Flexibility of Food Proteins As Revealed by Compressibility

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To elucidate the flexibility–structure–function relationships of food proteins, the adiabatic compressibility, $\bar{\beta}_s$, of 14 egg and milk proteins was determined by means of sound velocity and density measurements in aqueous solutions at 25 °C. All the proteins showed positive $\bar{\beta}_s$ values, indicating the large internal flexibility characteristic of the protein molecules. On the basis of statistical analyses of $\bar{\beta}_s$ previously reported for globular proteins (Gekko, K.; Hasegawa, Y. *Biochemistry* **1986**, *25*, 6563–6571), the flexibility–structure relationship is discussed in terms of the internal cavity and hydration, focusing on the hydrophobicity of proteins. It was found that the protease susceptibility, foaming capacity, and free energy of unfolding of proteins are positively correlated to $\bar{\beta}_s$. This result indicates that the flexibility of the structure plays an essential role in the conformational stability and functional properties of food proteins.

INTRODUCTION

Many studies have been carried out on the structure–function relationships of proteins and enzymes. At present, however, it seems difficult to predict precisely the functional property of a protein from its primary or tertiary structure. For example, α-lactalbumin shows good emulsifying and foaming properties as compared to those of lysozyme, although there is a high similarity in the primary and tertiary structures of the two proteins. One of the reasons for such discrepancy may be because the dynamic structure of a protein is not taken into account in the prediction. It has been demonstrated by some relaxational techniques such as hydrogen-exchange experiments that a protein molecule is fluctuating as to the relative positions of its constituent atoms (Woodward and Hilton, 1979; Karplus and McCammon, 1981). Recent X-ray analyses have revealed that there are some packing defects or cavities in a protein molecule that actually permit considerable internal motions in response to thermal or mechanical forces (Frauenfelder et al., 1987; Kundrot and Richards, 1987). A matter of concern is how such a fluctuation is related to the structural characteristics and functional properties of proteins.

An importance of the flexibility of food proteins has been pointed out from the correlation between the protease susceptibility and some properties such as foaming capacity (Nakai, 1983; Townsend and Nakai, 1983; Kato et al., 1985). In these studies, the flexible proteins are assumed to be more easily digested by protease than the rigid ones. Although this assumption may be useful as a measure of the relative flexibility at a given condition, it would be desirable to determine the absolute level of flexibility with physical meanings for further advanced understanding of the flexibility–structure–function relationships of food proteins. Among such physical quantities, compressibility is being discovered as a new light on protein dynamics since it is directly linked to the fluctuation of volume or internal cavities (Cooper, 1976; Pain, 1987). Although this type of fluctuation is a thermodynamic and macroscopic one, we found that compressibility sensitively reflects the structural characteristics of globular proteins (Gekko and Noguchi, 1979; Gekko and Hasegawa, 1986, 1989). In this paper, we will extend the compressibility study to discuss the flexibility–structure–function relationships of food proteins.

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MATERIALS AND METHODS

Materials. Most proteins used were commercial products from Sigma: turkey egg lysozyme (lot 75F-8040), turkey ovalbumin (lot 101F-8100), hen egg conalbumin with iron (lot 104F-8065), α-casein (lot 36F-9540), β-casein (26F-9760), and κ-casein (lot 46F-96201). Whole casein (Hammarsten casein) was prepared from fresh cow milk. Hen egg ovalbumin was prepared from fresh egg white by the ammonium sulfate method. *s*-Ovalbumin was prepared from ovalbumin according to the procedure of Smith and Back (1965). Origins of other proteins are listed in a previous paper (Gekko and Hasegawa, 1986). These proteins were exhaustively dialyzed against distilled water or a given dilute buffer solution at 4 °C (the buffers used are presented in Table I). Six sample solutions of different protein concentration (0.2–1.0%) were prepared by diluting the dialyzed stock solution with the dialysate after purification through a glass filter. The solutions were kept as still as possible to avoid surface denaturation or bubbling.

