Changes in Interfacial Properties of Hen Egg Ovalbumin Caused by Freeze-Drying and Spray-Drying

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Purified ovalbumin dialyzed against distilled water was freeze- or spray-dried. Foaming power, foam stability, and emulsion stability of the ovalbumin solution were increased by freeze- or spray-drying, but only slight changes in the intrinsic viscosity, the number of sulfhydryl residues, the spectrum of circular dichroism of the ovalbumin molecule, and the acceptability for subtilisin of the ovalbumin molecule were observed. The hydrophobicity of the molecule was increased by drying, measured by two distinct methods with poly(ethylene glycol) palmitate and with *cis*-parinaric acid. The time dependence of surface tension decay of the dried ovalbumin solution was different from that of the original purified ovalbumin. The drying of salt-free ovalbumin changed the interfacial properties, and the conformation of ovalbumin in the solutions prepared from freeze- or spray-dried powder obtained from salt-free ovalbumin was not the same as that of native ovalbumin.

Freeze- and spray-drying are conventional methods for concentrating liquids or for making them into powder or flour, in the food industry. Development of new equipment and the improvement of processes are giving sufficient products with suitable quality nowadays (Lorentzen, 1981; Masters, 1983). However, changes in functional properties of protein during such processes, e.g., egg albumen, as well as whole egg and yolk, have been reported by many researchers (Baldwin and Cotterill, 1967; Zabik and Brown, 1969; Galyean and Cotterill, 1979). The reduction of foaming ability of egg protein by dehydration with spray-drying was observed (Rolfes et al., 1955; Bergquist, 1964), while satisfactory whipping properties were retained after spray-drying (Joslin and Proctor, 1954; Carlen and Ayres, 1953) or after freeze-drying (Rolfes et al., 1955) of egg albumen. This controversy might be from the food constituents contained in the samples, that is, various proteins, fat, and others. The effects of heat experienced by the food materials during drying and the heat history must also influence the functionality of the final product. This study was undertaken to elucidate the influence of drying on the functional and physicochemical properties of protein using purified ovalbumin as the model protein.

Ovalbumin is the major constituent of egg white, an important food ingredient with high functionality including foam stability and emulsifying properties. Ovalbumin is constructed of a single peptide, and its purification in quantity is possible.

MATERIALS AND METHODS

Chemicals. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) and urea were purchased from Nakarai Chemical Co. Ltd. (Kyoto). Subtilisin BPN′ was obtained from Sigma Chemical Co. (St. Louis, MO). *cis*-Parinaric acid was obtained from Molecular Probes Inc. (Junction City, OR). Dextran T-70 (MW 70000) and poly(ethylene glycol 6000) were obtained from Pharmacia Fine Chemicals and Union Carbide (Somarset, NJ), respectively.

Preparation of Ovalbumin. Fresh hen eggs were purchased from the local producer. Ovalbumin was purified with the method reported previously (Kitabatake and Doi, 1987). Usually about 1700 mL of egg white from 50 eggs was used for purification. The crystallization of ovalbumin was repeated five times. Finally, about 25 g of purified ovalbumin was obtained. The distilled water, ammonium sulfate solution, and sulfuric acid used in this experiment were nitrogen-bubbled, and EDTA (0.1 mM) and sodium azide (0.03%) was added to them. The ovalbumin crystals were dissolved in 20 mM sodium phosphate buffer, pH 7.5, and were dialyzed against the same buffer at 4 °C, and then the dialysate was used in the following experiments as the original ovalbumin. The ovalbumin sample gave a single band on polyacrylamide gel electrophoresis with SDS.

Measurement of Ovalbumin Concentration. Ovalbumin concentration was calculated from the absorbance at 280 nm based on the value of $E^{1\%}_{1\,cm} = 7.12$ (Glazer et al., 1963).

