REVIEWS

Structure-Function Relationships of Food Proteins with an Emphasis on the Importance of Protein Hydrophobicity

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When hydrophobic parameters in addition to solubility were used, better correlations with protein functionality than using solubility alone were obtained. The hydrophobicity was measured fluorometrically before (S_0) and after (S_e) unfolding protein samples. Significant correlations were found for emulsifying capacity, emulsion stability, and fat binding capacity of heat-denatured proteins with surface hydrophobicity (S_0) and solubility. For foaming capacity, an exposed hydrophobicity (S_e) and viscosity played important roles; however, net charge was the most influential factor for foam stability. The S_e and available SH-group content showed good correlations with thermal functional properties of proteins, e.g., heat coagulation, gelation, and thickening. Protein hydrophobicity, the hydrophile–lipophile balance (HLB) value of surfactants, and polarity of partition chromatography seem to be closely related. Ultimately, protein functionality is dependent on hydrophobic, electrostatic, and steric parameters of the proteins, which are all essential for defining the protein structure. More advanced data processing techniques, e.g., multivariate analysis, are needed for studying these relations.

Elucidation of the mechanism of protein functionality has been attempted by many food protein chemists. The most recent monographs are the ones edited by Cherry (1981). However, the demand from the industry to predict the functionality of food proteins has not yet been met. In the case of general chemical compounds, it has been suggested that hydrophobic, electronic, and steric parameters can be independent variables for predicting their functional properties (Stuper et al., 1979).

Although many papers have been published to emphasize the importance of hydrophobicity and hydrophobic interactions in protein functionality, the quantitative administration of this parameter is not facile. The quantitation of protein hydrophobicity can be an essential step for accurate prediction of protein functionality.

The objective of this article is to discuss the relationship between hydrophobicity which effects structure and functionality of food proteins. However, the discussion is restricted to only quantitative data. Most general discussions without quantitative supporting data or with indirect evidence of the importance of hydrophobicity, e.g., data using dissociating agents, are therefore excluded.

Solubility and Hydrophobicity Relationship. Solubility is an important property governing the functional behavior of proteins and their potential application to food processing. Denaturation implicates damages to functionality and is usually measured as a loss of solubility.

Generally, soluble proteins possess superior functional attributes for most applications in food processing. However, there have been several contradictory reports showing that emulsifying properties and solubility are not closely correlated (Aoki et al., 1980; McWatters and Holmes, 1979; Smith et al., 1973; Wang and Kinsella, 1976).

Bigelow (1967) proposed that two structural features, namely, charge frequency and hydrophobicity, are major factors that control the protein solubility. The higher the charge frequency and lower the hydrophobicity, the higher would be the solubility.

Shen (1981) explained the formation of insoluble precipitate of soy protein as a combination of two reactions, i.e., reversible conversion of soluble monomers to aggregates followed by the irreversible conversion further into the insoluble precipitate. The balance between charge frequency and hydrophobicity of protein molecules can create forces for molecular repulsion and association, respectively.

Bigelow used the hydrophobicity scale for side chains of amino acid residues suggested by Tanford (1962) for his calculation of the average hydrophobicity, $H\phi_{av}$, of proteins. Meirovitch et al. (1980) reported the effects of protein size on the hydrophobic behavior of amino acids and suggested a new scale of hydrophobicity of amino acid residues. The most substantial differences in their values from that of Nozaki and Tanford (1971) are that proline, tyrosine, and lysine appear to be hydrophobic on Tanford's scale, whereas they are hydrophilic or ambivalent according to Meirovitch's classification. It should again be noted that there is a fundamental difference between the Nozaki–Tanford (1971) and Jones (1975) scales and the other hydrophobicity scales including the scale of Meirovitch et al. (1980). The latter are based on the empirical inspection of protein structures. They, therefore, reflect in a complex wasy the interaction between the hydrophobic interaction and other factors, such as local chain structure preference dictated by short-range interactions. The Nozaki–Tanford–Jones scale, which is based on experiments performed on isolated amino acids, should not be expected to give identical results.

Determination of Protein Hydrophobicity. For the determination of protein hydrophobicity, several attempts have been made, i.e., (a) reverse-phase chromatography (van Oss et al., 1979), (b) binding of hydrocarbons to proteins (Mohammadzadeh et al., 1969), (c) hydrophobic partition between two phases containing dextrans with

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Table I. Relationships of Emulsifying Ability and Fat Binding Capacity with Surface Hydrophobicity and Solubility of Heat-Denatured Proteins[a]

emulsifying activity index = $29.28 + 0.21S_0 + 0.23s$ ($n = 52$, $R^2 = 0.542$, $P < 0.001$)
emulsion stability index = $-14.31 + 0.28S_0 + 0.29s$ ($n = 49$, $R^2 = 0.434$, $P < 0.001$)
fat binding capacity = $4.90 + 0.45S_0 + 1.40s - 0.001S_0^2 - 0.014s^2$ ($n = 48$, $R^2 = 0.473$, $P < 0.001$)

[a] S_0 is surface hydrophobicity: fluorescence intensity/% protein. s is solubility index: soluble N/total N %. Emulsifying activity index: determined turbidimetrically, m^2/g. Emulsion stability index: the half-life (min) of the absorbance decay of the activity index samples. Fat binding capacity: fat bound to protein determined turbidimetrically, mL of oil/100 g of protein. Backward stepwise multiple regression analysis was carried out on an Amdahl 470 V/8 computer by using the UBC Triangular Regression Package (Voutsinas et al., 1983a).

poly(ethylene glycol) and with palmitic acid ester of poly(ethylene glycol) (Shanbhag and Axelsson, 1975), (d) fluorescence probe method for which the most popular hydrophobic probe is 8-anilinonaphthalene-1-sulfonate (Ans) (Horiuchi et al., 1978; Clarke and Nakai, 1972), and (e) salting-out effect and surface tension measurement (Melander and Horvath, 1977).

