Note

Thiol-dependent Gelation of Bovine Serum Albumin in the Presence of Ethanol

Eui-Sang Ji*, Jin Young LEE
and Masaaki HIROSE**

Research Institute for Food Science,
Kyoto University, Uji, Kyoto 611, Japan

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Bovine serum albumin (BSA) is an important source of high-quality food protein,^{1–5} and has been extensively studied for its functional properties including gelation.^{6–11} In previous reports, we have shown that BSA as well as ovotransferrin can form an opaque gel by incubating with a thiol under mild temperature and pH conditions.^{5,12–15} In the present study, we found that the hardness of the BSA gel was markedly enhanced by the presence of ethanol.

BSA (fraction V) was purchased from Wako Pure Chemical Industries Ltd, and ovotransferrin was purified as described previously.¹² Under standard conditions for gelation, BSA was incubated at 30 mg/ml in the presence of different concentrations (v/v) of ethanol with the indicated concentrations of 2-mercaptoethanol in 50 mM sodium phosphate buffer, pH 8.0 at 37°C for 16 hr. The gel hardness was determined with a rheometer (Yamaden Co., Rheoner type RE-3305), using a 5 mm-diameter plunger and an aluminum cup (24 mm in diameter and 7 mm in depth). The gelation of ovotransferrin was carried out under the same conditions, except that a lower protein concentration of 15 mg/ml was used because of the lower solubility of the egg white protein in an ethanol solution. All the data for gel hardness are expressed as averages of quadruplicate determinations, and the standard errors are indicated.

As shown in Fig. 1, the gel hardness of BSA that was induced by 70 mM 2-mercaptoethanol was slightly decreased in the presence of low concentrations of ethanol (5 to 20%), whereas at ethanol concentrations higher than 30%, the gel hardness was markedly increased. In the presence of 35% ethanol, the gel hardness of BSA was 2.4-fold higher than in the absence of the alcohol. At an ethanol concentration higher than 35%, due to non-specific aggregation of BSA, we were not able to induce a

thiol-dependent gel. With regard to the gelation of ovotransferrin, no stimulatory effect by ethanol was observed. The ethanol-dependent increase in gel hardness, therefore, may be specific for BSA.

Our previous data have shown that gelation proceeds in such a way that a thiol cleaves some of the 17 intra-chain disulfide bonds in BSA before the protein denatures,

Fig. 1. Effect of Ethanol Concentration on the Gel Hardness.

BSA (closed circles) and ovotransferrin (open circles) were incubated with 70 mM 2-mercaptoethanol in the presence of various concentrations (v/v) of ethanol. The gel hardness in the absence of ethanol was $1,580 \pm 90 \text{ N/m}^2$ for ovotransferrin and $8,350 \pm 380 \text{ N/m}^2$ for BSA.

Fig. 2. Effect of the Concentration of 2-Mercaptoethanol on the Gel Hardness.

BSA was incubated with different concentrations of 2-mercaptoethanol in the presence (open circles) and absence (closed circles) of 30% ethanol.

* Present address: Department of Food and Nutrition, Shin Heung Junior College, #115 Howon-dong, Euijungbu City, Korea.

** To whom correspondence should be addressed.

subsequently forming gel networks through inter-chain disulfide formation.⁵⁾ To investigate whether or not the increased gel hardness in the presence of ethanol can be accounted for by an increased accessibility of BSA against the thiol, we examined the effect of the concentration of 2-mercaptoethanol in the presence and absence of 30% ethanol. As shown in Fig. 2, the gel hardness was a maximum at 50 mM 2-mercaptoethanol, both in the presence and absence of ethanol. The increased gel hardness, therefore, may not be explained by an increased cleavage of the disulfide bonds in BSA. Although the mechanism for the enhancement of gel hardness is still not clear, the present findings appear useful for the utilization of BSA as a foodstuff.

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