Sound Velocity Measurements. The sound velocity in a protein solution was measured, with an accuracy of 1 cm/s, by means of a "sing-around pulse method" at 3 MHz. The apparatus and procedures were essentially the same as those used in previous studies (Gekko and Noguchi, 1979; Gekko and Hasegawa, 1986, 1989). The partial specific adiabatic compressibility of the solute, $\bar{\beta}_s$, was calculated with the equations

$$\bar{\beta}_s = -(1/\bar{v}^\circ)(\partial \bar{v}^\circ/\partial P) = (\beta_0/\bar{v}^\circ) \lim_{c\to 0}[(\beta/\beta_0 - V_0)/c] \quad (1)$$

$$V_0 = (d - c)/d_0 \quad (2)$$

$$\bar{v}^\circ = \lim_{c\to 0}[(1 - V_0)/c] \quad (3)$$

where P is the pressure; β and β_0 are the adiabatic compressibilities of solution and solvent, respectively; d and d_0 are the densities of solution and solvent, respectively; c is the protein concentration in grams per milliliter of solution; V_0 is the apparent volume fraction of the solvent in solution; and $\bar{v}^\circ$ is the partial specific volume of the protein. The values of β and β_0 can be calculated from the sound velocity, u, and the density, d, of the solution or solvent with the Laplace equation, $\beta = 1/du^2$.

Density Measurements. The densities of the solvent and solutions were measured with a precision density meter, DMA-02C (Anton Paar, Gratz). The partial specific volumes of the proteins were calculated from eqs 2 and 3 according to the standard procedure.

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Table I. Partial Specific Volume and Adiabatic Compressibility of Food Proteins in Water at 25 °C[a]

sample no.	protein	H_ϕ,[b] cal/mol	$\bar{v}°$, mL/g	du/dc, (m·mL)/(g·s)	$\bar{\beta}_s \times 10^{12}$, cm²/dyn
	egg proteins (hen)				
1	lysozyme	890	0.714	261.9	4.48
2	lysozyme (turkey)	910	0.709	274.9	1.97
3	ovalbumin	980	0.746	234.1	9.18
			(0.744)[c]	(253.8)[c]	(8.37)[c]
4	ovalbumin (turkey)	990	0.725	246.2	7.95
5	*s*-ovalbumin	980	0.743[c]	253.8[c]	8.94[c]
6	conalbumin (+Fe)	980	0.717	257.9	5.24
7	conalbumin (−Fe)	980	0.728	272.6	4.89
8	ovomucoid	830	0.696	268.7	3.38
	milk proteins (bovine)[d]				
9	whole casein		0.744	261.2	6.67
10	α-casein		0.739	256.3	7.74
11	α_s-casein	1050	0.732	270.0	5.68
12	κ-casein	1060	0.739	258.1	7.49
13	β-casein	1340	0.744	294.4	3.80
14	α-lactalbumin	1050	0.736	250.1	8.27
15	β-lactoglobulin	1060	0.751	276.2	8.45
	muscle proteins				
16	gelatin		0.689	316.4	−2.50
17	F-actin[e]		0.720		−6.3
18	myosin[e]		0.724		−18
19	tropomyosin[e]		0.733		−41

[a] Some data are taken from our previous paper (Gekko and Hasegawa, 1986). [b] The average hydrophobicity per mole of amino acid residue (Bigelow and Channon, 1976). [c] In 0.1 M phosphate buffer (pH 7.0). [d] In 0.02 M phosphate buffer (pH 7.0). [e] Data at 20 °C (Sarvazyan and Kharakoz, 1977).

Protein Concentration Determination. The concentrations of proteins were determined by absorption measurements with a Jasco UVIDEC-610C spectrophotometer after sound velocity and density measurements. The extinction coefficient of ovalbumin and *s*-ovalbumin was assumed to be 7.12 dL/(g·cm) at 280 nm in phosphate buffer (Glazer et al., 1963). The extinction coefficients of other proteins in a respective solvent were determined by a dry-weight method. In most cases, the results were in good agreement with those in the literature.

RESULTS AND DISCUSSION

The partial specific volume of a protein at infinite dilution, $\bar{v}°$, was determined by a linear extrapolation of the apparent specific volume, $(1 - V_0)/c$, to zero protein concentration, with an accuracy of ±0.002 mL/g. The $\bar{v}°$ values obtained are presented in Table I together with the average hydrophobicity of the proteins, H_ϕ (Bigelow and Channon, 1976). These $\bar{v}°$ values may be regarded as identical with (or close to) those in the literature, considering the experimental uncertainty and the different experimental conditions. For all the proteins studied, the sound velocity of the solutions increased in proportion to the protein concentration. The concentration dependence of the sound velocity, du/dc, calculated by the least-squares method, is shown in the fifth column of Table I. The du/dc values for most food proteins were in the range 250–270 m·mL/(g·s), while β-casein and gelatin showed considerably high values. The adiabatic compressibility, $\bar{\beta}_s$, was determined by a linear extrapolation of $(\beta/\beta_0 - V_0)/c$ to zero protein concentration, with an experimental error of less than 5% in most cases. The $\bar{\beta}_s$ values obtained for egg and milk proteins are listed in the last column of Table I, in which the data for muscle proteins (Sarvazyan and Kharakoz, 1977) are also included for comparison.

