Changes in the Emulsifying and Foaming Properties of Proteins during Heat Denaturation

Akio KATO, Yukiko OSAKO, Naotoshi MATSUDOMI and Kunihiko KOBAYASHI

Department of Agricultural Chemistry, Yamaguchi University, Yamaguchi 753, Japan

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Changes in the emulsifying and foaming properties of ovalbumin, 7S globulin, κ-casein, β-lactoglobulin and bovine serum albumin were followed during heat denaturation, and these surface properties were correlated with the corresponding surface hydrophobicity, in order to investigate the role of surface hydrophobicity in the surface properties of proteins. The surface hydrophobicity of ovalbumin, 7S globulin and κ-casein increased with heat denaturation, while that of β-lactoglobulin and bovine serum albumin decreased. The emulsifying activity and emulsion stability of proteins correlated linearly with surface hydrophobicity, although protein structure changed greatly during heat denaturation. On the other hand, the foaming power of proteins correlated curvilinearly with surface hydrophobicity during heat denaturation. No significant correlation was observed between the foam stability and the surface hydrophobicity of proteins.

On the basis of these results, the relationships between the surface properties and the structure of proteins are discussed.

We have already reported that the surface hydrophobicity of proteins showed good correlations with emulsifying and foaming properties; the more hydrophobic proteins, the better the functional properties.[1～3] Despite the hydrophilic nature of protein molecular surface, a significant number of hydrophobic amino acid groups are exposed at the molecular surface of such proteins as bovine serum albumin, β-lactoglobulin, casein and soy globulin. Bovine serum albumin has a large number of sites available for hydrophobic ligands.[4] The surface hydrophobicity of β-lactoglobulin is well established through its interaction with hydrocarbons and detergents.[5,6] κ-Casein is well known as a hydrophobic protein with a high concentration of nonpolar amino acid residues in the N-terminal region (segment 1～105).[7] In addition, soybean 7S globulin (abbreviated as 7S globulin) has also been found to have relatively high hydrophobicity by a screening test of various proteins. Therefore, these proteins may possess the amphiphilic properties to cause good emulsifying and foaming properties. It is reasonable to assume that surface hydrophobicity plays a governing role as the trigger of emulsification and foaming, because amphiphilic proteins possessed of high surface hydrophobicity are forcefully adsorbed at the interface between oil or air and water to cause a pronounced reduction of interfacial tension or surface tension that readily facilitates emulsification and foaming. However, structural factors other than surface hydrophobicity should be also considered to elucidate the relationship between the surface properties and the structure of proteins. The facility of protein-protein interaction and surface denaturation at the oil–water and air–water interface may be concerned in the surface properties, especially emulsion and foam stability.

This paper describes changes in the emulsifying and foaming properties of hydrophobic proteins during heat denaturation to elucidate further the relationship between the surface properties and structure of proteins.

MATERIALS AND METHODS

Ovalbumin was used as a standard protein for comparison. Ovalbumin was prepared from fresh egg white by the sodium sulfate procedure[8] and recrystallized five times. κ-Casein was prepared from fresh milk by the method of Zittle and Custer.[9] 7S globulin was prepared from soybeans by the methods of Thanh *et al.*[10] Bovine serum albumin was purchased from Sigma Chemical Co., St. Louis, MO. β-Lactoglobulin was from ICN Pharmaceuticals Inc., Cleveland, OH.

The heat denaturation of proteins was carried out as follows: 10 ml of 0.2% protein solution in 0.1 M phosphate buffer, pH 7.4, was heated in an incubator at an increasing rate of 1°C per min from 20°C to 80°C. Heat-denatured protein solution was then immediately cooled to 20°C after the rise to given temperatures. No precipitates were produced under these heating conditions. The surface hydrophobicity of proteins was determined by the fluorescence probe method using *cis*-parinaric acid.[3] *cis*-Parinaric acid was purchased from Wako Pure Chemical Industries Ltd.

