Functional Protein–Polysaccharide Conjugate Prepared by Controlled Dry-heating of Ovalbumin–Dextran Mixtures

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A functional ovalbumin–dextran conjugate was prepared by dry-heated storage at 60°C and 65% relative humidity for 3 weeks. The emulsifying properties of the ovalbumin–dextran conjugate were about three times higher than those of an ovalbumin–glucose conjugate. SDS-electrophoresis patterns showed that the ovalbumin–dextran conjugate obtained by dry-heating was not as polydisperse as that obtained by cyanogen bromide-activated dextran. The average molecular weight of the ovalbumin–dextran conjugate was about 200,000. The excellent emulsifying properties of ovalbumin–dextran conjugate were maintained even at pH 3 and were further improved at pH 10. In addition, the emulsifying properties of the ovalbumin–dextran conjugate were greatly enhanced by preheating the conjugate at 100°C. Thus, it is suggested that an ovalbumin-dextran conjugate prepared by controlled dry-heating can be used as a macromolecular emulsifier for food applications.

Many studies on the chemical and enzymatic modification of proteins have attempted to improve protein functionality.1–13 Among these studies, there is little information about the functionality of protein conjugates modified with macromolecular biomaterials. Although proteins are generally unstable against heating, organic solvents and proteolytic attack, the proteins may be converted into stable forms by binding with biopolymer templates such as polysaccharides. In addition, new functional properties may be endowed by attaching proteins with biopolymers. We found that a soluble protein–dextran conjugate prepared by coupling proteins to cyanogen–bromide activated dextran showed excellent emulsifying properties superior to commercial emulsifiers.14 This suggests that a covalently bound protein–polysaccharide conjugate can be used to make new functional biopolymers. The attachment of proteins to polysaccharides may enhance the stability and solubility of proteins. Therefore, this technique for conjugate formation can be utilized for medical and food applications, if the safety of the conjugates is ensured. The use of chemical reagents to make the conjugate should be avoided for this purpose. Thus, we have tried to prepare protein–polysaccharide conjugates without using chemical reagents. One of the most promising methods is to apply the naturally occurring Maillard reaction. Kato et al.15–17 have reported the formation of ovalbumin–oligosaccharide complexes by the Maillard reaction during storage at 50°C and 65% relative humidity. Since dextran also has an active reducing-end residue in a molecule, the formation of ovalbumin–dextran conjugates can be expected by the Maillard reaction. This paper describes the properties of an ovalbumin–dextran conjugate prepared by controlled dry-heating of ovalbumin–dextran mixtures.

Materials and Methods

Materials. Dextran (molecular weight, 60,000–90,000) was purchased from Wako Pure Chemical Industries, and Sephacryl S-300 was purchased from Pharmacia LKB. Ovalbumin was prepared from fresh egg white by the crystallization method in sodium sulfate, and recrystallized five times.18
Dry heating of ovalbumin–dextran mixture. Ovalbumin–dextran mixture was dissolved in water and freeze-dried in the weight ratio of 1:1 or 1:5. Ovalbumin–glucose solution was also freeze-dried in the weight ratio of 1:1 as a control sample. Freeze-dried ovalbumin–carbohydrate mixtures were stored at 50°C or 60°C and 65% relative humidity in a desiccator containing saturated KI solution for a given time (0–3 weeks). The stored samples were dissolved in distilled water and the absorbance was measured at 470 nm to follow the browning reaction.

Preparation of ovalbumin–dextran conjugate by dry heating. Freeze-dried ovalbumin–dextran mixtures in the weight ratio of 1:5 were stored at 60°C and 65% relative humidity for 3 weeks. To further purify ovalbumin–dextran conjugate, gel filtration of the conjugate was performed on a column (70 x 3 cm) of Sephacryl S-300. Elution was carried out with 50 mM acetate buffer, pH 5.0, containing 10 mM sodium chloride, and 3.0 ml fractions were collected. Fraction numbers 26 to 36 of the conjugate were collected together, dialyzed against deionized water and lyophilized.