Freeze- and Spray-Drying of Ovalbumin Solution. The ovalbumin crystals were dialyzed against distilled water exhaustively. To prepare the ovalbumin powder by freeze-drying, the dialysate was frozen with liquid nitrogen and then dried (freeze-dryer, Model VFD-510DM, Daia Co., Ltd., Tokyo). For spray-drying, the dialysate was spray-dried at 120 °C inlet temperature and 70 °C outlet temperature by using a laboratory-scale spray-dryer (Yamato Minispray, Type DL-21, Yamato Scientific Co., Ltd., Tokyo). The atomizer has a two-fluid nozzle with an orifice of 406-μm i.d. The atomization pressure was 0.5 kg/cm^2. The final moisture contents of the powder obtained with freeze-drying and spray-drying were 8.8% (w/w) and 9.7% (w/w), respectively, measured with a Karl-Fischer moisture meter (Model KF-05, Mitsubishi-kasei, Co. Ltd., Tokyo). The ovalbumin powders obtained by both methods were dissolved in 20 mM sodium phosphate buffer, pH 7.5, and then dialyzed against the same buffer at 4 °C. The dialysate was centrifuged at 3000 rpm (Kokusan Co., Ltd., Tokyo). The supernatant was used in the following experiments as freeze- or spray-dried ovalbumin solution.

Foaming Power and Foam Stability. The foaming power of ovalbumin was measured as follows. Fifteen milliliters of ovalbumin solution, in a 50-mL stainless steel container, was homogenized in a homogenizer (Ace Type, Nihon Seiki Kaisha Ltd.) with a constant-temperature cell compartment (25 °C), using a rotating six-bladed knife at 10000 rpm. After 1 min, enough foam to fill an Erlenmeyer flask of known weight and volume was transferred immediately by pipet and weighed. This operation was completed in 7 s. Foaming power was defined by $100(G/L)$. G and L are the gas and liquid volumes in the foam, respectively. Measurements of the foaming power were repeated three times with each sample solution. To measure the foam stability, the foam prepared as described above was transferred to a graduated cylinder up to the 10.00-mL mark, and then the drainage from the foam and change in the foam height with time were followed for 3 h. The top of the graduated cylinder containing foam was tightly closed with a film (Parafilm).

Emulsion Stability. Emulsions were prepared by ultrasonic treatment of ovalbumin solution and soybean oil (Haque and Kito, 1983). To 30 mL of ovalbumin solution (0.5–5.0 mg/mL of 20 mM sodium phosphate buffer, pH 7.5) was added 6.0 mL of soybean oil. The mixture was sonicated at 25 °C with a Kaijo Denki ultrasonic oscillator at 15 kHz for 1 min, stirring with a magnetic stirrer. Ten microliters of the emulsion was added to 10 mL of 1% (w/w) SDS solution. After gentle stirring, the absorbance of the emulsion diluted with SDS solution was measured at 600 nm, which was used as the emulsion index of the emulsifying ability (Pearce and Kinsella, 1978). To find the stability of the emulsion, the prepared emulsion was left for 24

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h at room temperature, 10 μL was taken and added to the SDS solution and then the absorbance was measured as described above.

Measurement of Reduced Viscosity. The viscosity was measured on an Ostwald capillary viscometer, with an efflux time of 140 s for water at 25.0 ± 0.2 °C. NaCl was added to all samples at the concentration of 0.1 M. The sample was diluted with buffer.

Measurements of Sulfhydryl Levels. To 0.1 mL of ovalbumin solution was added 4.9 mL of 40 mM sodium phosphate buffer, pH 8.0, containing SDS (0.48%), urea (8 M), and EDTA (1 mM). Samples were incubated at 50 °C, then 33 μL of 1 mM DTNB solution was then added to the mixture, and the whole mixture was incubated for 60 min at 50 °C. After the mixture was cooled, the absorbance at 412 nm was measured. Distilled water was used as the reference instead of ovalbumin solution. The molar extinction coefficient of 13 600 M^{-1} at the sulfhydryl groups in the ovalbumin molecule modified by DTNB was used (Ellman, 1959).

Measurement of Circular Dichroism Spectrum. The circular dichroism measurement of ovalbumin solution (0.02 mg/mL of 20 mM sodium phosphate buffer, pH 7.5) was taken on a Jasco J-500 spectropolarimeter, using a cell of 1-mm path length at 25 °C. The cell chamber was swept with N_2 gas during measurement.

