Chapter 4

Quantitation of Hydrophobicity for Elucidating the Structure–Activity Relationships of Food Proteins

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Hydrophobicity is a major structural variable used for predicting the functionality of food proteins, such as emulsification and foaming abilities. However, the published data for protein hydrophobicity often show inconsistencies, due to differences in the principles underlying the various methods currently employed for quantitation of hydrophobicity. New approaches using nuclear magnetic resonance and Raman spectrophotometry are proposed which may help to clarify the definition of "surface" or "available" hydrophobicity of proteins important for function, and should be investigated for quantitating the extent of exposure of aliphatic and aromatic hydrophobic side chains of protein molecules. Once reliable and quantitative measurements of protein hydrophobicity are obtained, these parameters may be incorporated into QSAR equations, for use in computer-aided optimization of food formulations.

Quantitative structure-activity relationship (QSAR) techniques use molecular structure and physical property data to make predictions about activity or reactivity of compounds. These techniques have gained wide acceptance and application especially in toxicological or pharmacological research. Various molecular structure and property descriptors are used for the formulation of QSAR. For example, hydrophobicity, topological descriptors, electronic descriptors and steric effects have been suggested to predict therapeutic response or toxicity of chemicals. Polarity has also been a commonly used concept in the field of chemistry to explain behavior such as solubility of compounds and the mechanism of chromatographic behavior. The terms "polarity" and "hydrophobicity" are commonly used as antonyms, and the term "hydrophobic" is often synonymously used with "lipophilic" or "nonpolar".

0097–6156/91/0454–0042$06.00/0

For small compounds, relative solubility in a nonpolar solvent versus a polar solvent is commonly used as an indication of hydrophobicity. For example, octanol/water partition coefficients are often measured as a hydrophobicity parameter. In the case of proteins, which are macromolecules, solubility has been postulated to be one of the most important factors in functionality. Therefore, polarity or hydrophobicity must also have an important role in food protein functionality. However, despite the increasing recognition of its importance in protein function, the quantitation of hydrophobicity for use as a descriptive parameter in QSAR equations is not as straightforward for proteins as it is for small molecules. The difficulty arises from the lack of sound theoretical rules to define a hydrophobicity parameter which considers the influences of steric effects of protein structure.

The objectives of this chapter are to discuss the important role of hydrophobicity in elucidating the structure-function relationships of food proteins, to review current trends and propose new approaches in quantitating protein hydrophobicity for QSAR, and to illustrate the potential benefits of applying the derived QSAR equations for optimization of food formulations.

Selection of Important Descriptors for QSAR of Food Proteins

Charge, hydrophobicity and steric parameters were originally proposed as the three major classes of descriptors for QSAR investigation for smaller compounds, as described by Hansch and Clayton (1) and Rekker (2). These parameters include measurement or calculation of Hammett constants, dipole moments, molar refractivities and ionization potentials as electronic descriptors; octanol/water partition coefficients as hydrophobicity descriptors; and Taft constants, van der Waals radii and total surface area as steric descriptors.

The importance of the three major classes of descriptors also extends to QSAR for elucidating functionality of proteins in food systems, such as emulsifying and foaming ability and stability, gelation, coagulation, film formation, water and fat binding properties. However, the traditional types of parameters mentioned above have not been used to measure these descriptors due to the complexity of food proteins in terms of co-existence of several types of proteins and changes induced upon processing. Instead, simpler alternatives have been sought which give empirical measures of parameters related to charge, hydrophobicity and steric effects. For example, solubility is recognized in the food industry as one of the most influential properties of a protein molecule which affects its other functions. In fact, solubility is a reflection of the balance of charge and hydrophobicity of the protein molecule (3, 4) which affects its interaction with the solvent (→ "soluble") and with other protein molecules (→ "insoluble"). Measurement of solubility thus indirectly provides a descriptor for QSAR which incorporates both charge and hydrophobicity effects. Other examples of parameters used to predict food protein functionality include viscosity to reflect steric effects and intermolecular interactions, and sulfhydryl and disulfide group contents to give an indication of molecular flexibility or ability for crosslink formation.