Hydrophobic (reverse-phase) chromatography and hydrophobic partition were used first by Keshavarz and Nakai (1979). It was found that there was no significant correlation between the measured hydrophobicity and the $H\phi_{av}$ values of Bigelow, while this hydrophobicity showed a significant correlation ($P < 0.05$) with interfacial tension, which is important for emulsification.

Due to the propensity of nonpolar amino acid residues to position themselves in the interior of protein molecules in solutions, thus avoiding contact with the aqueous surroundings, only a portion of them participate in the emulsification of oil into an aqueous phase. Hydrophobicity thus measured would be "surface" or "effective" hydrophobicity which does not directly correlate with the $H\phi_{av}$ or "total hydrophobicity". Keshavarz and Nakai (1979) used the term "effective hydrophobicity" for a relative hydrophobicity value measured by hydrophobic chromatography or hydrophobic partition since this represented the hydrophobicity of protein effectively involved in the interfacial tension depression. Melander and Horvath (1977) used the term "relative surface hydrophobicity" for a parameter derived from the salting-out constant.

Simplification of the hydrophobicity measurement was attempted, since the above methods are time consuming. To achieve this, a fluorescence probe, *cis*-parinaric acid, which has the formula $CH_3CH_2CH{=}CHCH{=}CHCH{=}CHCH{=}CH(CH_2)_7COOH$ and which fluoresces under a hydrophobic environment, was used (Kato and Nakai, 1980). *cis*-Parinaric acid is a natural polyene fatty acid and thus can readily simulate natural lipid–protein interacting systems. Advantages of *cis*-parinaric acid compared to Ans as a fluorescence membrane probe were discussed by Sklar et al. (1976).

Emulsifying Ability. To form an emulsion, proteins acting as emulsifiers spread around the surface of oil droplets as a thick skin (Friberg, 1976). Due to their amphiphilic nature, proteins are adsorbed to the interface between oil and water, causing a pronounced reduction of the interfacial tension. The ability of proteins to bind lipids is important for such applications as meat replacers and extenders (Rand, 1976).

The emulsifying properties of proteins ultimately depend on a suitable balance between the hydrophile and lipophile and do not necessarily increase as the proteins become more lipophilic (Aoki et al., 1981). According to them, the excessive denaturation of the soy protein by 1-propanol resulted in lower emulsion stabilization properties.

A significant correlation ($P < 0.01$) was obtained between the emulsifying capacity and the hydrophobicity of proteins determined fluorometrically as shown in Figure 1 (Kato and Nakai, 1980). These results suggest that the emulsification of oil with protein can be explained based on protein hydrophobicity. A relative hydrophobicity measured by the method of Kato and Nakai (1980), S_0, could be a measure of "surface hydrophobicity".

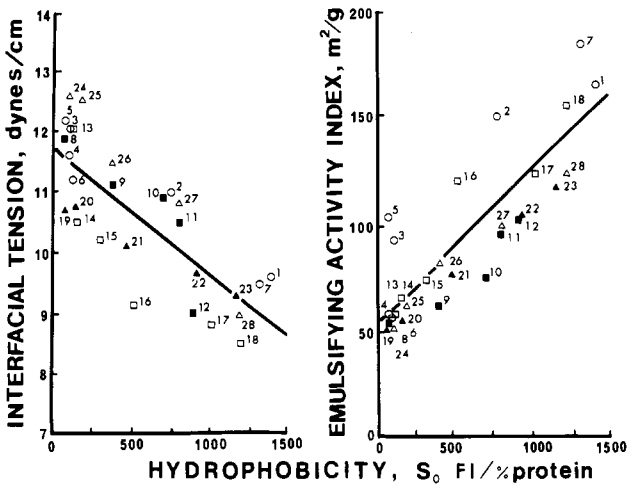

Figure 1. Relationships of S_0 with interfacial tension and emulsifying activity of proteins. 1, bovine serum albumin; 2, β-lactoglubulin; 3, trypsin; 4, ovalbumin; 5, conalbumin; 6, lysozyme; 7, κ-casein; 8, 9, 10, 11, and 12, denatured ovalbumin by heating at 85 °C for 1, 2, 3, 4, and 5 min, respectively; 13, 14, 15, 16, 17, and 18, denatured lysozyme by heating at 85 °C for 1, 2, 3, 4, 5, and 6 min, respectively; 19, 20, 21, 22, and 23, ovalbumin bound with 0.2, 0.3, 1.7, 5.7, and 7.9 mol of dodecyl sulfate/mol of protein, respectively; 24, 25, 26, 27, and 28, ovalbumin bound with 0.3, 0.9, 3.1, 4.8, and 8.2 mol of linoleate/mol of protein, respectively. Interfacial tension: measured at corn oil/0.2% protein interface with a Fisher Surface Tensiomat Model 21. Emulsifying activity index: calculated from the absorbance at 500 nm of the supernatant after centrifuging blended mixtures of 2 mL of corn oil and 6 mL of 0.5% protein in 0.01 M phosphate buffer, pH 7.4. S_0: initial slope of fluorescence intensity (FI) vs. percent protein plot. 10 μL of 3.6 mM *cis*-parinaric acid solution was added to 2 mL of 0.002–0.1% protein in 0.01 M phosphate buffer, pH 7.4, containing 0.002% $NaDodSO_4$. FI was measured at 420 nm by exciting at 325 nm [adapted from Kato and Nakai (1980)].