Digestion by Subtilisin. Subtilisin digestion of ovalbumin was done as follows. To 2.0 mL of ovalbumin solution (10 mg/mL of 20 mM sodium phosphate buffer, pH 7.5) was added 0.1 mL of subtilisin (0.1 mg/mL) dissolved in the sodium phosphate buffer described above. After incubation for 1–4 h at 25 °C, 4.0 mL of trichloroacetic acid (TCA) solution (5%) was added to the reaction mixture, and then the mixture was left for 30 min at 25 °C. The precipitate was removed by centrifugation at 3000 rpm. The absorbances of the supernatant were measured at 280 nm.

Titration with *cis*-Parinaric Acid. The fluorescence intensity from the complex of *cis*-parinaric acid and ovalbumin was measured according to the method reported by Kato and Nakai (1980). To 2.0 mL of ovalbumin solution (10 mM sodium phosphate buffer, pH 7.4, containing 0.002% SDS) was added 10 μL of *cis*-parinaric acid. The *cis*-parinaric acid bound to ovalbumin molecules in the mixture was excited at 325 nm, and the emission was measured at 420 nm using a fluorescence spectrophotometer (Hitachi MPF-4, Tokyo).

Synthesis and NMR Analysis of Poly(ethylene glycol) Palmitate. Poly(ethylene glycol) palmitate (P-PEG) was synthesized by the method of Shanbhag and Johansson (1974) from poly(ethylene glycol) (PEG) and palmitic acid as starting materials. The recrystallization from absolute ethanol was repeated three times. The degree of the introduction of palmitate into the poly(ethylene glycol) molecule was examined by nuclear magnetic resonance (NMR). Before the NMR analysis, both P-PEG and PEG were dried well with use of diphosphorus pentoxide under reduced pressure with heating to remove the ethanol and water completely. The dried samples were analyzed with NMR to calculate the number of protons in the PEG molecule.

Partition in Aqueous Biphasic Systems Containing Dextran and PEG or P-PEG. Partition of ovalbumin in biphasic systems containing dextran and PEG or P-PEG was measured by the method of Shanbhag and Axelsson (1975). To 1.5 mL of ovalbumin solution were added 1.0 g of 40% (w/w) dextran T-70 and 2.5 g of 16% (w/w) PEG or P-PEG. Ovalbumin and PEG were dissolved in 2 mM potassium phosphate buffer containing 100 mM K_2SO_4. The mixture was gently stirred and centrifuged at 15000*g* for 5 min at room temperature. The absorbance at 280 nm of the upper and lower layers of the mixture was measured. The difference in partition of ovalbumin in phase systems of PEG and P-PEG, $\Delta \log K$, where K is the partition coefficient, was taken as a measure of hydrophobic interaction. The partition coefficient of ovalbumin was defined as $K = C_u/C_l$, where C_u and C_l are its concentrations at equilibrium in the upper phase and the lower phase, respectively, C_u' and C_l' are the concentrations of ovalbumin in the upper and lower phases when PEG was used, and C_u'' and C_l'' were when P-PEG was used.

$$\Delta \log K = \log K'' - \log K' \quad K' = C_u'/C_l \quad K'' = C_u''/C_l''$$

Surface Tension Decay of Ovalbumin Solutions. The surface tension of ovalbumin solutions was monitored with a Wilhelmy plate-type surface tensiometer (Kyowa Model CBVP surface tensiometer Type A3, Kyowa Kaimenkagaku Co., Ltd., Tokyo) with a digital printer (Yokogawa portable, Model 2543, Yokogawa Co. Ltd., Tokyo) and a constant-temperature cell compartment. The vessel containing the solution was a dish 5.5 cm in diameter and 1.5 cm in depth. The solution was introduced into the vessel, and after the solution stood for 30 min for temperature equilibration, a fresh surface was formed by removal of the surface of the solution by decantation. The surface tension was measured at the formation of the new surface, and the change in surface tension was recorded for 1 h after the new surface formation. The sample temperature was kept at 25 ± 0.2 °C.

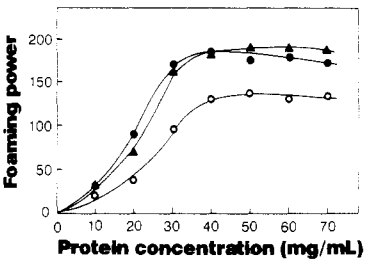

Figure 1. Foaming power of the ovalbumin solutions at various concentrations: original purified ovalbumin (○), freeze-dried ovalbumin (●), and spray-dried ovalbumin (▲).