Table I shows examples of equations describing the relationships between physicochemical descriptors (solubility, hydrophobicity, viscosity, sulfhydryl or disulfide group content) and functionality (emulsifying ability, foaming capacity, thermally induced coagulation and gelation, and fat binding capacity) of some food proteins (5-8). As shown in these equations, hydrophobicity is an important parameter to explain diverse functional properties of food proteins. Various parameters have been used to represent hydrophobicity in these equations (ANS, CPA, $CPAS_e$, and $H\phi_{avg}$) and different parameters have been used by other workers in the area for correlation to functionality (9). In most cases, these parameters measure hydrophobicity of protein molecules in dilute solutions. Whether or not the measurement of hydrophobicity of proteins at concentrations typically encountered in food applications would improve the accuracy of equations for QSAR elucidation is not clear. However, comparison of the equations for predicting coagulability and gel strength of ovalbumin solutions at 0.5 and 5.0% concentration, respectively, shows that the relative importance of various physicochemical parameters to describe functionality is concentration dependent. ANS hydrophobicity and charge frequency expressed as zeta potential (ZP) were found to be important in predicting coagulability upon heating of 0.5% ovalbumin solutions, whereas ANS hydrophobicity and sulfhydryl (SH) group content were significant in explaining gel strength of 5% heated solutions (8). Similarly, emulsifying activity of soluble proteins was dependent on the concentration of the protein solution, as shown in Figure 1 (10). At protein concentrations approaching zero, emulsifying activity expressed as turbidity of the emulsion, A_{500}, was correlated with CPA hydrophobicity of the proteins:

$$A_{500} = 0.387 + 8.391\ CPA \qquad (r=0.932,\ P<0.05).$$

However, at protein concentrations of 2.5% or greater, a parameter describing the complex interaction of CPA hydrophobicity with viscosity (η_0) of the continuous phase became a significant factor in emulsion formation:

$$A_{500} = 0.382 + 16.52\ \eta_0^2 CPA^{\frac{1}{2}} \qquad (r=0.922,\ P<0.05).$$

For application of QSAR equations to explain and predict functionality of food proteins, the quantitative measurement of relevant physicochemical parameters is crucial. Of the various physicochemical parameters identified to be important in explaining food protein functionality, methods have been well established to measure most of them, such as solubility, viscosity and sulfhydryl or disulfide groups. However, consensus has not yet been reached on a method for measuring hydrophobicity of a protein which can explain functionality.

Comparison of Current Methods for Measuring Protein Hydrophobicity

Methods proposed for quantitative estimation of protein hydrophobicity can be roughly categorized into (1) calculated values using data of hydrophobicity scales of the individual amino acids

Table I. Examples of regression equations reported in the literature describing the relationships between physicochemical descriptors and functionality of some food proteins

Functional Property	Regression Equation	Reference
Emulsifying Activity Index (EAI) of native and heated proteins	$EAI = 16.9 + 0.21CPA + 0.93s - 0.007s^2$ ($n=52$, $R^2=0.583$, $P<0.001$)	Voutsinas et al. (5)
Emulsion Stability Index (ESI) of native and heated proteins	$ESI = -69.5 + 0.565CPA + 2.03s - 0.004CPA\ s - 0.012s^2$ ($n=49$, $R^2=0.584$, $P<0.001$)	Voutsinas et al. (5)
Fat Binding Capacity (FBC) of native and heated proteins	$FBC = 4.90 + 0.45CPA + 1.40s - 0.001CPA^2 - 0.014s^2$ ($n=48$, $R^2=0.473$, $P<0.001$)	Voutsinas et al. (5)
Foaming Capacity (FC) of native proteins	$\ln(FC+30) = 0.039 + 0.0041H\emptyset_{avg}$ ($n=11$, $r^2=0.677$, $P<0.01$) or $FC = -1775 + 0.1493\eta + 25.93\ \ln S_e$ ($n=19$, $R^2=0.779$, $P<0.01$)	Townsend & Nakai (6)
Water Absorption (AM_b) of minced meat in brine	$AM_b = -0.26 + 0.0021CPA - 0.0000017CPA^2$ ($n=58$, $R^2=0.439$, $P<0.001$)	Li-Chan et al. (7)
Coagulability (C) of native and heated 0.5% ovalbumin solutions	$C = -4.77 + 0.476ANS - 0.000404ANS^2 - 0.0137ANS\ ZP$ ($n=26$, $R^2=0.794$, $P<0.001$)	Hayakawa & Nakai (8)
Gel Strength (G) of native and heated 5.0% ovalbumin solutions	$G = 821 - 0.0628ANS - 8.91SH$ ($n=26$, $R^2=0.621$, $P<0.001$)	Hayakawa & Nakai (8)

Abbreviations:
ANS = hydrophobicity determined using 1-anilinonaphthalene-8-sulfonic acid.
CPA = hydrophobicity determined using cis-parinaric acid.
$CPAS_e$ = hydrophobicity determined using cis-parinaric acid after first treating the protein solution by heating in the presence of sodium dodecyl sulfate (6).
$H\emptyset_{avg}$ = average hydrophobicity value calculated according to the method of Bigelow (3).
η = viscosity.
SH = sulfhydryl group content.
ZP = net charge determined as zeta potential.