The partial specific volume of a protein in water consists of three contributions (Kauzmann, 1959): (1) the constitutive volume estimated as the sum of the constitutive atomic or group volumes (V_c); (2) the volume of the cavity in the molecule due to imperfect atomic packing (V_{cav}); and (3) the volume change due to solvation or hydration (ΔV_{sol}).

$$\bar{v}° = V_c + V_{cav} + \Delta V_{sol} \quad (4)$$

Here, V_{cav} involves not only the incompressible cavity formed on the closest packing of atoms but also the compressible void space generated on the random close packing of atoms. This volume has been estimated to be 0.02–0.05 mL/g, which corresponds to 3–6% of $\bar{v}°$ (Zamyatnin, 1972; Gekko and Noguchi, 1979). ΔV_{sol} is ascribed to three types of hydration, electrostriction around the ionic groups, hydrogen-bonded hydration around the polar groups, and hydrophobic hydration around the nonpolar groups. Each of them produces a negative volume change, and the resulting negative ΔV_{sol} is known to cancel out almost completely the positive V_{cav} (Gekko and Noguchi, 1979). Since the constitutive atomic volumes may be assumed as incompressible, differentiation of eq 4 with pressure, P, under isoentropic (adiabatic) conditions yields the equation for $\bar{\beta}_s$:

$$\bar{\beta}_s = -(1/\bar{v}°)(\partial\bar{v}°/\partial P) = -(1/\bar{v}°)[\partial V_{cav}/\partial P + \partial\Delta V_{sol}/\partial P] \quad (5)$$

Thus, the experimentally determined adiabatic compressibility of a protein would be mainly due to the contributions of cavity and hydration. The first term on the right-hand side of eq 5 contributes positively and the second term negatively to $\bar{\beta}_s$ due to the smaller compressibility of waters of hydration compared with those of free water.

As shown in Table I, the $\bar{\beta}_s$ value is positive for all the egg and milk proteins examined as well as for other globular proteins (Gekko and Hasegawa, 1986), indicating the existence of the highly compressible cavities in these protein molecules. According to our estimation (Gekko and Noguchi, 1979; Gekko and Hasegawa, 1986), the apparent compressibility of cavity is on the order of a few hundred × 10^{-12} cm²/dyn, e.g., (540–750) × 10^{-12} cm²/dyn for lysozyme. These values are more than 10 times the compressibility of free water (45 × 10^{-12} cm²/dyn), so that the cavity effect could compensate for the hydration effect to make $\bar{\beta}_s$ positive. In contrast to egg and milk proteins, muscle proteins show the negative $\bar{\beta}_s$ values (Sarvazyan and Kharakoz, 1977). For these fibrous proteins, the hydration effect may overcome the cavity effect due to the larger accessible surface area compared with globular proteins. A noteworthy point is that $\bar{\beta}_s$ varies over a considerably wide range depending on the structural characteristics of individual proteins. This may allow us to examine the relationships of compressibility with the structure and function of food proteins. At present, however, such discussion would be limited for globular proteins since $\bar{\beta}_s$ of fibrous proteins seems to be determined by the specific structure, which is outside the scope of statistical analysis.

Compressibility–Structure Relationship. The compressibility–structure relationships of proteins have not been much discussed at a molecular level because of the complicated contributions of hydration. However, some important factors determing $\bar{\beta}_s$ of globular proteins have been recently revealed by the statistical analyses of the data (Gekko and Hasegawa, 1986): (1) $\bar{\beta}_s$ increases with increasing partial specific volume and hydrophobicity of proteins; (2) the helix element also appears to be a dynamic domain to increase $\bar{\beta}_s$; and (3) four amino acid residues (Leu, Glu, Phe, and His) greatly increase $\bar{\beta}_s$, but another four (Asn, Gly, Ser, and Thr) decrease it. These rela-