The surface hydrophobicity index S_0 was also correlated with the emulsifying capacity of heat-denatured proteins (Voutsinas et al., 1983a). As solubility was progressively decreased by heat denaturation, the solubility became an increasingly important controlling factor for emulsification in addition to hydrophobicity. This implies that both hydrophobicity andd solubility should be taken into consideration to explain the emulsifying phenomena of heat-denatured protein (Table I).

A simple turbidimetric method for determining the fat binding capacity was developed (Voutsinas and Nakai, 1983). By use of this method, a close relationship between fat binding capacity and surface hydrophobicity was also observed (Table I).

Foaming Ability. It is commonly accepted that good foaming agents must have a mixture of hydrophilic and

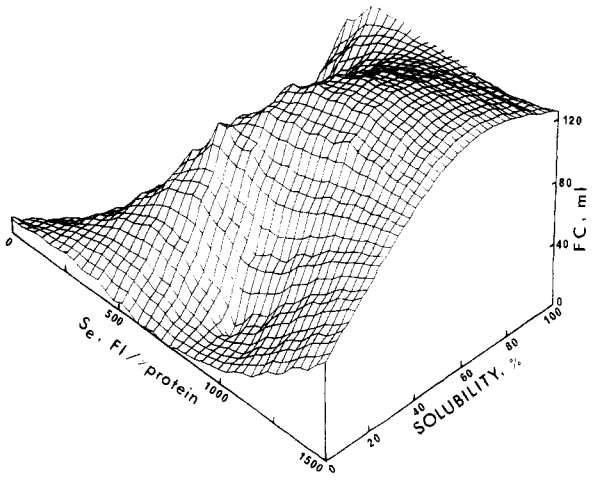

Figure 2. Relationship of hydrophobicity and solubility with foaming capacity of proteins. Proteins: bovine serum albumin, ovalbumin, lysozyme, β-lactoglobulin, ovomucoid, trypsin, ribonuclease A, conalbumin, β-casein, and κ-casein. S_e: S_0 measured after 1.5% protein solutions with 1.5% $NaDodSO_4$ were heated for 10 min in boiling water and diluted. Solubility: percent Kjeldahl N of the supernatant in total N after centrifuging 1% protein in 0.1 M phosphate buffer, pH 7.0, stirred on a magnetic stirrer for 10 min. Foaming capacity: volume (mL) of foam from 15 mL of 0.1% protein solution by air sparging through a sintered glass disk. The three-dimensional plot was drawn by an Amdahl 470 V/8 computer by using the UBC SURFACE program (Townsend and Nakal, 1983).

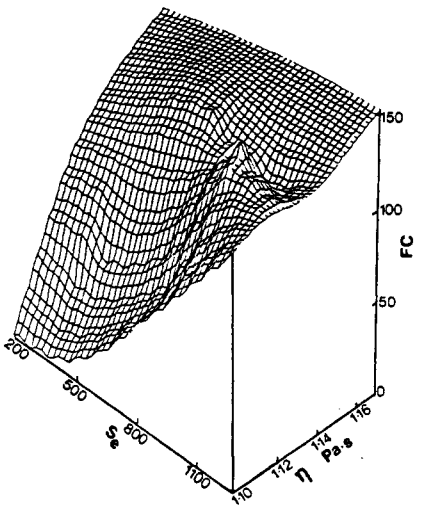

Figure 3. Relationship of hydrophobicity and viscosity to foaming capacity of proteins. Viscosity: measured by using an Ostwald viscometer (Townsend and Nakai, 1983).

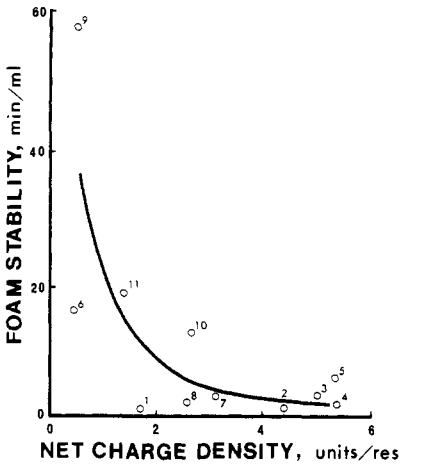

Figure 4. Relationship between net charge density and foam stability of proteins. 1, ribonuclease A; 2, ovomucoid; 3, trypsin; 4, lysozyme; 5, pepsin; 6, conalbumin; 7, ovalbumin; 8, bovine serum albumin; 9, κ-casein; 10, β-lactoglobulin; 11, β-casein. Foam stability: $2t/(50V_m)$, where V_m is the maximum volume (mL) and t is the time (min) required for the foam to collapse to $V_m/2$. Net charge density: measured by hydrogen ion titration of 0.5% protein in 6 M guanidine solution (Townsend and Nakai, 1983).

hydrophobic groups in their molecules (Cherry and McWatters, 1981; Rosen, 1972). According to Phillips (1981) the dynamic dilatational modulus

$$\epsilon = -A(d\pi/A) \quad (1)$$

which is the change in the surface pressure ($d\pi$) caused by a relative change in surface area (dA/A) is important for foamability as well as foam stability. Since the surface pressure is the decrease in surface tension produced by adsorbed protein films at the surface and the adsorption is due to their amphiphilic nature, a close relation between the hydrophobicity and foaming ability of proteins can be expected. Horiuchi et al. (1978) were able to correlate the foam stability of five enzyme-hydrolyzed proteins with the content of surface hydrophobic regions of the molecules which was fluorometrically measured using Ans as a hydrophobic probe.