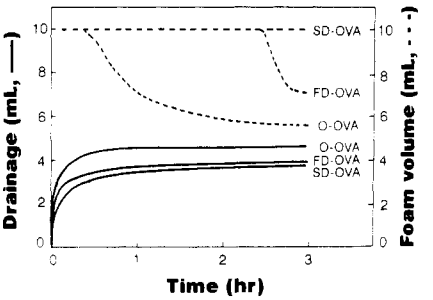

Figure 2. Changes in foam volume (dotted line) and drainage from the foam (solid line) with standing time. The foams were prepared from the original purified ovalbumin (O-OVA), the freeze-dried ovalbumin (FD-OVA), and the spray-dried ovalbumin (SD-OVA).

RESULTS

Foaming and Emulsification Properties. Figure 1 shows the foaming power of the ovalbumin at various concentrations. Up to 7%, freeze- and spray-dried ovalbumins gave higher values of foaming power than those of the original ovalbumin. The characteristics of the plot of the foaming power against protein concentration can be expressed by two terms, Fp(max) and Pc(1/2) (Kitabatake and Doi, 1982a,b). Fp(max) is the maximum value of foaming power for the protein solution and Pc(1/2) is the protein concentration that gives half of Fp(max). Fp(max) values for the original ovalbumin and the freeze- or the spray-dried ovalbumin were 135 and 180, respectively. Pc(1/2) values for the original ovalbumin and the freeze- or the spray-dried ovalbumin were both 2.35%. This means that the foamability of ovalbumin solution was increased by freeze- or spray-drying, especially in the maximum foaming power. However, the ovalbumin concentrations giving half of Fp(max) were not different.

Figure 2 showed the change in the column volume of the foam prepared with the original, spray-dried, and freeze-dried ovalbumin solutions with the foam standing in the graduated cylinder for 3 h. The bubbles in the foam column prepared from the original purified ovalbumin solution started to rupture after about 20 min of standing. After the mixture was allowed to stand for 1 h, most of the bubbles had disappeared. On the other hand, the heights

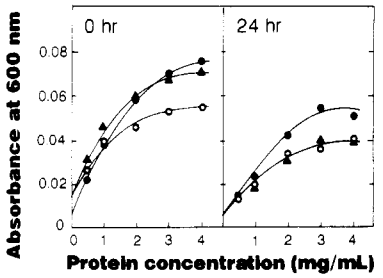

Figure 3. Absorbance of ovalbumin-stabilized emulsions as a function of the ovalbumin concentration. Absorbance of the emulsion was measured immediately after the preparation of the foam (0 h) and after the mixture was allowed to stand for 24 h at room temperature (24 h). Key: original purified ovalbumin (○), freeze-dried ovalbumin (●), and spray-dried ovalbumin (▲).

of the foam columns prepared from the freeze-dried and the spray-dried ovalbumin solutions were stable until 2 h of standing and until 3 h and more, respectively.

Figure 2 also depicted the increase in the drainage volume from the foam in the graduated cylinder. While the drainage volume reached an almost constant value in every case after 30 min of standing, the total drainage volume from the foam of the original ovalbumin solution was larger than that of the freeze- and the spray-dried ovalbumin foams, indicating that the foams prepared from the freeze- and the spray-dried ovalbumin solutions could hold more water in the lamella of the bubbles. This may be one of the reasons why the foam made from the dried ovalbumin solution gave more stable foam than that from the original ovalbumin solution. While the foam column stood in a graduated cylinder whose top was tightly closed with a film (Parafilm), a substantial amount of drainage from the foam column occurred without a change in foam volume. This is because the film of each lamella of the bubbles became thinner with time, and in some instances, that is, when the film became too thin to keep the structure of foam column, it collapsed and the foam volume started to decrease.

Figure 2 shows considerable foam instability of the native ovalbumin after 1 h; hence, in a food system, it might be that a stable foam prepared from whipping of egg white resulted from the complex formation of ovalbumin with other proteins in egg white at the air–water interface (Johnson and Zabik, 1981). Ovalbumin provides heat-denaturable protein to egg white foam; thereby, it seems that the ovalbumin denatured with heat in a foam gives a more stable foam structure through making a tight network structure together with other proteins at the air–water interface. Ovalbumin is in itself not a particularly good foamer, but it should give a stable foam.