The emulsifying properties of proteins were determined by the method of Pearce and Kinsella.[11] To prepare emulsions, 1.3 ml of corn oil and 4 ml of 0.2% protein solution in 0.1 M phosphate buffer, pH 7.4, were homogenized in an Ultra Turrax (Hansen & Co., West Germany) at 12,000 rpm for 1 min at 20°C. 0.1 ml of each emulsion was taken from the bottom of the container after different times and diluted with 0.1% SDS solution. The turbidity of diluted emulsions was then measured at 500 nm. Emulsifying activity and emulsion stability were determined by the method of Pearce and Kinsella.[11]

Foaming properties were determined by measuring the electric conductivity of foams when air was introduced into 5 ml of 0.1% protein solution in 0.1 M phosphate buffer, pH 7.4, in a glass filter (G-4) at a constant flow rate, 90 cm^3/min, for 15 sec.[12] Foaming power was determined by measuring the conductivity of foams produced immediately after air was introduced into protein solution for 15 sec. Foam stability was determined by measuring the conductivity of foams 5 min after foam production.

The values of emulsifying and foaming properties were represented as the ratios to those of ovalbumin.

RESULTS AND DISCUSSION

Table I shows the emulsifying and foaming properties of bovine serum albumin, β-lactoglobulin, κ-casein and 7S globulin. The emulsifying and foaming properties of these hydrophobic proteins were much better than those of ovalbumin used as a non-hydrophobic standard protein. Except for κ-casein, good correlations were observed for surface hydrophobicity with the emulsifying and foaming properties; the more hydrophobic proteins, the better the functional properties. The relationship between the surface properties and hydrophobicity were more closely investigated as below.

Changes in the emulsifying and foaming properties of hydrophobic proteins were followed during heat denaturation, correlating with the corresponding changes in hydrophobicity. The surface hydrophobicity of proteins greatly changed during heat denaturation, as shown in Fig. 1. The surface hydrophobicity of 7S globulin and κ-casein increased with heat denaturation as well as ovalbumin, while that of β-lactoglobulin and bovine serum albumin decreased with heat denaturation. These changes in surface hydrophobicity must be due to the conformational changes of proteins, because big differences in surface hydrophobicity were observed at the melting points for thermal denaturation. This was confirmed from CD analysis of ovalbumin and bovine serum albumin during heat denaturation. Therefore, if surface hydrophobicity is a main factor governing the surface properties, a good correlation may be obtained

TABLE I. EMULSIFYING AND FOAMING PROPERTIES OF HYDROPHOBIC PROTEINS

The values of emulsifying and foaming properties are represented as the ratios to those of ovalbumin.

	Surface hydrophobicity	Emulsifying activity	Emulsion stability	Foaming power	Foam stability
Ovalbumin	10	1.00	1.00	1.00	1.00
7S globulin	260	3.46	3.07	1.24	2.85
κ-Casein	430	5.90	6.43	2.18	3.45
β-Lactoglobulin	2700	5.34	6.01	2.38	4.03
Bovine serum albumin	3200	6.44	8.51	2.62	4.61

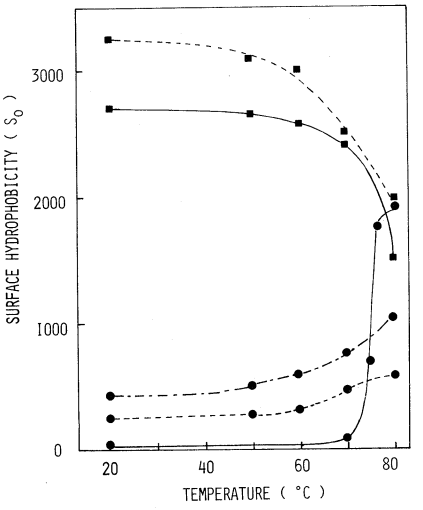

FIG. 1. Changes in the Surface Hydrophobicity of Heat-denatured Proteins.
●—●, ovalbumin; ●---●, 7S globulin; ●–·–●, κ-casein; ■—■, β-lactoglobulin; ■---■, bovine serum albumin.