Several attempts, however, to correlate protein surface hydrophobicity with foaming capacity of protein have been unsuccessful (Townsend and Nakai, 1983). Alternatively, it was found that the Bigelow hydrophobicity significantly ($P < 0.01$) correlated with foaming capacity. The $H\phi_{av}$ values of Bigelow were experimentally reproduced by exposing proteins to intensive dissociating conditions, i.e., heating a 1.5% protein solution at 100 °C for 10 min in the presence of 1.5% sodium dodecyl sulfate ($NaDodSO_4$), prior to the application of the fluorescence probe method for hydrophobicity determination.

Three-dimensional graphics demonstrates the relationships of hydrophobicity and solubility (Figure 2) and of hydrophobicity and viscosity (Figure 3) with foaming capacity of proteins. The normalized regression coefficient indicated that the degree of contribution to foaming capacity was viscosity, hydrophobicity (S_e), and solubility, in descending order.

There is a significant negative relationship between foam stability and net charge density (Figure 4). As the net electrical charge on protein molcule increases, the electrical potential barrier to surface adsorption is intensified. In order to adsorb, a charged molecule must do work equal to $\int_0^{\psi} q\, d\psi$, where q is the charge on the molecule and ψ is the electrical potential in the plane of the charged groups at the interface (MacRitchie, 1978). Hydrophobicity and viscosity may also play roles in foam stability since the correlation coefficients were significant ($P < 0.01$) but only when they were correlated with foam stability independently.

Kato et al. (1981) observed an increase in surface hydrophobicity and a decrease in surface tension upon heat denaturation of ovalbumin and lysozyme under the conditions that did not coagulate the proteins. As a result, the emulsifying and foaming capacities and stabilities were all considerably improved. These results suggest that there is a similarity in the mechanisms between surface denaturation and heat denaturation. The difference in the reaction conditions for measuring hydrophobicity to elucidate the emulsifying and foaming capacities of protein is indicative of more extensive uncoiling of the protein molecules at an air/water interface than an oil/water interface. This may be related to the fact that tension at the air/water interface (73 dyn/cm) is far greater than that

Table II. Relationships between Hydrophobicity and Thermal Functional Properties of Eight Food Proteins[a]

gelation = $-6.54 + 0.0065S_e + 0.012[SH]^2$ ($R^2 = 0.740, P < 0.05$)
gelation = $-7.20 + 0.006S_e + 0.022[TSH]$ ($R^2 = 0.810, P < 0.05$)
thickening = $-202.7 + 0.75S_e + 0.016S_e[SH] - 0.001S_e^2$ ($R^2 = 0.961, P < 0.01$)
thickening = $2.47 + 0.35S_e - 2.29[TSH] - 0.0036S_e[TSH] - 0.0005S_e^2 + 0.0043[TSH]^2$ ($R^2 = 0.995, P < 0.01$)
heat coagulation = $-5.62 - 2.50[SH] + 0.009S_e[SH]$ ($R^2 = 0.740, P < 0.05$)

[a] TSH = SH plus reduced SS. Gelation: the score visually judged on the rating scale −4 to +3 after 10% protein solutions are heated at 100 °C for 40 min. Thickening: viscosity difference (mPa s) after heating 8% protein solutions at 90 °C for 5 min and before heating. Heat coagulation: sedimentable protein by centrifugation (%) vs. soluble protein in 5% protein suspensions determined after heating at 100 °C for 30 min. Backward stepwise multiple regression analysis was carried out on an Amdahl 470 V/8 computer by using the UBC Triangular Regression Package (Voutsinas et al., 1983b).

at the oil/water interface (13–19 dyn/cm). These results are in good agreement with those reported by Graham and Phillips (1979).

Thermal Functional Properties. A marked increase in the effective hydrophobicity was observed upon heating of proteins, indicating unfolding of the molecules. When too many hydrophobic sites are exposed due to unfolding, protein solubility usually decreases, then hydrophobic interactions are actuated between the exposed hydrophobic sites causing aggregation of the protein molecules. This may be a mechanism of heat coagulation. However, covalent cross-linking formation, e.g., disulfide linkage formation and sulfhydryl–disulfide exchange reaction, may also be involved. Shimada and Matsushita (1980), based on turbidity studies, reported that the mechanism of protein thermocoagulation is largely dependent on hydrophobic interactions among proteins. The hydrophobicity they used was calculated from the contents of hydrophobic amino acid residues in the proteins. Ishino and Okamoto (1975) worked on alkali-treated soybean proteins and interpreted their observations to be indicative of the involvement of hydrogen, hydrophobic, and disulfide bonds in gelation. They used the heptane binding method of Mohammadzadeh et al. (1969) for determining exposed hydrophobic regions of the proteins.

Relationships between protein hydrophobicity and thermal functional properties of eight food proteins were investigated (Voutsinas et al., 1983b). The exposed hydrophobicity S_e was more closely related to the thermal properties than the surface hydrophobicity S_0. Significant correlations ($P < 0.05$) were observed for gelation with S_e and SH groups or SH plus reduced SS groups, respectively (Table II). Similar relationships were observed for thickening of the proteins upon heating. Heat coagulability was also significantly ($P < 0.05$) correlated with S_e and SH groups.

