Note

Conformational Stability of Ovalbumin Reacted with Glucose in a Maillard Reaction

Yasuko Kato, Kenji Watanabe* and Yasushi Sato*†

Women’s College of Tokaiigakuen, Tenpaku-ku, Nagoya 468, Japan
*Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

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The stabilization of protein has been widely investigated thermodynamically and/or kinetically in the presence of sugars,1-3 and it has been demonstrated that they stabilize protein against heat denaturation as determined by the scanning calorimetric method.1,3 On the other hand, soluble enzyme-carbohydrate conjugates, which were prepared by coupling enzymes to cyanogen bromide-activated dextran4) and to lactose,5) have been reported to be more stable to heat than the respective native enzymes. Thus, sugars which coexist or conjugate with proteins play an important role in protein stabilization against heat.

In our previous studies,6-8) egg white proteins- and ovalbumin-glucose complexes, which were produced in dried egg white and ovalbumin mixed with glucose, were shown to possess higher solubility and heat-stability. In the course of 10 day storage (50°C, 65% relative humidity) of the mixture of ovalbumin with glucose (protein : glucose = 10 : 3 in dry weight), the sample stored for 4 days showed the highest denaturation temperature, 7.8°C higher than that (76.6°C) of native ovalbumin (OV), in differential scanning calorimetric determinations. In this study, we present further information on conformational stabilities of the Maillard-reacted OV.

A 2% solution of the sample prepared for 4 days as described in the previous paper8) was prepared by dissolving it in 0.02 m sodium chloride. The solution was adjusted to pH 3.0 and heated at 50°C for 90 min to remove OV unreacted with glucose according to the method of Smith and Back.9) After removing the precipitated protein, the monomer type of the Maillard-reacted ovalbumin complex (mOVG) in the supernatant was separated from the polymer by Sephadex G-150 column chromatography, dialyzed and freeze-dried.8) The polyacrylamide gel electrophoretic patterns of mOVG gave a broad band which had fewer positive charges than OV (Fig. 1.). The free ε-amino group content in mOVG compared with OV (lysine: 20 residues) was shown to be about 7.6 residues, as determined by the method of Bohlen et al.10) The quantity of lysine present as fructoselysine converted from the bound glucose in mOVG was approximately estimated to be 6.4 residues using an amino acid analyzer by the method of Möller et al.,11) according to the procedure of Finot and Mauron.12) That value showed that about half of the bound lysine in mOVG was transformed into the Amadori rearrangement product. So mOVG could be recognized to be a mixture of products in the initial stage of the Maillard reaction with respect to modification with glucose. The helix content of mOVG, estimated to be 27% from circular dichroism (CD) spectra, was smaller than that of OV (33%). mOVG was only partially denatured, even though 62% of the ε-amino groups were blocked.

Conformational changes of OV and mOVG were observed by means of CD at 225 nm. Sample solutions (2 mg/ml of phosphate buffer, pH 7.0, I = 0.1), in the absence and presence of 2 M guanidine hydrochloride (Gdn·HCl, reagent of ultrapure grade), were put in a stoppered cuvette and placed in a thermostatically controlled cell-holder. The thermal denaturation was registered from 20 to 88°C at a heating rate of 1.3°C/min. The denaturation profile in the absence of Gdn·HCl showed that the transition temperature between the native and denatured forms of OV was 68.0°C, whereas that of mOVG could not be determined in these experimental conditions (20~88°C). For a more definitive comparison of the OV and mOVG transition temperatures, both samples were heated in the presence of 2 M Gdn·HCl (Fig. 2). The transition temperatures of

† Present address: Faculty of Agriculture, Meijo University, Tenpaku-ku, Nagoya 468, Japan.
OV and mOVG were 62.5 and 73.0°C, respectively. Thus, a difference in the two transition temperatures, from the combined effects of temperature and 2 M Gdn·HCl, was clearly recognized as well as the difference determined by the differential scanning calorimetry described above. Therefore, it can be said that the mOVG structure was more stable than OV, though the mOVG structure at 22°C was a little less ordered than that of OV. To determine the effect of the Gdn·HCl concentration on the conformations of OV and mOVG, a stock solution (8 M) of Gdn·HCl was added slowly, with continuous stirring, to the protein solution (phosphate buffer, pH 7.0, I = 0.1, final concentration 0.253 mg/ml) to obtain the desired Gdn·HCl concentration between 0 to 7.5 M. Changes in CD at 225 nm were obtained after 24 hr at room temperature. The degree of denaturation with increasing Gdn·HCl concentration was determined by observation of the CD at 225 nm (Fig. 3). As seen from the derivative plots in Fig. 3, the stability of mOVG was confirmed by the fact that the transition occurred at 2.5 M Gdn·HCl, whereas that of OV occurred at 1.75 M Gdn·HCl.

The ordered structure in partially denatured mOVG may well be stabilized by intrachain noncovalent interactions between the protein moiety and the carbohydrates bound in the forms of Schiff's base and Amadori rearrangement product in mOVG. Differences in OV and mOVG hydration were also assumed to be one reason for the resistance of the mOVG structure to thermal- and Gdn·HCl-induced denaturation, as seen in the case of glycoprotein.4)

REFERENCES