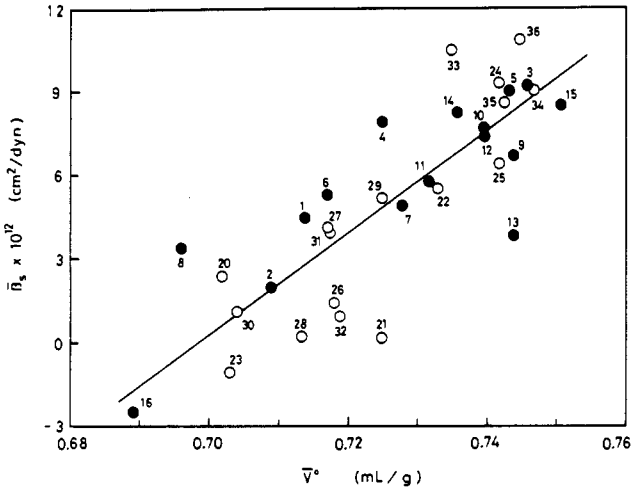

Figure 1. Plots of the adiabatic compressibility, $\bar{\beta}_s$, against the partial specific volume, $\bar{v}°$, of proteins. The numbers 1–16 of the points (●) correspond to the sample numbers of food proteins in Table I. The other numbers 20–36 (○) refer to the following proteins (Gekko and Hasegawa, 1986): (20) peroxidase, (21) cytochrome c, (22) catalase, (23) subtilisin BPN′, (24) insulin, (25) carbonic anhydrase, (26) trypsinogen, (27) α-chymotrypsin, (28) soybean trypsin inhibitor, (29) α-amylase, (30) ribonuclease A, (31) α-chymotrypsinogen, (32) trypsin, (33) bovine serum albumin, (34) myoglobin, (35) pepsin, and (36) hemoglobin. The solid line represents the least-squares linear regression for all 33 points (correlation coefficient of 0.82), $\bar{\beta}_s = (173.31\,\bar{v}° - 120.80) \times 10^{-12}$ cm²/dyn.

tionships would essentially hold true for food proteins. As shown in Figure 1, the $\bar{\beta}_s$ values of egg and milk proteins appear to be a linear increasing function of $\bar{v}°$ as well as those of other globular proteins including a fibrous protein (gelatin). Such positive correlation could be expected from the positive contribution of cavity and the negative contribution of hydration to $\bar{v}°$ and $\bar{\beta}_s$. The least-squares linear regression of all the plots yields the following equation with a high correlation coefficient of 0.82:

$$\bar{\beta}_s = (173.31\bar{v}° - 120.80) \times 10^{-12} \text{ cm}^2/\text{dyn} \quad (6)$$

If four proteins (bovine serum albumin, cytochrome c, ovomucoid, and β-casein) showing a deviation of more than 3 units in $\bar{\beta}_s$ from this equation are omitted in regression, the correlation coefficient increases to 0.91 for the improved equation:

$$\bar{\beta}_s = (192.01\bar{v}° - 134.34) \times 10^{-12} \text{ cm}^2/\text{dyn} \quad (7)$$

Therefore, it would be possible to predict the $\bar{\beta}_s$ value of an unknown protein from these equations if its $\bar{v}°$ value is known. Although the reason for the deviation of these four proteins is not clear, the large positive deviation of ovomucoid may be attributed to its high sugar content (22%), which would extensively reduce $\bar{v}°$ relative to $\bar{\beta}_s$. The small $\bar{\beta}_s$ value of β-casein despite the largest hydrophobicity in caseins (Table I) may reflect its extended structure (proline-type structure) as expected from the exceptionally high proline content (17%).

In relation to the effect of amino acid composition, it is a matter of interest whether the flexibility of a protein depends on its origin or species. As shown in Table I, the $\bar{\beta}_s$ values of lysozyme and ovalbumin from turkey are smaller than those of the respective proteins from hen, although there is only 7% substitution of amino acid residues between both species. These $\bar{\beta}_s$ values for turkey proteins, 1.97×10^{-12} cm²/dyn (lysozyme) and 7.95×10^{-12} cm²/dyn (ovalbumin), are very close to the respective $\bar{\beta}_s$ values, 1.68×10^{-12} and 7.62×10^{-12} cm²/dyn, predicted by using the empirical equation (Gekko and Hasegawa, 1986)

$$\bar{\beta}_s = (40.9X_1 + 10.1X_2 - 6.83) \times 10^{-12} \text{ cm}^2/\text{dyn} \quad (8)$$

where X_1 represents the sum of the volume fraction of the four residues, Leu, Glu, Phe, and His, and X_2 that of the another four, Asn, Gly, Ser, and Thr. This supports the idea that the flexibility of proteins is predominantly determined at the level of amino acid composition of the eight residues as listed above.