Hydrophobicity–HLB–Polarity Relationship. The term "polarity" has been loosely defined. According to Reichardt (1979), polarity is the sum of the permanent dipole moment and dielectric constant of a compound which is responsible for the interaction forces between solvent and solute molecules. An important aspect of the solvent polarity is its overall solvation ability. Many empirical parameters have been submitted to quantify the solvent polarity.

The hydrophile–lipophile balance (HLB) value of surfactants is a useful index for the systematic approach to emulsifier selection. Gas chromatography has been used for comparing the retention index and the HLB value of surfactants. As seen in Figure 5, good correlations are observed between log ρ and HLB (Olano and Martinez, 1975). Since the value ρ is an index of polarity, it is reasonable to state that there is a good correlation between the HLB values and the polarity of surfactants.

Meanwhile, the hydrophobicities of amino acids were correlated with their polarity and expressed as a retention coefficient from a reverse-phase liquid chromatograph

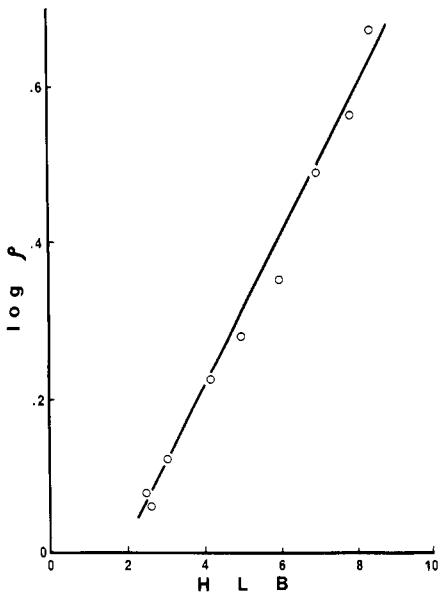

Figure 5. Relationship between retension time ratio of methanol to hexane and HLB of surfactants. ρ: relative retention time, $(R_{EtOH} - R_{air})/(R_{hexane} - R_{air})$ [adapted from Olano and Martines (1975)].

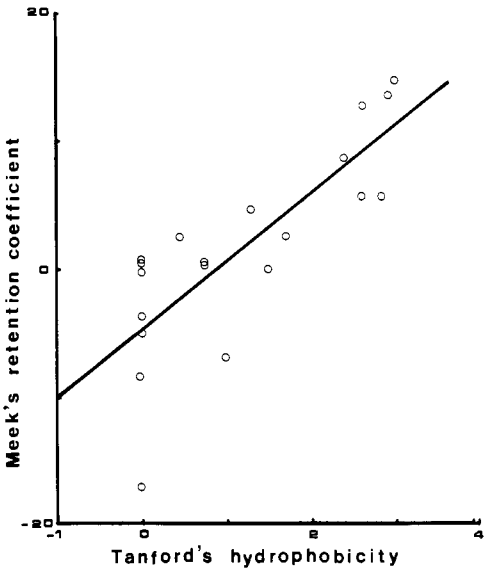

Figure 6. Relationship between hydrophobicity and retention coefficient of amino acids. Retention coefficient: index representing the contribution to relation of each of the common amino acids and end groups. Regression equation: $y = 5.31x - 4.53$ ($r = 0.798, P < 0.01$) [adapted from Meek (1980)].

column as shown in Figure 6 (Meek, 1980). For peptides containing up to 20 amino acid residues, the retention time could be predicted solely on the basis of their amino acid composition (Figure 7). Some steric or conformational effects were observed (Meek, 1980). Therefore, there are reasons to believe the existence of similarity between

Table III. Relationship among Hydrophobicity, HLB, and Polarity

hydrophobicity (protein)	HLB (surfactant)	polarity[a] (chromatography)
$\frac{[\text{hydrophobic AA}^b]}{[\text{hydrophobic AA}] + [\text{hydrophilic AA}]}$	$\frac{[\text{hydrophile}]}{[\text{hydrophile}] + [\text{lipophile}]}$	$p' = \log (K_g'')_{EtOH} + \log (K_g'')_{dioxane} + \log (K_g'')_{nitromethane}$ where K_g'' = solute solubility constant corrected for M_r
$\frac{[\text{nonpolar}]}{[\text{nonpolar}] + [\text{polar}]}$	$\frac{[\text{polar}]}{[\text{nonpolar}] + [\text{polar}]}$	for nonpolar, $p' = 0$ for polar, $p' = 10$

[a] Polarity from Snyder (1978). [b] Amino acid residues.

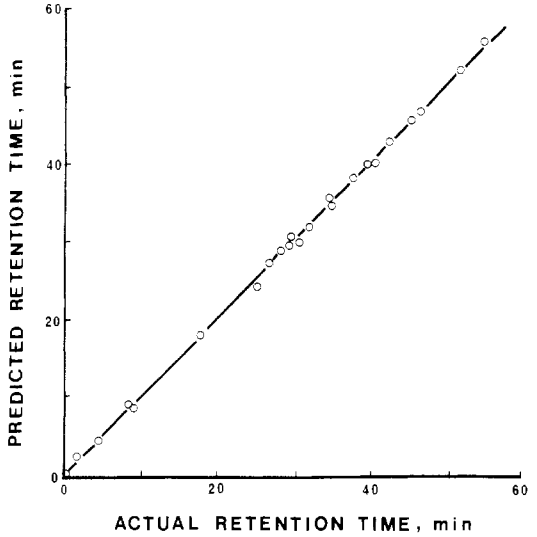

Figure 7. Relationship between actual retention times of peptides and times predicted by summing retention coefficients of the amino acids and end groups (pH 7.4) [adapted from Meek (1980)].

