Hydrophobicity and Emulsifying Activity of Milk Proteins

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Hydrophobicity is thought to be one of the most important properties which affect the functionalities of proteins. The correlation between the hydrophobicity and such functional properties as the foaming, emulsifying and gel forming properties has been demonstrated by many investigators.1-3) The importance of the surface (effective) hydrophobicity of proteins in such functionalities was particularly emphasized and many methods for determining the surface hydrophobicity of proteins have been proposed. The fluorescent probe methods involving 8-anilinonaphthalene-1-sulfonate (ANS)4) and cis-parinaric acid (cis-PnA)5) have been widely used to determine the surface hydrophobicity because of their simplicity. However, it is still questionable as to whether or not the hydrophobicity values obtained by these methods reflect the true hydrophobicities of the proteins. In this study, the hydrophobicity values for milk proteins obtained by the fluorescent probe methods were compared with those obtained by another three methods, hydrophobic affinity chromatography (reverse phase-high performance liquid chromatography), hydrophobic partition and heptane binding methods.

Five major milk proteins (asl-casein, β-casein, α-lactalbumin, β-lactoglobulin and serum albumin), whose molecular structures are markedly different from each other, were used for the analyses. αsl-casein (αsl-CN), β-casein (β-CN), α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) were isolated from fresh skim milk by ordinary methods, and then purified by DEAE-Sephacel chromatography (αsl, β-CN) or Sephadex G-100 gel filtration (α-La and β-Lg). Crystallized bovine serum albumin (BSA) was purchased from Seikagaku-Kogyo Co., Ltd., and used without further purification.

Emulsification was carried out by homogenizing a 1% (w/v) protein solution in 5mM phosphate buffer (pH 7.0) with 20% (w/v) soybean oil at 30°C with a Polytron PTA-7 (Kinematica, Switzerland) for 3 min. The emulsifying activity of the proteins was determined by the procedure of Pearce and Kinsella,6) and expressed as the Emulsifying Activity Index (EAI). The hydrophobicity of the proteins was evaluated by the following five methods, (i) A fluorescent probe method involving cis-parinaric acid (cis-PnA): according to the procedure of Kato and Nakai,7) other methods, and then purified by DEAE-Sephacel chromatography (αsl, β-CN) or Sephadex G-100 gel filtration (α-La and β-Lg). Crystallized bovine serum albumin (BSA) was purchased from Seikagaku-Kogyo Co., Ltd., and used without further purification.

Fig. 1. Hydrophobicity of Milk Proteins Determined by Different Methods.

Emulsifying Activity Index (EAI) values for the proteins are also shown. 1, αsl-casein; 2, β-casein; 3, α-lactalbumin; 4, serum albumin; and 5, β-lactoglobulin. The hydrophobicity value, So, was calculated according to Kato and Nakai.7) Δ log K was calculated according to Keshavarz and Nakai.20)

* The low value is due to the low solubility of β-casein in the polyethylene glycol solution.
buffer (pH 7.0) for the analysis. (ii) A fluorescent probe method involving 8-anilinonaphthalene-1-sulfonate (ANS) according to the procedure of Creamer et al.\textsuperscript{8)} except that the proteins were dissolved in 5 mM phosphate buffer (pH 7.0). (iii) Reversed phase-high performance liquid chromatography (RP-HPLC): a Trirotor SR-2 (Jasco) equipped with a Senshupak-SSS-SC4 (butylated silica column; \( \phi 4.6 \times 150 \) mm) was used. The column was equilibrated with 10 mM phosphate buffer (pH 7.0). The proteins were eluted at a linear gradient of isopropanol at room temperature with monitoring at 280 nm. (iv) Hydrophobic partitioning between two phases, the palmitic acid ester of polyethylene glycol and dextran: according to the procedure of Shanbhag and Johansson.\textsuperscript{9)} (v) Heptane binding method: according to the procedure of Mohammadzadeh et al.,\textsuperscript{10} except that the proteins were dissolved in 5 mM phosphate buffer (pH 7.0) for the analysis.

Figure 1 shows the hydrophobicity values for the five milk proteins determined by the different methods. No correlation was observed among the results with the five determination procedures, suggesting that the methods determined different properties of the proteins. With binding procedures involving cis-PnA, ANS and heptane, globular proteins, such as \( \alpha_\text{La} \), BSA and \( \beta\text{-Lg} \), showed relatively high hydrophobicity values compared to \( \alpha_\text{s1} \) and \( \beta\text{-CN} \). The order of their hydrophobicities, however, was different for each of the three methods. The high affinities of cis-PnA to \( \beta\text{-Lg} \), ANS to BSA and heptane to \( \alpha_\text{La} \) suggest that the binding of these hydrophobic ligands to proteins is based on some specific interactions including ionic interactions as well as the hydrophobic interaction. For example, the serum albumin molecule is known to have strong binding sites for hydrophobic components such as fatty acids, bilirubin and various drugs.\textsuperscript{11,12} \( \beta\text{-Lg} \) has been found to have a retinol-binding site.\textsuperscript{13} The presence of such specific binding sites might account for the high values obtained with the hydrophobic probe methods. The existence of two types of protein hydrophobicities, aliphatic and aromatic, might also affect the binding of various hydrophobic probes to proteins, as recently suggested by Hayakawa and Nakai.\textsuperscript{14} \( \alpha_\text{s1}\text{-CN} \) and \( \beta\text{-CN} \) are known to be rather hydrophobic since the hydrophobicity values calculated from their amino acid compositions are relatively high.\textsuperscript{15} However, the hydrophobicity values for the caseins determined by probe-binding methods were very low. This might suggest that highly flexible non-globular proteins such as caseins have few binding sites for the probes. With the hydrophobic partition method, \( \alpha_\text{s1}\text{-CN} \) showed a high hydrophobicity value. A high hydrophobicity value for \( \beta\text{-CN} \) was obtained on RP-HPLC. These methods might be useful for evaluating the hydrophobic properties of flexible proteins like caseins.

Nakai and coworkers have reported that functional properties such as the emulsifying activity of proteins are significantly correlated with the surface hydrophobicity, especially with the values obtained by fluorescent probe methods.\textsuperscript{7,16–19} However, we could not find such a correlation for the major milk proteins with any of the determination methods investigated. This might indicate the diversity in the structure of milk proteins and also the complex features of the protein hydrophobicity and emulsification.

REFERENCES