The compressibility data of some proteins may be discussed in the difference of the structures beside the statistical analysis. There is a high homology between hen egg lysozyme and bovine milk α-lactalbumin in the amino acid sequence (45% homology) and the secondary structure (29–33% α-helix and 16–17% β-sheet). As can be seen in Table I, however, α-lactalbumin is highly flexible compared with lysozyme. This result is consistent with findings by other techniques: (1) NMR studies show about 82% of the amide protons can be exchanged in lysozyme, while in α-lactalbumin almost 100% of the amide protons are exchanged on dissolution of the proteins (Poole and Finney, 1983) and (2) small-angle X-ray analysis shows α-lactalbumin is more expanded in solution than lysozyme (Krigbaum and Kügler, 1970). A possible origin for such a large $\bar{\beta}_s$ value of α-lactalbumin may be its large hydrophobicity (H_ϕ = 1050 cal/mol) compared with lysozyme (H_ϕ = 890 cal/mol) since the cavity would be mainly generated by imperfect packing of nonpolar amino acid residues localized in the interior of protein molecules, and the nonpolar surface would cause the decrease in hydration.

Transformation of ovalbumin to s-ovalbumin has been extensively studied from physicochemical aspects. Although the thermal stability is largely raised by the transformation, only small changes in structure have been observed between both proteins at native state: (1) Raman spectra indicate the conversion of a small part (3–4%) of α-helix to β-sheet (Kint and Tomimatsu, 1979) and (2) the sedimentation coefficient and intrinsic viscosity slightly decrease in the transformation (Smith and Back, 1965; Nakamura and Ishimura, 1981). Although these results suggest the more compact and rigid structure for s-ovalbumin, there is no remarkable difference in $\bar{\beta}_s$ values of both proteins (Table I). Then the average compactness of internal atoms of the protein molecules would not be significantly different, although the molecular shape may be modified in such a transformation. This is also expected from the observation that the transformation to s-ovalbumin is not affected by a hydrostatic pressure of 2000 atm, that is, the activation volume of the process is negligibly small (Gekko and Yamagami, unpublished data).

As shown in Table I and Figure 1, caseins have values of $\bar{\beta}_s$ comparable to those of typical globular proteins such as whey proteins. Since casein molecules consist of only a small amount of secondary structure, such a high compressibility may be predominantly brought about by the large hydrophobicity of caseins. Although an intermolecular hydrophobic interaction may partially contribute to increase $\bar{\beta}_s$, the substantial effect of such self-association would be limited to β-casein, since the solution turbidity of β-casein is pressure-dependent while those of κ- and α_{s1}-caseins are not (Ohmiya et al., 1989). Considering the overestimation of $\bar{\beta}_s$ for β-casein, we could conclude that casein molecules are more flexible in the order β-casein < α_s-casein < whole casein < κ-casein < α-casein (Table I). The larger $\bar{\beta}_s$ value of α-casein compared with those of its constituents, α_s- and κ-caseins,

may reflect some interaction between both components (Clark and Nakai, 1971). From a similar aspect, the $\bar{\beta}_s$ value of whole casein was calculated by assuming a simple additivity of the contributions of its four main components, α_s-, β-, κ-, and γ-caseins, from the respective compositions 45% (α_s), 30% (β), 15% (κ), and 10% (γ) (these weight fractions correspond to the volume fractions since the $\bar{v}°$ values of caseins are very close to each other). The $\bar{\beta}_s$ value of γ-casein, not measured in this study, was assumed to be identical with those of β-casein since the amino acid sequences of both caseins are very similar. The $\bar{\beta}_s$ value thus estimated for whole casein is 4.2×10^{-12} cm^2/dyn, which is considerably smaller than the experimentally observed value, 6.67×10^{-12} cm^2/dyn. This result suggests that the cavity increases and/or hydration decreases in whole casein due to the intermolecular interactions of each of its components and that the compressibility data might be useful for studying the micelle structure of caseins.