Determination of the molecular weight of ovalbumin–dextran conjugate. 0.1% ovalbumin–dextran hybrid solution in 67 mM sodium phosphate buffer (pH 7.0) was applied to a high performance gel chromatography system, connected with a TSK gel G3000 SW column (Toyo Soda Co., 0.75 x 60 cm) at a flow rate of 0.3 ml/min. Elution from the columns was monitored with a low-angle laser light scattering photometer (LS-8, Toyo Soda Co.) and then with a precision differential refractometer (RI-8, Toyo Soda Co.). The molecular weight of ovalbumin–dextran conjugate was estimated from the ratio of total area in the peak of a low-angle laser light scattering photometer (LS) to that of a refractometer (RI) by the following equation:

\[ M_w = M_{std} \frac{(dn/dc)_{sam}(LS/RI)_{sam}}{(dn/dc)_{sam}(LS/RI)_{std}} \]

where \( M_{std} \) is molecular weight of standard protein, \((dn/dc)_{sam}\) is the refractive index increment of standard protein, \((dn/dc)_{sam}\) is the refractive index increment of ovalbumin–dextran conjugate, \((LS/RI)_{sam}\) is the ratio of total area in the peak of a low-angle laser light scattering to that of refractometer of standard protein, and \((LS/RI)_{sam}\) is that of ovalbumin–dextran conjugate.

Measurement of emulsifying properties. The emulsifying properties were determined by the method of Pearce and Kinsella. SDS-slab polyacrylamide gel electrophoresis was carried out by the method of Laemmli, using 10% acrylamide separating gel, and 3% stacking gel containing 0.1% SDS. Protein samples (20 μl, 0.1%), were prepared in Tris–glycine buffer, pH 8.8, containing 1% SDS and 1% mercaptoethanol. Electrophoresis were carried out at constant current of 10 mA for 5 hr using electrophoretic buffer of Tris–glycine containing 0.1% SDS. The gel sheets were stained for proteins and carbohydrates with Coomassie blue G-250 and Fuchsin, respectively.

Results and Discussion

Figure 1 shows the browning color development of ovalbumin–carbohydrate mixtures during dry-heated storage at 65% relative humidity. Although the browning color development of ovalbumin–glucose mixtures greatly
increased during dry-heated storage at 50°C, that of the ovalbumin–dextran mixture only slightly increased under the same conditions, suggesting the lack of a Maillard reaction. To enhance the Maillard reaction, the ovalbumin–dextran mixture was stored at 60°C in the weight ratio of 1:5. A considerable browning color was observed under these conditions, reflecting the progress of Maillard reaction. Although the data are not shown, the presence of dicarbonyl compounds was also observed in the foregoing conditions. Thus, in the case of the ovalbumin–dextran mixture, the Maillard reaction seems to occur at 60°C and 65% relative humidity.

Figure 2 shows the emulsion turbidity (relative emulsifying activity) of ovalbumin–carbohydrate mixtures obtained under various dry-heated conditions. The emulsifying activity of ovalbumin–dextran mixtures was much higher than that of ovalbumin–glucose mixture or ovalbumin alone during dry-heated storage. The highest emulsifying activity was obtained by dry-heated storage at 60°C for 3 weeks of ovalbumin–dextran mixture in the weight ratio of 1:5.

The ovalbumin–dextran mixture thus obtained was applied to a column of Sephacryl S-300 (Fig. 3). The ovalbumin–dextran mixture was apparently converted to a high molecular fraction, suggesting that ovalbumin was co-

![Graph](image_url)

**Fig. 2.** Changes in the Emulsifying Properties of Ovalbumin–Carbohydrate Mixtures during Dry-heated Storage at 65% Relative Humidity. ▲, ovalbumin–glucose mixture in the weight ratio of 1:1 at 50°C; □, ovalbumin–dextran mixture in the weight ratio of 1:1 at 50°C; ■, ovalbumin–dextran mixture in the ratio of 1:5 at 60°C; ●, ovalbumin stored at 60°C.