In Interactions of Food Proteins; Parris, Nicholas, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1991.

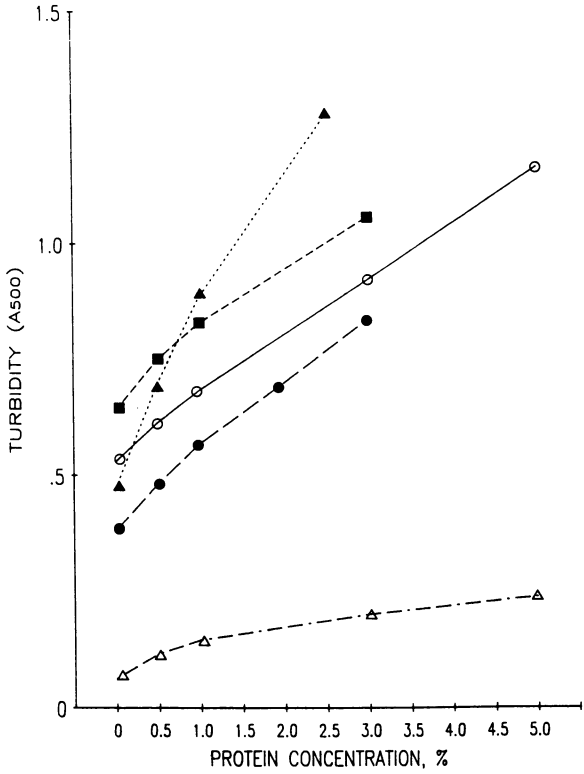

Figure 1. Effect of protein concentration on emulsifying activity. ○, bovine serum albumin; △, ovalbumin; ●, casein; ▲, gelatin; ■, β-lactoglobulin.

and the amino acid composition of the protein; (2) partition methods, including relative solubility in polar and nonpolar solvents, or relative retention on reverse phase or hydrophobic interaction chromatography; (3) binding methods, including the binding of aliphatic and aromatic hydrocarbons, sodium dodecyl sulfate, simple triglycerides and corn oil; (4) contact angle measurement; and (5) spectroscopic methods, including intrinsic fluorescence, derivative spectroscopy, and use of fluorescence probes. These methods have been recently reviewed (9, 11), and some of the published data for protein hydrophobicity using different methods are compared in Table II.

Considerable variations are observed for the values obtained by different methods, as shown in Table II. Some inconsistencies in these values could be attributed to the fact that the sample proteins used for analyses may not have been from the same source, or that the conditions such as pH, ionic strength or concentration were not necessarily the same. Nevertheless, dramatic differences are apparent. For example, large values for hydrophobicity were reported for both chicken albumin and lysozyme when measured by hydrophobic interaction chromatography and for chicken albumin by calculation of average hydrophobicity, compared to the small values obtained by the fluorescence probe, binding and partition methods. It is probable that differences in principles underlying the various methods of measuring hydrophobicity are the cause of the significant variation in the reported values.

Even for methods within a class, such as reverse phase (RP) and hydrophobic interaction chromatography (HIC), discrepancies have been observed. For example, there was no correlation between the retention times of 12 proteins eluted by RP and HIC (12). Recently, anomalous elution behavior of proteins on HIC columns was explained by salt binding, which may alter the number and distribution of protein surface groups, including charged groups (13). Steadman et al. (14) stated that it is important to distinguish between those methods which estimate hydrophobicity by measuring aggregation from those relying on differential solubility as well as those estimating the adherence of substances. According to the Gibbs adsorption isotherm, the adsorption of solutes is negatively correlated to the difference in surface tension between solute and solvent, and the polarity of the solute seriously affects this difference. It is reasonable therefore to assume that hydrophobic attraction plays a major role in adsorption chromatography.

Wilson et al. (15) investigated RP chromatographic behavior of 96 peptides, ranging in length from 2 to 65 residues. Hydrophobic constants of amino acid residues were computed from the retention properties of these peptides, and these constants were compared with hydrophobic constants published in the literature obtained by other methods. Correlation coefficients between these constants and peptide retention times were also computed. Table III shows that the best correlation was obtained for the constants derived by RP chromatography by Wilson et al. (15). It was thus concluded that chromatographic behavior of peptides could be explained based on polarity of the constituent amino acid residues.