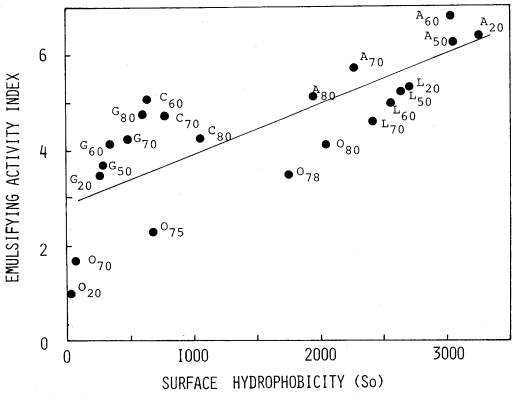

FIG. 2. Correlation of Emulsifying Activity Index with Surface Hydrophobicity of Proteins.
R=0.76 ($p<0.01$). O, ovalbumin; G, 7S globulin; C, κ-casein; L, β-lactoglobulin; A, bovine serum albumin. The values on the right of O, G, C, L and A indicate heating temperature (°C) at a increasing rate of 1°C per min from 20°C to 80°C.

between the surface hydrophobicity and the surface properties of denatured hydrophobic proteins. On the other hand, if no significant correlations are observed, this will suggest the presence of structural factors other than surface hydrophobicity.

Figure 2 shows the plots of emulsifying

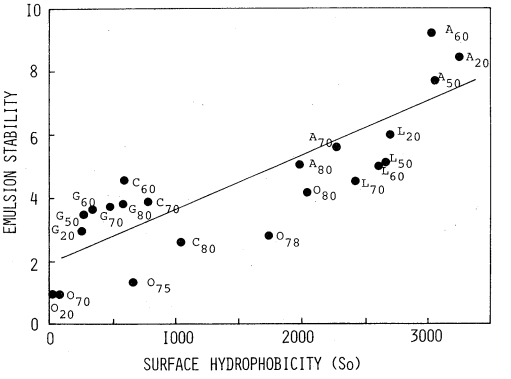

FIG. 3. Correlation of Emulsion Stability with Surface Hydrophobicity of Proteins.
R=0.82 ($p<0.01$). O, G, C, L and A are the same as Fig. 2.

activity index against surface hydrophobicity of denatured hydrophobic proteins. The plots of ovalbumin were added as a standard. Close correlations were obtained between the emulsifying activity and the surface hydrophobicity of 7S globulins ($r=0.92$), β-lactoglobulins ($r=0.99$) and bovine serum albumins ($r=0.93$), but not for κ-caseins. Similar good correlations were observed in the case of denatured ovalbumin and lysozyme.[3] Interestingly, the emulsifying activity of β-lactoglobulin and bovine serum albumin decreased in proportion to heat denaturation by which the surface hydrophobicity of these proteins was lowered. The negative correlation in κ-casein may have occurred because denatured molecules easily associate[13] due to an increase in surface hydrophobicity during heat denaturation which causes the interaction of denatured κ-casein with oil to lower. Although five protein systems were combined, a good correlation was observed between the emulsifying activity and the surface hydrophobicity. The correlation coefficient is 0.76 and significant ($p<0.01$). Figure 3 shows the relationship between the emulsion stability and the surface hydrophobicity of denatured hydrophobic proteins. As in the case of emulsifying activity, good correlations were obtained between the emulsion stability and the surface hydrophobicity of denatured 7S globulins ($r=0.74$), β-lactoglob-

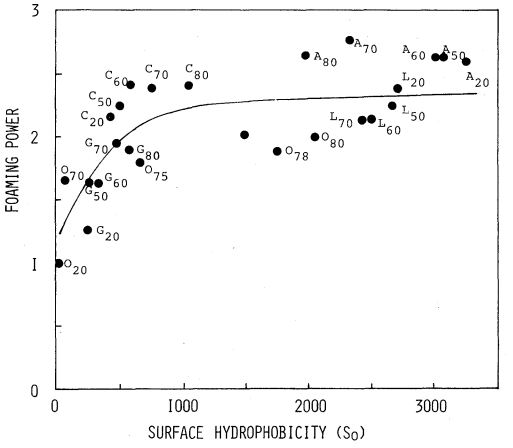

FIG. 4. Correlation of Foaming Power with Surface Hydrophobicity of Proteins.

O, G, C, L and A are the same as Fig. 2.