Foaming properties are due to the surface-active properties of protein. Emulsification properties of protein also depend on the interfacial properties of protein. The properties of emulsions prepared from the three kinds of ovalbumin were examined. Figure 3 depicts the turbidity of the emulsions prepared at various ovalbumin concentrations. The emulsions prepared with the freeze- and the spray-dried ovalbumins gave higher absorbances. That is, the turbidity values in the range measured were higher than those of the original ovalbumin solution. After the emulsion was left for 24 h, the absorbance decreased in any concentrations of ovalbumin, but the emulsion prepared with the freeze-dried ovalbumin showed a higher value than the emulsion prepared with the original ovalbumin. The emulsion prepared from spray-dried ovalbumin gave a similar curve to that of the original ovalbumin. These results apparently indicated that freeze- or spray-drying increased both the emulsifying activity and the emulsion

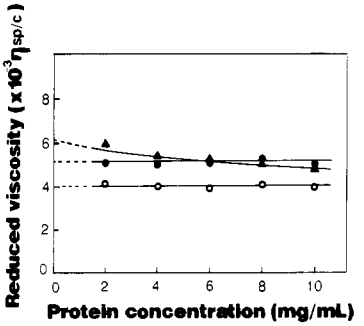

Figure 4. Reduced viscosity of ovalbumin solution as a function of the ovalbumin concentration: original purified ovalbumin (○), freeze-dried ovalbumin (●), and spray-dried ovalbumin (▲).

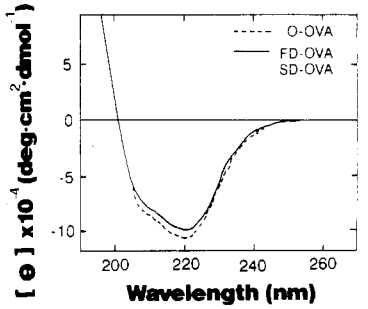

Figure 5. Circular dichroism spectra of ovalbumin solutions: original purified ovalbumin (dotted line); freeze-dried and spray-dried ovalbumins (solid line).

Table I. Sulfhydryl Contents of Original Purified, Freeze-Dried, and Spray-Dried Ovalbumin with and without Denaturants

ovalbumin soln	SH found, mol/mol	
	without urea and SDS	with urea and SDS
orig	0	3.92
freeze-dried	0.08	3.75
spray-dried	0.02	4.08

stability of the ovalbumin solution; that is, the surface activity of the ovalbumin molecule was increased by such drying treatments. The cause of this change in the functional properties seemed to be derived from the alteration of the protein conformation. Several physicochemical properties of ovalbumin before and after drying were examined.

Physicochemical Properties. Figure 4 shows the reduced viscosity of the original and the freeze- and spray-dried ovalbumin solutions at various concentrations. The intrinsic viscosity, obtained by the extrapolation of the line to the vertical axis, of the original ovalbumin was about 4.0, which was consistent with the values in other reports (McKenzie et al., 1963; Kitabatake and Doi, 1987). The intrinsic viscosities of the freeze- and spray-dried ovalbumins were a little higher than that of the original ovalbumin.

Table I showed the number of sulfhydryl residues of three kinds of ovalbumin with and without denaturant. In the presence of the denaturant, in both cases little difference among the original, freeze-dried, and spray-dried ovalbumins was observed.

Figure 5 showed the circular dichroism spectrum of each ovalbumin. The spectra of the freeze- and spray-dried ovalbumins were similar, but a slight difference was observed from that of the original ovalbumin.

Figure 6 showed the acceptability of the ovalbumin by subtilisin. Under the assay conditions used, the freeze- or spray-dried ovalbumin was more easily hydrolyzed by

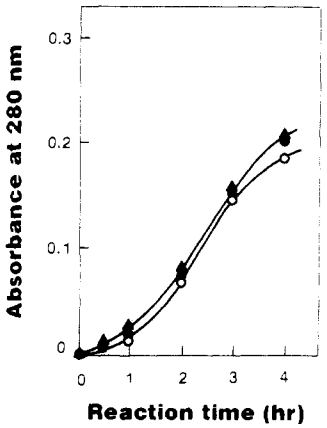

Figure 6. Absorbance at 280 nm of the supernatant of the TCA precipitated protease reaction mixture as a function of the reaction time. Subtilisin was used. Key: original purified ovalbumin (○), freeze-dried ovalbumin (●), and spray-dried ovalbumin (▲).