Although chromatographic behavior of peptides could be successfully predicted, the situation is not as straightforward for

Table II. Relative hydrophobicity values of some proteins measured by different methods [a]
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Proteins	$H\emptyset_{ave}$	fluorescence probes			DPH probe	chromatography		binding			partition
		S_0ANS	S_0CPA	S_eCPA	TG binding	RPC	HIC	heptane	SDS	TG	Δlog K
albumin, bovine	1120	1000	100	100	100	100	100	100	100	100	100
albumin, chicken	1110	7	2	96	15	0	9-86	2	14	3	1-25
casein, α-	1200	57	6-30	/	150	400	/	/	/	160	/
casein, β-	1320	60	50	107	/	315	/	/	/	/	/
casein, κ-	1210	83	13-93	89	/	340	/	/	30	/	/
chymotrypsin, α-	1030	0	3	/	41	/	57-139	9	/	3	9-33
globulin, soy 7S	1090	47	9-77	/	50	165	/	/	21	/	/
globulin, soy 11S	950	27	2-17	/	25	150	/	/	/	/	/
lactalbumin, α-	1150	33	9-54	/	98	225	10	/	/	/	/
lactoglobulin, β-	1230	13	54-146	80	49	0	67-102	100	62	130	41-78
lysozyme	970	0.7	7	/	/	/	42-113	0	19	1	0
ovomucoid,chicken	920	/	0	22	/	/	/	24	18	108	/
pepsin	1063	0.7	2	57	32	/	92	/	/	95	/
ribonuclease A	870	/	1	24	/	/	4-71	17	18	2	/
transferrin,chicken	1080	/	4	108	/	/	16-31	29	20	118	9-20
trypsin	940	8	3	34	/	/	/	/	13	/	9-27
trypsin inhibitor	1040	0	0	/	1	0	/	/	/	/	/

[a]Adapted from ref 11. The majority of the data are expressed relative to "100" for bovine albumin to facilitate comparison. Where varying values were reported by investigators using essentially the same method, a range of values is presented.
[b] 530 and 800 for α- and β-casein, respectively, at the isoelectric pH.

Abbreviations: $H\emptyset_{ave}$=average hydrophobicity calculated by Bigelow's method (3); S_0=initial slope of relative fluorescence intensity versus protein concentration plot, using native proteins; S_e=S_0 measured for protein solutions after treatment with 1.5% SDS at 100°C for 10 min; ANS=1-anilinonaphthalene-8-sulfonic acid; CPA=cis-parinaric acid; DPH=1,6-diphenyl-1,3,5-hexatriene; TG=triglyceride; RPC=reverse phase chromatography; HIC=hydrophobic interaction chromatography; SDS=sodium dodecyl sulfate; /=no data available.

In Interactions of Food Proteins; Parris, Nicholas, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1991.

Publication Date: February 19, 1991 | doi: 10.1021/bk-1991-0454.ch004

Table III. Amino acid hydrophobicity constants and correlation with peptide chromatographic retention times (Adapted from Ref. 9. Reproduced with permission. Copyright 1988 CRC Press.)

	Amino Acid Hydrophobicity Constants					
amino acid	Bigelow-Chapman	Meek	Pliska-Fauchere	Rekker	Segrest-Feldman	Wilson et al.
Ala	0.5	-0.1	0.38	0.53	1.0	-0.3
Arg	0.75	-4.5	-1.23	-0.82	-	-1.1
Asn	-	-1.6	-0.27	-1.05	-1.5	-0.2
Asp	0.0	-2.8	-1.23	-0.02	-	-1.4
Cys	-	-2.2	-	1.11	0.0	6.3
Gln	-	-2.5	-0.09	-1.09	-1.0	-0.2
Glu	0.0	-7.5	-1.20	-0.07	-	0.0
Gly	0.0	-0.5	0.0	0.0	0.0	1.2
His	0.50	0.8	-1.3	-0.23	1.0	-1.3
Ile	2.95	11.8	1.56	1.99	5.0	4.3
Leu	1.80	10.0	1.66	1.99	3.5	6.6
Lys	1.50	-3.2	-0.93	-0.52	-	-3.6
Met	1.30	7.1	1.39	1.08	2.5	2.5
Phe	2.50	13.9	1.80	2.24	5.0	7.5
Pro	2.60	8.0	0.56	1.01	1.5	2.2
Ser	-0.30	-3.7	0.04	-0.56	-0.5	-0.6
Thr	0.40	1.5	-0.33	-0.26	0.5	-2.2
Trp	3.40	18.1	1.87	2.31	6.5	7.9
Tyr	2.30	8.2	1.70	1.70	4.5	7.1
Val	1.50	3.3	1.06	1.46	3.0	5.9
Correlation coefficient[a]						
(1)with RT	0.536	0.681	0.713	0.693	0.826	0.831
(2)with 15	0.741	0.757	0.868	0.841	0.778	-

[a]Correlation coefficients were computed (1) between experimental and predicted retention times RT of peptides, calculated using the amino acid hydrophobicity constants in each column; and (2) between amino acid hydrophobicity constants in each column compared to those of Wilson et al. (15).