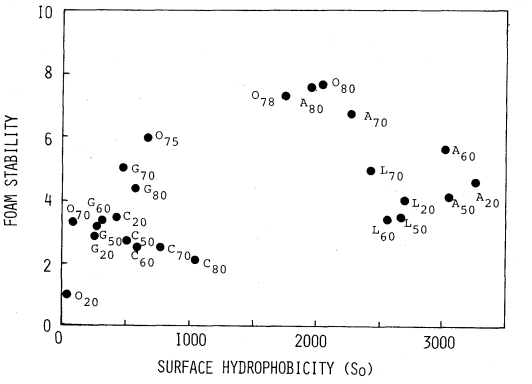

FIG. 5. Correlation of Foam Stability with Surface Hydrophobicity of Proteins.

O, G, C, L and A are the same as Fig. 2.

ulins ($r=0.87$) and bovine serum albumins ($r=0.93$), but not for κ-caseins. Although five protein systems were combined, a good correlation was likewise observed between emulsion stability and surface hydrophobicity. The correlation coefficient is 0.82 and significant ($p<0.01$). Thus, good correlations between surface hydrophobicity and emulsifying properties were observed not only for the same protein systems but also for the protein systems in which the physicochemical properties are different from each other. These results suggest that the surface hydrophobicity is certainly a main factor governing the emulsifying properties of proteins. That is, the emulsifying properties of proteins change at a rate depending on the surface hydrophobicity, although protein structure changes markedly during heat denaturation. However, this is not the case for a glycoprotein such as κ-casein. Further studies should be done to investigate this.

The role of surface hydrophobicity on the foaming properties of proteins was also investigated. Figure 4 shows the relationship between the foaming power and the surface hydrophobicity of denatured hydrophobic proteins. The plots of ovalbumin were added as a standard. Unlike the case of emulsifying properties linear correlations were not observed, but curvilinear correlations existed between the foaming power and the surface hydrophobicity of denatured 7S globulins and κ-caseins. This curvilinear correlation between foaming power and surface hydrophobicity has also been reported in the case of denatured ovalbumins and lysozymes.[3] On the other hand, the foaming power of highly hydrophobic proteins, β-lactoglobulin and bovine serum albumin did not significantly change, despite big changes in the surface hydrophobicity during heat denaturation. This result suggests that the foaming power of proteins increases due to a slight increase in surface hydrophobicity, because of a lowering of surface tension, and remains at a maximum value beyond the definitive value for surface hydrophobicity. This tendency was also observed in the plots of foaming power against surface hydrophobicity which combined the five protein systems. On the other hand, as shown in Fig. 5, no significant correlation was observed between the foam stability and surface hydrophobicity of proteins, although a curvilinear correlation was observed for denatured 7S globulins as well as for denatured ovalbumins and lysozymes.[3] It is probable that the foam stability of proteins is related to the extent of denaturation rather than surface hydrophobicity, for the foam stability of proteins increases as heating temperature increases, except for κ-casein. Since the signifi-

cance of surface tension on foaming has been widely accepted, it is reasonable to assume the existence of a good correlation between the surface hydrophobicity and the foaming properties of proteins. However, this is only the case for foaming power, *i.e.*, the ease of foaming, and not the case for foam stability. The ability to associate and form a film due to denaturation may be essential for the foam stability of proteins.

Differences in the conditions for measuring the surface hydrophobicity and the surface properties should be taken into account in elucidating their relationships. Surface denaturation may have occurred during measurement of the surface properties. However, the considerable differences in emulsifying and foaming properties between native and heat-denatured proteins suggest that the surface denaturation of native proteins does not occur in the conditions used for the experiment. Nevertheless, differences in the dependence upon surface hydrophobicity between the emulsifying and foaming properties are suggestive of more extensive unfolding in protein molecules at the air–water interface than at the oil–water interface. Since partially denatured proteins with slightly increased surface hydrophobicity might cause more extensive unfolding at the air–water interface than at oil–water interface, a curvilinear correlation may be observed between the foaming power and the surface hydrophobicity of proteins. This may also be related to the fact that the surface tension at air–water interface (73 dyn/cm) is much greater than that at oil–water interface ($13 \sim 19$ dyn/cm).[14,15]

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