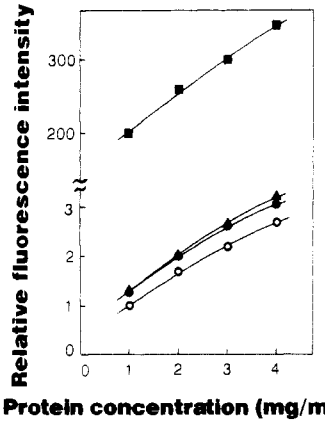

Figure 7. Relative fluorescence intensity of the complex of ovalbumin and *cis*-parinaric acid as a function of the reaction time: original purified ovalbumin (○), freeze-dried ovalbumin (●), spray-dried ovalbumin (▲), and ovalbumin heated at 80 °C for 10 min (■).

subtilisin than the original ovalbumin, though the difference was slight.

From these results, slight differences in physicochemical properties were found between the original and freeze- or spray-dried ovalbumin, indicating little conformational change with freeze- or spray-drying. However, these differences are very small, compared with the thermal or other denaturations.

Hydrophobicity of Ovalbumin Molecules. From the results above, freeze- or spray-drying made little alteration in the physicochemical properties of ovalbumin, but the functional properties such as foaming and emulsification were changed and improved. This suggested that the drying process resulted in some other change in the ovalbumin molecules without a large alteration in protein conformation as examined above. To obtain evidence for a change in the protein structure, the hydrophobicity of ovalbumin molecules was measured with two different methods.

Figure 7 shows the relative fluorescence intensity from the complex of *cis*-parinaric acid and ovalbumin molecules, measured at various ovalbumin concentrations. Although the difference between the dry-treated ovalbumins and the original ovalbumin was small, compared to heat denaturation, this difference was significant (measurement in triplicate). This result indicated that the hydrophobicity of the ovalbumin was increased by the drying process. Several ways to measure the hydrophobicity of protein

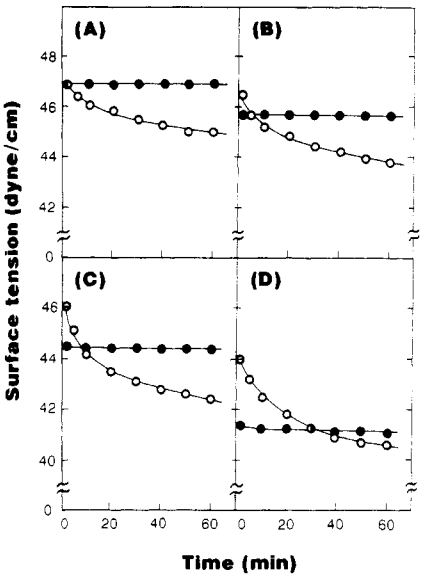

Figure 8. Surface tension decay of various concentrations of ovalbumin solutions with time: original purified ovalbumin (○) and freeze-dried ovalbumin (●). The protein concentrations of each sample were 5 mg/mL (A), 10 mg/mL (B), 20 mg/mL (C), and 40 mg/mL (D).

molecules have been reported as well as the method above, one of which is the measurement by partition in aqueous two-phase systems (Shanbhag and Axelsson, 1975).

As for the P-PEG preparation, the proton ratio of the PEG moiety and palmitate moiety, measured with NMR, was 500:31, which showed that a P-PEG molecule contained 0.6 of a palmitoyl group. The hydrophobic coefficients (Δ log K) of the original ovalbumin and the freeze-dried ovalbumin were −0.05 and 0.08, respectively. The original ovalbumin had a negative hydrophobic coefficient, and the freeze-dried ovalbumin was positive, indicating that the freeze-dried ovalbumin was more hydrophobic than the original ovalbumin, which corresponded to the results of the titration experiment using *cis*-parinaric acid described above. Changes in foaming and emulsifying activity should be reflected in the surface behavior of the protein solution.