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the behavior of amphiphilic macromolecules such as proteins. In the latter case, the retention times cannot usually be accurately determined based on hydrophobicity values calculated by simply summing up hydrophobic constants of the constituent moieties of the macromolecules, due to the possible existence of steric effects. The problem is further compounded by the influence of highly nonpolar or organic solvents often used in reverse phase chromatography or partition methods, which can destroy the so-called native structure of proteins, thus changing the steric effects.

A significant limitation of values calculated from hydrophobicity scales of amino acids is their lack of consideration of the effect of tertiary and quaternary structures of proteins on the extent of exposure or "effective" hydrophobicity of residues in individual proteins. It is generally agreed by protein chemists that charged residues are located preferentially at the surface of the molecule, where they can interact with water; residues in the interior are close packed and burial of hydrophobic groups away from the surface can be a major source of stabilization of tertiary structure. It is therefore likely that the groups which can participate in protein functionality are those hydrophobic residues which are located on the surface of the native protein molecules or become exposed during processing such as heating or whipping - in other words, "surface" or "available" hydrophobicity.

Methods such as the fluorescence probe or the various ligand binding techniques measure hydrophobic groups on the surface of the protein molecule which are able to bind the probes or ligands, and thus are expected to yield parameters which correlate with the functionality of proteins. In terms of simplicity of methodology, hydrophobic probe methods using anilinonaphthalenesulfonate (ANS), cis-parinaric acid (CPA) and other fluorescence probes are probably the most popular for hydrophobicity determination. Good correlation has been observed between the surface hydrophobicity of proteins measured by these probes and functionality. However, criticism has arisen against using ANS and CPA as strictly hydrophobic probes because of the coexistence of charge bearing moieties on these probe molecules which can interact with the protein. Considering this, diphenyl hexatriene (DPH) may be a more suitable probe as it is nonpolar and nondissociable. To overcome the insolubility of DPH in water, Tsutsui et al. (16) first dissolved DPH in corn oil, then measured the fluorescence associated with oil bound to proteins. Although strictly speaking, this method measures the oil-binding capacity rather than hydrophobicity of proteins, it may yield a useful physicochemical parameter in elucidating food protein functionality because of the importance of lipid- or oil-protein interactions in food systems, eg. emulsifying and fat binding properties. However, despite its potential relevance for explaining food protein functionality, this method has not been widely used probably due to the rather tedious and time-consuming nature of the procedure.

As shown in Table I, various hydrophobicity parameters have been used to develop equations explaining functionality. Although the fluorescence probe methods with ANS and CPA have proved most popular due to ease of measurement and ability to predict functionality, application of the hydrophobicity values obtained by

these methods may be limited due to (1) the presence of the anionic group on these probes which may interact with the protein molecule through charge effects, and (2) the low protein concentrations used for measurement of fluorescence, which is in contrast to the much higher concentrations usually encountered in real food systems.

New Approaches to Determination of Protein Hydrophobicity

Since Wilson et al. (15) have shown that the hydrophobic constants of individual amino acids can be successfully used to predict behavior such as retention on reverse phase chromatography for peptides in which steric effects are negligible, one approach to determination of protein hydrophobicity would be to quantitate the extent of exposure of different types of amino acid residues in the protein and then use established amino acid hydrophobicity scales to calculate the total hydrophobicity of the exposed residues. Two methods are currently being investigated in our laboratory for their ability to quantitate the extent of exposure of side chains in proteins, namely proton magnetic resonance (PMR) and laser Raman spectroscopy.

Proton Magnetic Resonance Spectroscopy. According to McDonald and Phillips (17) who developed a procedure to compute PMR spectra of random coil proteins from their amino acid composition, protein molecules in 6M guanidine at 40°C appear to be in a configuration close to random coil; all hydrocarbon side chains then appear at 1-1.7 ppm and aromatic at 6-8 ppm. Wurthrich and Wagner (18) also stated that the spatial folding of the polypeptide chain is responsible for the differences between PMR spectrum observed for a native globular protein and the spectrum observed for the random coil form of the polypeptide chain, which corresponds closely to the computed spectrum.