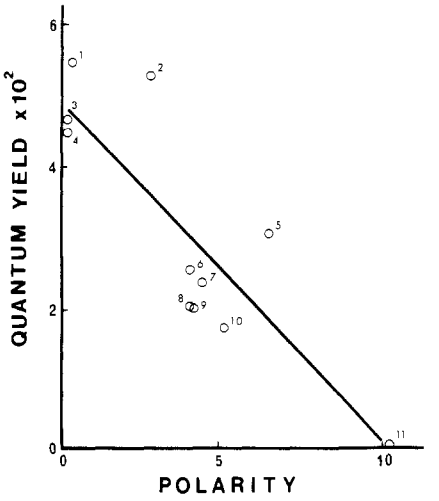

Figure 8. Relationship between *cis*-parinaric acid quantum yield and polarity of solvents. 1, decane; 2, ethyl ether; 3, hexane; 4, cyclohexane; 5, dimethylforamide; 6, butanol; 7, ethanol; 8, chloroform; 9, propanol; 10, methanol; 11, water. Quantum yield values from Sklar et al. (1977). Polarity values from Snyder (1978).

protein hydrophobicity and HLB, which are both based on polarity of the compounds.

It is noteworthy that as a peptide becomes larger in molecular size, the steric or conformational effect cannot be ignored as suggested in a concept of surface or effective hydrophobicity. The extent of unfolding of protein molecules at an interface becomes, therefore, critical, unlike the case of small molecular surfactants.

As seen in Figure 8, a significant correlation ($P < 0.01$) is observed between the quantum yield of *cis*-parinaric acid in different solvents and the polarity scale of the solvents. The empirical parameter p' of solvent polarity suggested by Snyder (1978) is defined tin Table III. It is reasonable, therefore, that the protein hydrophobicity measured by the fluorescence method using *cis*-parinaric acid as a hydrophobic probe has good correlations with emulsifying and foaming ability since hydrophobic proteins readily migrate to an interface, decreasing interfacial tension and facilitating emulsion or foam formation.

The well-known rule of "like dissolves like" is the basis of the polarity of solvents, i.e., solvent strength. For the highest affinity, in other words, for the best dissolution, the polarity of the solute should match the polarity of the solvent. The polarity p', in addition to selectivity (x_e, x_d, and x_n for ethanol, dioxane and nitromethane, respectively) relating to the chemical structure of the solvent, is an important factor for chromatographic separation (Snyder, 1978). This explains reasons why there is an optimum protein hydrophobicity for a functionality instead of a simple linear relation between them, i.e., the higher the hydrophobicity the better the functionality. This situation is quite similar to the optimum HLB value of surfactants, i.e., the HLB value of the surfactant should match the required HLB value of fat.

Conceptual similarity among protein hydrophobicity, surfactant HLB value and polarity in partition chromatography is summarized in Table III. Polar and nonpolar compounds or radicals are defined based on the size of polarity values. Protein hydrophobicity and emulsifier HLB are defined as the proportion of nonpolar side chains and polar radicals in the molecules of protein and emulsifier, respectively.

Secondary Structure of Protein Molecules. The variability of physicochemical and functional properties of proteins can be ascribed to differences in the structure of protein molecules that are caused by a variety of their building blocks, i.e., 20 different amino acids. This is one reason for the unique functions of proteins and enzymes, which are different from other macromolecular compounds, e.g., starch of which the unit compound is a single glucose.

Molecular flexibility must be important for protein function especially at an interface, e.g., as an emulsifier or foaming agent. Chemical forces are involved in the rigidity of protein molecules as well as in interaction with other protein molecules or small molecular ligands.

Graham and Phillips (1976) ascribed the ready foaming of β-casein to a flexible random coil structure compared to bovine serum albumin and lysozyme being more rigid in structure and thus less effective at stabilizing air bubbles. Morr (1979) similarly ascribed the better emulsifying capacity of casein than whey proteins to greater flexibility of casein molecules.

Hydrophobic, electrostatic and steric parameters are three important parameters that describe molecules. Attempts have been made, based on these parameters, to compute the prediction formula for elucidating the functional mechanisms of many chemical compounds, e.g., drug and olfactory stimulants (Stuper et al., 1979). A similar attempt was made to elucidate the action of enkephalin-like peptides on opiate receptors (Do et al., 1981). The importance of the charge frequency of protein for foam

stability was stated by Townsend and Nakai (1983). During the study of effects of heating lysozyme and ovalbumin, Kato et al. (1981) found that the helix content, measured as elipticity $[\Theta]_{222nm}$ of the proteins, linearly correlated with S_0, indicating a close relationship between the structure and the extent of exposure of hydrophobic sites of the protein molecules which in turn improved the emulsifying and foaming ability of the proteins.

Although X-ray crystallography is the best method for obtaining three-dimensional structure information on proteins, many food proteins do not crystallize; therefore, this technique is not applicable. As a substitute, a computer program was written (Pham, 1981) for predicting the secondary structure of protein, i.e., α-helix, β-sheet, β-turns, and random structure, from their amino acid sequence according to the rule of Chou and Fasman (1978).

The rule of Chou and Fasman was selected mainly because of its simplicity; thus, it is easier to revise or modify the computer program when necessary in the future. There are more complicated programs for prediction of the structure of proteins. Momany et al. (1975) used empirical interatomic potentials, i.e., the potentials of geometric, nonbonded interaction, hydrogen bond, and intrinsic torsional, for calculating the energetically most favored conformation. On the other hand, Nagano (1977) tested the probability of the appearance of two amino acid residues r and r' which were separated by m residues for possible secondary structure using χ^2 test.