![Graph](image_url)

**Fig. 3.** Elution Pattern of Ovalbumin–Dextran Conjugate Obtained by Dry-heated at 60°C for 3 Weeks on Sephacryl S-300 Column.

(---), absorbance at 280 nm; (-----), absorbance at 470 nm after color development by phenol–sulfate method. Vertical arrow indicates the position of elution peak of native ovalbumin.
valently attached to dextran and formed ovalbumin–dextran conjugate. This was confirmed by SDS-polyacrylamide gel electrophoresis, as shown in Fig. 4. The electrophoretic patterns of ovalbumin–dextran hybrid showed a single band for protein and carbohydrate stains near the boundary between stacking and separating gels. This indicates that ovalbumin was covalently attached to dextran and formed the conjugate. Peak 1 (fraction numbers 26 to 30 in Fig. 3) showed a sharper band than peak 2 (fraction numbers 31 to 36 in Fig. 3). On the other hand, ovalbumin–dextran conjugate prepared from CNBr-activated dextran showed broad high molecular weight bands in stacking gel, indicating a polydispersed high molecular distribution. The average molecular weight of ovalbumin–dextran conjugate prepared by dry-heated storage was determined by low-angle laser light scattering equipment connected with a high performance liquid chromatography. The average molecular weight of ovalbumin–dextran conjugate was 200,000. This molecular weight is much lower than that of ovalbumin–dextran conjugate from CNBr-activated dextran. This may be because the active site of dextran in the Maillard reaction is less than that of CNBr-activated dextran.

### Table 1. Binding Ratio of the Ovalbumin–Dextran Conjugate

<table>
<thead>
<tr>
<th>Binding ratio</th>
<th>Ovalbumin</th>
<th>Dextran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Molar</td>
<td>1</td>
<td>1.6–2.2</td>
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### Fig. 5. Emulsifying Properties of Ovalbumin–Dextran Conjugates Obtained by Dry-heated Storage at 60°C for 3 Weeks.

- □, peak 1 (fraction Nos. 26–30 in Fig. 3) of ovalbumin–dextran conjugate;
- ■, peak 2 (fraction Nos. 31–36) of ovalbumin–dextran conjugate;
- △, ovalbumin–dextran conjugate obtained by CNBr-activated dextran;
- ●, native ovalbumin.
and the formation of cross-linkage with ovalbumin results in a limited number. This is well consistent with the binding molar ratio of ovalbumin–dextran conjugate (1:1.6–2.2) shown in Table I.

The emulsifying properties of ovalbumin–dextran conjugate are demonstrated in Fig. 5. Peaks 1 and 2 showed higher emulsifying properties than the ovalbumin–dextran conjugate obtained by CNBr-activated dextran. As only a slight difference was observed between peaks 1 and 2, the peak of fraction numbers 26 and 36 was collected and used in the experiments described below.

Figure 6 shows the effect of various pH on the emulsifying properties of the ovalbumin–dextran conjugate obtained by dry-heated storage. The excellent emulsifying properties of ovalbumin–dextran conjugate were maintained even at pH 3, and were further improved at pH 10. The stable emulsifying properties in the acidic pH region are available for industrial application, because the emulsifying properties of most commercial emulsifiers are greatly reduced at low pH region.

Figure 7 shows the effect of heating the sample on the emulsifying properties of ovalbumin–dextran conjugate obtained by dry-heated storage. The emulsifying properties of ovalbumin–dextran conjugate were greatly increased by preheating the hybrid at 100°C, no insoluble matter being observed. Thus, another advantage of this ovalbumin–dextran conjugate is its heat resistance which enables it to be pasteurized.

We have reported that an ovalbumin–dextran conjugate prepared by CNBr-activated dextran showed good emulsifying properties superior to commercial emulsifiers. As described in this paper, ovalbumin–dextran conjugate prepared by dry-heated storage also showed excellent emulsifying properties in a similar manner. The latter conjugate is more suitable for industrial applications than the former, because of its preparation without chemical reagents. This con-
jugate preparation can be used not only as an emulsifier but also as a protein food additive requiring heat stability.

References