Kason et al. (19) in their interaction study of α_{s1}-casein showed sharpening effects of pH and concentration of the protein on its PMR spectra (Figure 2). The manually computed PMR spectrum (Figure 3) showed good agreement with that of the most dissociated spectra at 2.5% protein concentration and pH 9.9. A BASIC program of the procedure of McDonald and Phillips (17) was written for a PC computer as shown in the same figure for comparison.

The differences in the measured spectrum of a protein solution from the theoretical or computed spectrum are due to the deviation from a completely random coil structure in the protein solution, i.e. not all of the amino acid residues are exposed. Computation of a spectrum which matches the real spectrum thus should give information of the number of exposed amino acids in the protein. The program for PMR spectrum computation was incorporated into a computer program for simplex optimization to find the amino acid composition yielding a computed spectrum most closely resembling the measured protein spectrum. The differences in amino acid compositions computed to match spectra of the protein solution measured under various conditions such as pH, temperature or denaturing agent should correspond to differences in extent of exposure of the amino acid residues of the protein molecules. The optimization program being used in this study is a slight

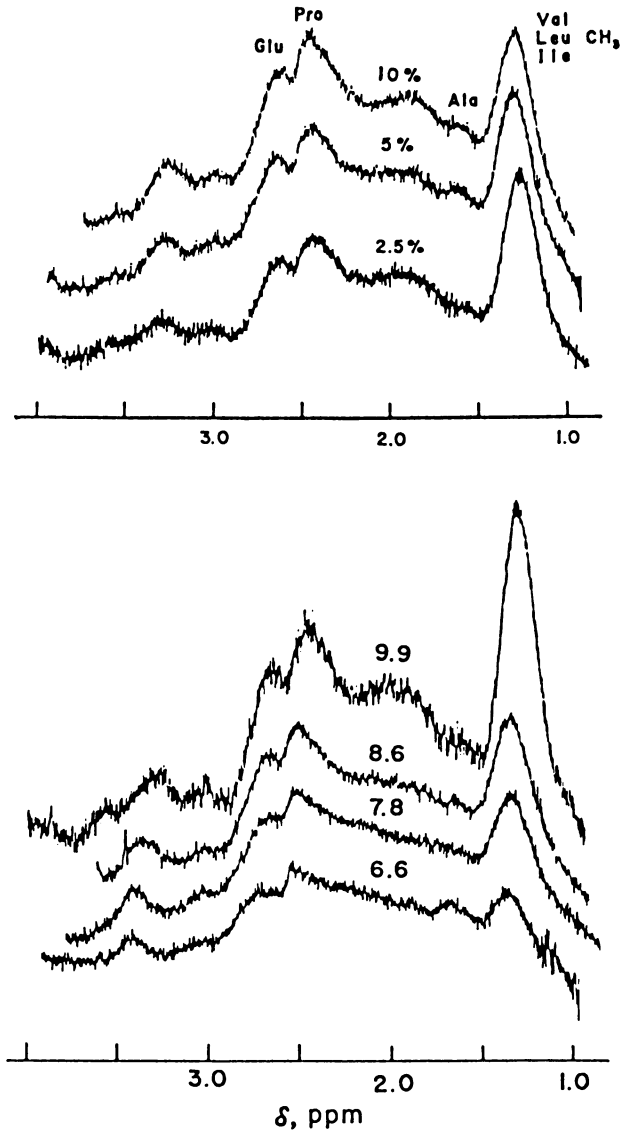

Figure 2. PMR spectra of α_{s1}-casein at 100 Mc/sec. Top, effect of protein concentration at pH 10; bottom, effect of pH. (Reprinted with permission from Ref. 19. Copyright 1971 American Dairy Science Association.)

