosin gel prepared with gluconate salt was very little affected by prolongation of heating

time. Fig.2 shows the scanning electron microscopic images of the myosin gels prepared with potassium salt. Fine network structure was observed in the gel prepared with potassium gluconate (Fig.2(B)) compared with that of the gel with potassium chloride (Fig.2(A)). The pore size of the network appears different about 2 fold or more. Similar results were obtained for the gels with sodium salt. The mechanical toughness of the gel with gluconate salt described above may be possibly due to the dense matrix of protein.

In order to understand the effect of gluconate salt on gelation of myosin, it is important to know what kind of influence is caused on a myosin molecule itself by gluconate salt. Therefore, we investigated specific volume of myosin in a solution of gluconate salt. We found that the apparent specific volumes of myosin in gluconate solution were small compared with that in chloride solution. The partial specific volumes obtained by extrapolation of them to zero protein concentration are 0.725 (KCl), 0.712 (NaCl), 0.662 (K-gluconate) and 0.655 cm³/g (Na-gluconate), respectively. The partial adiabatic compressibility of myosin was calculated from both the sound velocity of myosin solution and the partial specific volume. The obtained values were 8.31 cm²/dyn for myosin in KCl and -8.27 cm²/dyn for myosin in K-gluconate, respectively.

Fig.2 Scanning electron microscopic images of the heat-induced myosin gels prepared with KCl in (A) and K-gluconate in (B). Magnifications; x5,000. Myosin concentration was 15mg/ml in both (A) and (B). The white bar represents a length of 10μm.

DISCUSSION

The partial specific volume of myosin determined in this work, 0.655-0.662 cm³/g, are much smaller than those of other proteins previously reported⁶⁾. There are two possible explanations for the small partial specific volume. The first is a decrease of the cavity of myosin upon immersion into gluconate solution. Since the cavity of protein is very important for its function, such a significant decrease of the cavity must cause inactivation of the protein. But this is not the case, because ATPase activity of myosin was still remained in gluconate solution (data not shown). Another possibility is an increase of hydration of myosin molecule by gluconate salt. The fact that the partial adiabatic compressibility of myosin in gluconate solution showed negative value strongly supports it. It seems reasonable that the increase of hydrated water stabilizes myosin and results in stiffening heat-induced myosin gel.

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