According to Geisow and Roberts (1980), if it is feasible to assign a protein to one of α-helical, all β, and mixed (α/β) protein classes on the evidence of circular dichroism (CD) spectra or low-resolution X-ray analysis, more accurate prediction of secondary structure can be obtained.

Higher Order Structures of Protein Molecules. A possible criticism against the secondary structure study is that the steric parameters, which better correlate to the functionality, should be of higher order (tertiary and quaternary) than the secondary structure. Several computer programs have been published for predicting tertiary structure (Geol and Ycas, 1979; Kuntz et al., 1976).

Effect of local hydrophobicity to stabilize protein secondary structure was stated by Kanehisa and Tsong (1980). To improve the efficiency of the prediction using the rule of Chou and Fasman, the distribution of the hydrophobic residues was incorporated into the rule (Busetta and Hospital, 1982). The average affinity of each amino acid side chain group was determined in an attempt to utilize for construction of the side chain interaction within the tertiary structure of protein (Warme and Morgan, 1978). The importance of chemical forces for studying protein structure should be emphasized.

Chemical Forces. A recent review written by Ross and Subramanian (1981) on thermodynamic parameters characterizing self-association and ligand binding of protein concluded that (a) the only contributions to positive entropy and enthalpy changes arise from ionic and hydrophobic interactions and (b) the only sources of negative enthalpy and entropy changes arise from van der Waals interactions and hydrogen-bond formation. They concluded also that $\Delta G°$, $\Delta H°$, $\Delta S°$, and ΔC_p are often all of negative sign and, therefore, it is not possible to account for the stability of an association complex of protein on the basis of hydrophobic interactions alone. The importance of hydrogen bonds and van der Waals interactions contributing to the stability of a protein association complex was described.

Barford et al. (1982) discussed the mechanism of protein retention in reverse-phase high-performance liquid chromatography and confirmed the theory of van Oss et al. (1979). According to them, the protein retention by alkylsilicas may be explained by van der Waals forces. van Oss et al. (1979) defined the hydrophobic effect as an interaction, frequently through van der Waals attractions, between macromolecules with low surface energy sites when immersed in a high-energy liquid such as water. There is a similarity between the hydrophobic effect defined by Hildebrand (1979) and the polarity defined by Snyder (1978). The hydrophobic effect is a result of deactivation or destruction of hydrogen bonds, whereas polarity is a relative ability to engage in hydrogen bonding or dipole interactions. According to Wolf (1969), groups increasing polarity are ionized groups and groups capable of entering into hydrogen bonding.

Considering all arguments and situations, an interpretation by van Oss et al. (1979) appears reasonable since this hypothesis can conveniently explain the protein hydrophobicity–HLB–polarity relationship suggested in this article. According to them, the hydrophobic effect can be explained by a relative balance between repulsive and attractive effects of van der Waals interactions, which are dependent on the size of Hamaker coefficients of solutes and solvents.

Since noncovalent foreces are difficult to measure and even more difficult to calculate, hydrophobicity measurement can be useful for explanation of the mechanism of food protein functionality mainly due to its relative simplicity. However, similar to the situation in polarity measurement, ambiguity in defining what is really analyzed by the present hydrophobicity measurement will remain.

Modification of Proteins. It is theoretically feasible to modify food proteins chemically or enzymatically to alter the charge frequency, hydrophobicity, and, furthermore, their structure, thus changing the functionality of the proteins (Table IV). However, the effects of these modifications on the steric parameters have not been fully investigated. It would be useful if a prediction program similar to the ones stated above could predict the possible changes of protein structure as a result of chemical or enzymatic modification.

One example is milk clotting enzymes. About half of the amino acid sequences are identical in calf chymosin and porcine pepsin; however, the predicted secondary structures are considerably different (Figure 9). Meanwhile, X-ray crystallography has shown that all carboxyl proteinases have great similarities in the folding of their peptide chains (Foltman, 1981). Prediction programs may be able to detect what differences in the sequence cause these structural differences. These studies will lead to the manipulation of chemical and enzymatic modification to alter or improve the function of proteins and enzymes in the future. A sequence deletion technique was applied to glucagon to investigate its structure–function relationships (Frandsen et al., 1981).

Statistical Consideration. Data handling techniques are very important after obtaining results from analysis. Even though expensive and sophisticated instruments are used, without proper data handling we would miss important information or simply waste valuable information.

As the number of structure-relating data (predictor variables) available increases by using modern analytical instruments, new problems arise in correlating these variables to the function-relating parameters. Although multiple regression analysis has been frequently used for this purpose, it is well documented that the accuracy of the correlation coefficients calculated quickly decreases when the predictor variables are highly correlated. A va-

Table IV. Chemical and Enzymatic Modifications To Alter Charge, Hydrophobic, and Steric Parameters of Proteins

to change the charge frequency
  (a) to increase the net negative charge
    acylation, e.g., succinylation
    alkylation, e.g., acetylation
    hydrolysis of amide groups
    plastein or chemical reaction to bind acidic amino acids
  (b) to increase the net positive charge
    plastein or chemical reaction to bind basic amino acids or amines
to change the hydrophobicity
  attaching fatty acids (Torchilin et al., 1980; Haque et al., 1982)
  alkylation with hydrophobic aldehydes
  reductive alkylation
  plastein or chemical reaction to bind hydrophobic amino acids (Watanabe et al., 1981)
  surfactant treatment (Nakai et al., 1980)
  alcohol modification (Aoki et al., 1981)
to change the steric parameters
  blocking, oxidation, and attachment of SH groups (Benesch and Benesch, 1958)
  promoting SH-SS interchange reaction
  cross-linking formation, e.g., reacting with disuccinimidyl tartrate (Smith et al., 1978)
  peptide bond cleavage