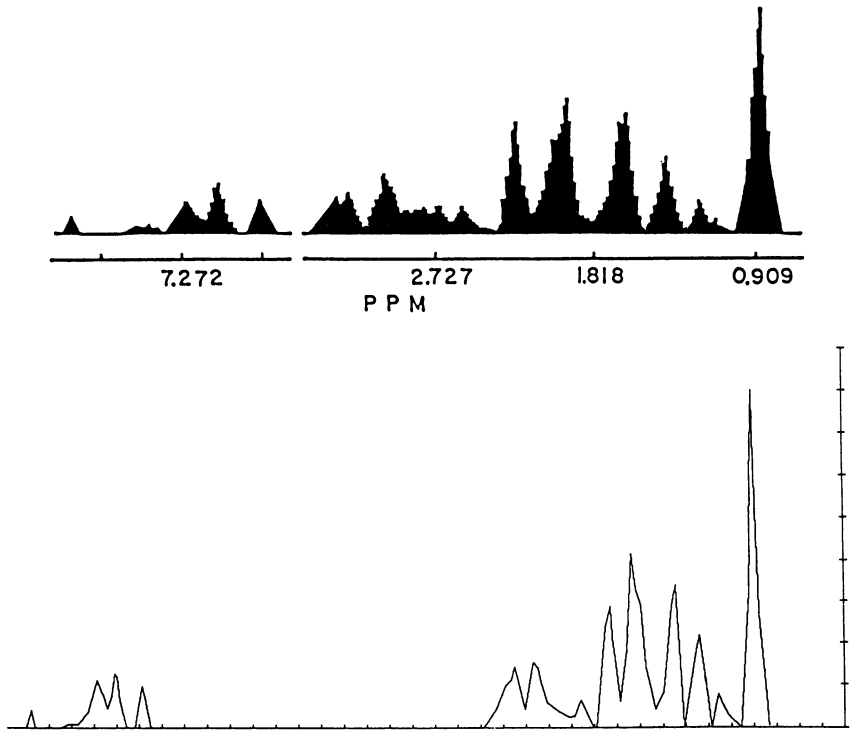

Figure 3. PMR spectrum of α_{s1}-casein computed by the procedure of McDonald and Phillips (17). Top, manually computed; bottom, computer-drawn.

modification of the program we have used for determining the blending ratio of ingredients to yield a GC pattern for the blended sample most similar to that of a reference standard, by maximizing a pattern similarity constant (20). The current optimization of PMR spectra is being carried out separately for aromatic and aliphatic regions of the spectrum. This approach has been validated using model PMR spectra, as the computed spectra satisfactorily matched with the original model spectra, and application to measured PMR spectra of a variety of proteins is underway.

Raman Spectroscopy. Raman spectroscopy has been used for determining the microenvironment of aromatic side chains in protein molecules (21,22). The intensity ratio I_{850}/I_{830} is used for estimating the exposure of tyrosine side-chains to water, and the appearance of peaks at 760, 880 and 1360 cm^{-1} are used as a sign for tryptophan side-chains in hydrophobic environment. Changes in C-C and C-H stretch bands at 950 and 1449 cm^{-1} were observed by thermal denaturation of egg white (23) and could represent indirect evidence of a change in the microenvironment of hydrocarbon side chains. Increase in the CH_3-stretching region at around 2930 cm^{-1} was suggested to arise from the insertion of previously buried aliphatic side chains into water (24). A significant difference in the application of Raman compared to other forms of spectroscopy is its ability to measure these changes in protein solutions of high concentration, or in solid or gelled states.

Use of Raman spectroscopy in the study of hydrophobic interaction is exemplified in our recent work (25). A Jasco model NR-1100 equipped with a 488 nm Argon laser was used for investigating interaction between 7S soy protein and soy lecithin. The difference spectrum between 7S-lecithin complex and their noninteracting mixture is shown in Figure 4, and characteristic peak assignments are shown in Table IV. The difference spectrum indicates decreases in intensity of peaks corresponding to α-helix and vibration of C-N stretch in 7S protein, and to C-C and hydrocarbon vibration in the phosphatidylethanolamine component, while increases in intensity are observed for peaks corresponding to β-sheet in 7S and to hydrocarbon vibration in phosphatidylcholine. Exposure of hydrophobic amino acid side chains thus observed was in agreement with the increase in hydrophobicity analyzed fluorometrically using CPA as a probe.

Application of QSAR to Formula Optimization

Once the structure-function relationships have been established, the best area for their use may be found in food formulation.

Linear programming (LP) is the most popular computer-aided technology currently being used for food formulation. For example, the processed meat industry uses LP in determination of the least cost formula that will meet predetermined product specifications using available ingredients. The specifications that are used as constraints in the LP computer program include proximate composition, ingredient content, and quality in the form of bind constants (26). However, the least cost programs place excessive emphasis on cost reduction and unduely deemphasize the product