Surface Tension Decay. Changes in the surface tension of the original and the freeze-dried ovalbumin solution with time after making the new surface were followed for 60 min (Figure 8). The surface tension of the original ovalbumin solution was decreased with time for 60 min, and the surface tension value at 60 min decreased with increases in the ovalbumin concentration. In the case of the freeze-dried ovalbumin solution, the surface tension immediately reached a constant value and little change in the surface tension was observed after that for 60 min. The surface tension value at 60 min decreased with increases in the ovalbumin concentration. The surface tension of protein solutions decreased with time, which might correspond to adsorption and conformational changes in molecules at the air–water interface, that is, to the surface denaturation of protein (Graham and Phillips, 1979; Phillips, 1981). The protein solution, the surface tension of which reaches a constant value quickly after formation of a new solution surface, has a high foamability (Kitabatake and Doi, 1982a,b, 1988), and this protein must have a flexible or amphipathic structure and be susceptible to surface denaturation. Increases in the hydrophobicity of ovalbumin molecules by freeze-drying seemed to enhance the surface activity of the molecule.

As shown in this study, the freeze- or spray-dried ovalbumin had a higher foamability and emulsifying ability

than the original ovalbumin solution, although no great alteration of protein conformation was found. However, a slight change in hydrophobicity was observed, which seemed to bring about the change in the interfacial activity of ovalbumin, resulting in the increase of foamability and emulsifying ability.

DISCUSSION

Ovalbumin has been recognized as an unstable protein (Osuga and Feeney, 1977), while the thermal denaturation temperature of ovalbumin was found at around 80 °C, which was not a very low value, compared with the denaturation temperature of other proteins (Donovan et al., 1975), and in the circular dichroism spectrum after thermal treatment, only a little change was observed, compared with that of denaturation by guanidine hydrochloride or urea (Doi et al., 1987). From this study, it was apparent that ovalbumin was susceptible to change in molecular hydrophobicity by drying. Although this change was not so large as that caused by heat denaturation, it was enough to increase the foamability, emulsifying ability, and their stabilities. Changes in molecule structure were clearly reflected in the surface tension decay of ovalbumin solutions. These properties might result in the discrepancy about the denaturation of ovalbumin mentioned above.

The conformational change of ovalbumin molecules occurs by foaming. At the air–water interface (Kitabatake and Doi, 1988), hence, it is probable that such changes might occur during freeze- or spray-drying at the air–water interface in some ovalbumin molecules of the sample solution. The concentration of ovalbumin molecules by drying might increase protein–protein interaction, and the ovalbumin molecules were liable to aggregate under this condition. However, it does not seem that all of the ovalbumin molecules in the sample solution were denatured at the air–water interface during drying, because no distinct alteration of physicochemical properties between native and dried ovalbumins was found. Even if small amounts of ovalbumin molecules, whose conformation was changed by drying, were retained in the supernatant as a soluble state after centrifugation, they could give the characteristic surface properties to the total. It is likely that these molecules were surface-active and could immediately adsorb to the air–water interface to decrease the surface tension of the solution quickly and that the formability and emulsifying ability increased (Kitabatake and Doi, 1982a,b, 1988).

In some instances, we know that spray-drying of protein is accompanied by heat denaturation during drying, because the control of the actual material temperature during drying is difficult. In our study, the spray-dried ovalbumins showed slightly different properties from those of freeze-dried ovalbumins: foam stability, intrinsic viscosity, subtilisin acceptability, and hydrophobicity measured with *cis*-parinaric acid. These might be brought about from the effects of heat during processing.

It could be concluded from this study that the drying of salt-free ovalbumin changes the interfacial properties, and the conformation of ovalbumin in the solutions prepared from freeze- or spray-dried powder obtained from salt-free ovalbumin is not the same as the conformation of native ovalbumin. Therefore, such solutions are not suitable for use in the studies of functional properties of native ovalbumin.