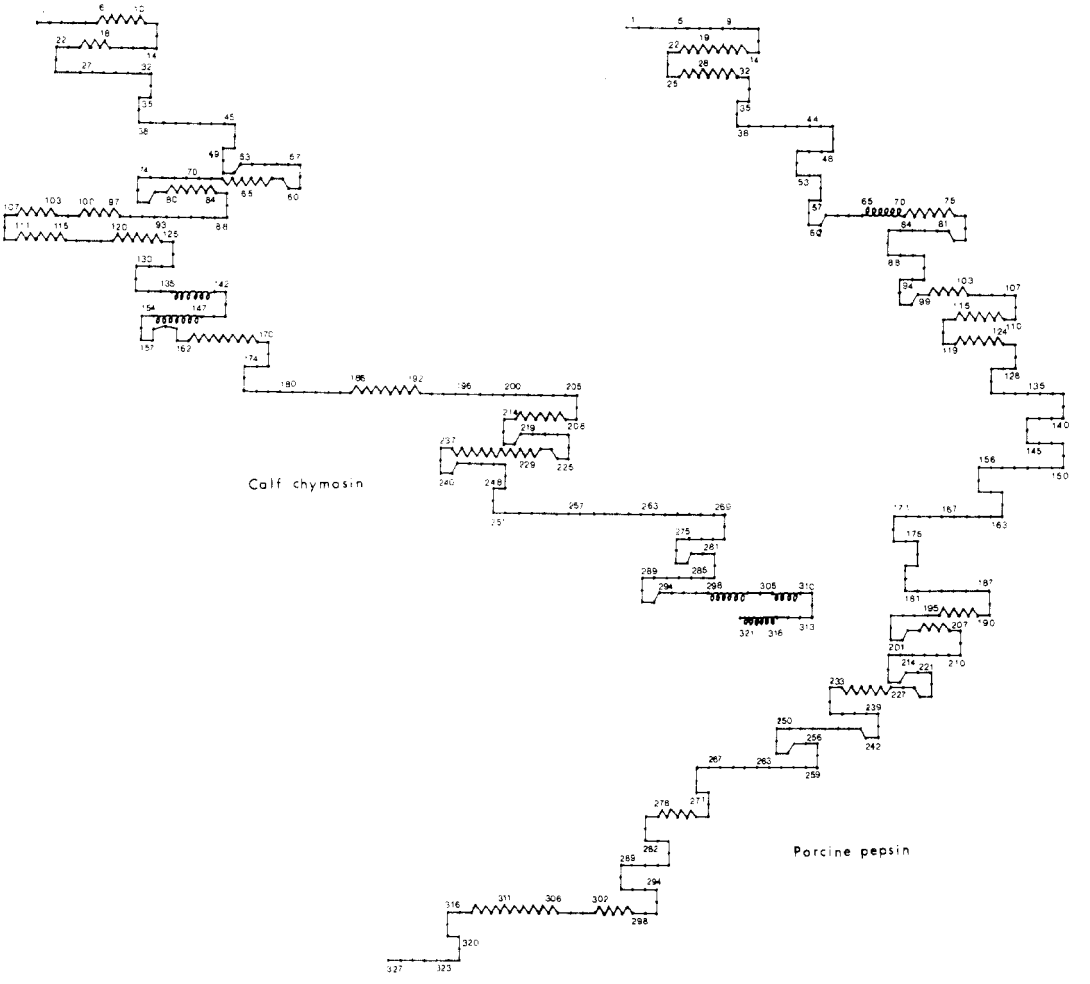

Figure 9. Schematic diagram of predicted secondary structure of porcine pepsin and calf chymosin (Pham, 1981).

riety of mathematical strategies were discussed by Stuper et al. (1979) for computer-assisted study on chemical structure and biological function. The important algorithms used are multivariate analysis. According to Kendal (1980), multivariate analysis finds simpler ways of representing the complex under study by transforming a set of "interdependent" variables to "independent" variables or reducing the dimensionality of a complex. Cluster analysis, factor analysis (principal component analysis), and linear discriminant analysis, of which the basic algorithm is eigenvector analysis, may be most useful for the study of structure–function relationships of food proteins.

Considering the large number of parameter data possibly available in the future, the need for selecting the best sample from various protein samples for certain functional purposes should make these data processing techniques useful.

Conclusion. (1) By use of hydrophobic parameters in addition to solubility, better correlations with emulsifying and foaming ability of proteins were obtained. (2) For thermal functional properties of protein, SH and SS group contents were required for good correlations in addition to the hydrophobicity data. (3) The extent of molecular unfolding was crucial for protein molecules since it might

have affected the availability of hydrophobic sites. (4) Protein hydrophobicity, HLB value of surfactants, and polarity of partition chromatography seem to be closely related and the major forces involved are hydrogen bonds, van der Waals forces, and probably electrostatic forces. (5) With an assistance of more accurate information on protein structure in the future, the function of proteins and enzymes may be elucidated on the basis of hydrophobic, electrostatic, and steric parameters. (6) Statistical data processing techniques would certainly enhance their roles in this structure–function relation study in the future.

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