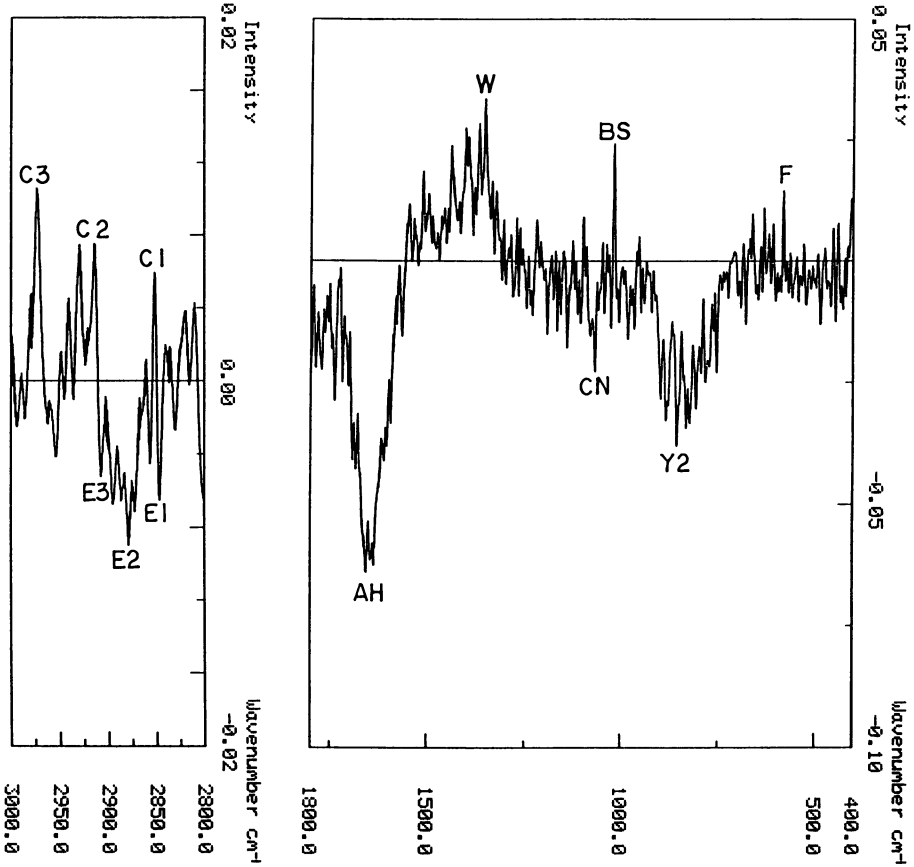

Figure 4. Difference spectrum between 7S protein-lecithin complex and noninteracted mixture. The symbols are explained in Table IV.

Table IV. Peak characteristics of difference spectrum between 7S-lecithin complex and noninteracted mixture shown in Figure 4

Symbol	Frequency (cm^{-1})	Assignment
F	624	Phe
Y1	830	Tyr
Y2	850	Tyr
BS	1002	Beta-sheet
CN	1110	C-N
W	1361	Trp
AH	1650	Alpha-helix
E1	2848	CH_2 in PE[a]
C1	2850	CH_2 in PC[b]
E2	2855	CH_2 in PE
E3	2900	CH_3 in PE
C2	2920	CH_2 in PC
C3	2960	CH_3 in PC

[a] PE = phosphatidylethanolamine.
[b] PC = phosphatidylcholine.

quality, by dealing with quality parameters as constraints rather than objective functions. The bind constants frequently used as quality parameters by the meat processors are relative variables, and linear relationships with the ingredients are assumed. However, the structure-functionality relationships of meat proteins are described by non-linear functions (7). Furthermore, relationships between ingredient composition and final product quality have also been shown to be nonlinear, making the incorporation of the prediction equations into the LP program difficult. It was found that the constrained simplex optimization (Complex) of Box (27) is more appropriate to use for this kind of formula optimization (28). Prediction equations are required in this formula optimization to define the objective functions as well as any imposed constraints. QSAR equations can best be used for this purpose. However, as discussed above, QSAR for food protein functionality are currently limited by the lag in progress to obtain an accurate or functionally relevant quantitative parameter to describe hydrophobicity in these equations.

Advances in this area will be a major step towards achieving the long term goal of elucidation and prediction of the functional properties of ingredients by QSAR analysis to ensure high standards of quality in food products.

Conclusions

The establishment of equations which can describe the quantitative structure-activity relationship (QSAR) of food proteins depends on accurate quantitation of hydrophobicity values for use as a descriptive parameter to elucidate functionality. New approaches to measure extent of exposure of amino acid residues, using computer-aided curve fitting of PMR spectra or monitoring changes in the intensity of peaks in Raman spectra, are proposed to complement current methods of hydrophobicity measurement. Once quantitative structure-functional property relationships are known, they can be used for optimization of food formulations.

Acknowledgments

The authors are indebted to Mrs. N. Helbig and L. Kwan for their technical assistance. This work was supported by an operating grant from the Natural Science and Engineering Council of Canada.

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RECEIVED June 20, 1990