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Terminal Apiose: A New Sugar Constituent of Grape Juice Glycosides

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Apiose [3-(hydroxymethyl)-D-erythrofuranose] has been identified as a component of the carbohydrate moiety of glycosides from the juice of Muscat grapes by acid hydrolysis, alditol acetate derivatization of liberated sugars, and GC–MS. Permethylation of the bulk of glycosides and subsequent GC–MS analysis of the partially methylated alditol acetates showed that apiose was under terminal nonreducing position.

Grapes from the group of Muscat cultivars (Frontignan, Alexandria, etc.) contain glycosides (e.g., arabinosyl and rhamnosyl glucosides) (Williams et al., 1982; Gunata et al., 1988; Strauss et al., 1988) of monoterpenols (mainly geraniol, nerol, and linalool) that might liberate by enzymic hydrolysis their strongly aromatic aglycon moieties. During the purification of the bulk of monoterpenyl disaccharide glycosides from a Muscat juice, other unidentified glycosides were detected, behaving in most separative techniques as the aromatic precursors that rendered them impossible to separate and yielded additional unknown peaks in GC (Gunata et al., 1988) and HPLC (Bitteur et al., 1989).

After acid hydrolysis of the carbohydrate moieties of the bulk of glycosides, a new compound appeared in addition to expected arabinose, rhamnose, and glucose (Gunata et al., 1988). The purpose of the present work was to identify this unknown sugar for a better knowledge of the constitution of the glycoside mixture with the aim of fully elucidating the GC and HPLC chromatograms.

RESULTS AND DISCUSSION

When the mixture of grape glycosides was submitted to strong-acid hydrolysis, TLC of released monosaccharides revealed rhamnose, arabinose (R_{rha} 0.52), glucose (R_{rha} 0.42), and an unknown component (R_{rha} 0.93) stained green or gray by naphthoresorcinol depending on its concentration (Gunata et al., 1988). Alditol acetate derivatives of liberated sugars were separated on a fused silica capillary column bonded with OV-225, and an unknown peak emerged hardly separated from xylose (RRt/xyl 0.98). The relative proportions were Rha:Ara:unknown:Xyl:Glc = 5.4:10.3:13.0:trace:71.3 (Gunata et al., 1988).

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Methylation analysis of carbohydrate moieties of glycosides in admixture using sodium (methylsulfinyl)-methanide and methyl iodide and subsequent alditol acetate derivatization of partially methylated sugars revealed (GC) an unknown peak hardly separated from 2,3,4-Me_3-rha (RRt 0.99) on an OV-1 column but well isolated from all other ethers on an OV-225 column (Gunata et al., 1988); its relative proportion was similar to that observed by direct sugar analysis.

According to the retention times of both the alditol acetate and the partially methylated alditol acetate derivatives of this unknown component, it was suspected to be a pentose under terminal nonreducing position. The alditol peracetate gave a chemical ionization spectrum showing intense ions at m/e 380 $(M + NH_4)^+$ and 303 $(M - OAc)^+$ corresponding to a molecular weight of 362 for a pentitol peracetate. The electron impact fragmentation pattern of the alditol acetate (Figure 1) was identical with the spectrum of apiitol peracetate obtained by acid hydrolysis of apiin and alditol acetate derivatization of liberated apiose; furthermore, it coeluted on the OV-225 column with apiitol peracetate from apiin.

The electron impact mass spectrum of the partially methylated alditol acetate (PMAA), shown in Figure 2, exhibited all the characteristic ions expected from 2,3,3[1]-tri-*O*-methyl-1,4-di-*O*-acetylapiitol and was identical with the spectrum of the PMAA from terminal apiose obtained by permethylation of apiin, acid hydrolysis, and alditol acetate derivatization. The primary and secondary fragmentation patterns of this last component are shown in Figure 3. It coeluted with the PMAA from apiin on both OV-1 and OV-225 capillary columns.

Finally, our unknown compound migrated similarly to apiose (R_{rha} 0.93) obtained by acid hydrolysis of apiin and gave the same staining with naphthoresorcinol.

Thus, it can be concluded from the above results that apiose [3-(hydroxymethyl)-D-erythrofuranose] is present in our mixture of grape glycosides under terminal nonreducing position. Although apiose has been detected in a great number of plants (Watson and Orenstein, 1975), it is the first report of its presence in grape juice, most likely linked to a glucopyranosyl moiety, since a large amount of 6-linked glucose was also detected in grape glycosides

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