Compressibility–Function Relationship. What role does the flexibility of proteins play in their functions and properties? This is an important problem in protein physics, but there are only limited studies to derive a general principle for the flexibility–function relationships of food proteins. Since compressibility or volume fluctuation is a bulk thermodynamic quantity, it should be primarily related to the macroscopic or physicochemical properties averaged over the entire protein molecule rather than the microscopic or physiological functions. As seen in Figure 1, most proteases and protease inhibitors are less compressible compared with nutrient (storage) proteins such as egg and milk proteins. Proteases and protease inhibitors may defend themselves against autolysis through depression of the fluctuations, while nutrient proteins must be more flexible to be easily digested by proteases. An extremely high flexibility may be required for the transport proteins such as bovine serum albumin, myoglobin, and hemoglobin to bind and carry specific molecules or ions from one organ to another. Although these interpretations are highly speculative, it is probable that the individual proteins have the flexibility desirable for their functions.

An important function of food proteins is protease digestibility. Such a property has been used as a measure of the flexibility of proteins by assuming that the flexible proteins would be more easily digested by proteases (Kato et al., 1985). This assumption would be more directly confirmed by examining the correlation between the protease susceptibility and compressibility of proteins. In Figure 2A, the digestion velocities of some proteins by α-chymotrypsin and trypsin, taken from a paper of Kato et al. (1985), are plotted against the $\bar{\beta}_s$ values. Evidently, the highly compressible proteins are more easily digested by both proteases. This is the case for digestion of milk caseins by rennet and pepsin as shown in Figure 2B; the digestion velocity increases in the order β-casein < α_s-casein < whole casein < κ-casein < α-casein (Tam and Whitaker, 1972; Vanderpoorten and Weckx, 1972), which corresponds to an increase in $\bar{\beta}_s$. These good correlations could not be explained without taking into consideration the flexibility of protein structure. The peptide bonds in a flexible protein molecule would be frequently exposed to solvent so that they could be easily attached by protease.

The foaming capacities of some proteins (Townsend and Nakai, 1983) are plotted against $\bar{\beta}_s$ in Figure 3. Clearly, the highly compressible proteins show high foaming capacity. This result essentially supports the positive correlation between the digestion velocity and the foaming

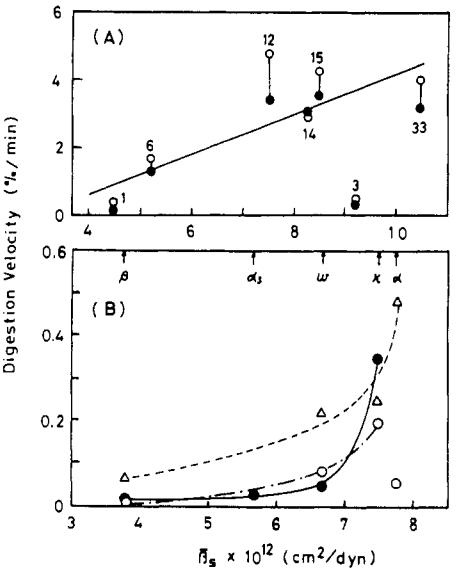

Figure 2. Plots of the digestion velocity against the adiabatic compressibility, $\bar{\beta}_s$, of proteins. (A) Digestion by α-chymotrypsin (○) and trypsin (●) at pH 8 and 38 °C (Kato et al., 1985). The numbers of the points correspond to the sample numbers of the proteins in Table I except for bovine serum albumin (33). The solid line represents the least-squares linear regression for all the points except for ovalbumin (3) (correlation coefficient of 0.80). (B) Digestion of caseins at 30 °C by rennet at pH 6 (○) (Tam and Whitaker, 1972) and pH 6.7 (●) (Vanderpoorten and Weckx, 1972), and by pepsin at pH 3 (△) (Tam and Whitaker, 1972). The letters in the figure, β, α_s, w, κ, and α represent β-casein, α_s-casein, whole casein, κ-casein, and α-casein, respectively.

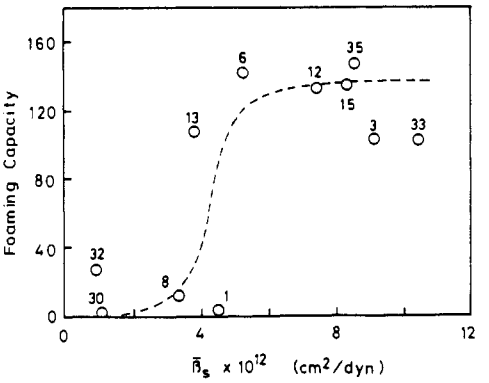

Figure 3. Plots of the foaming capacity against the adiabatic compressibility, $\bar{\beta}_s$, of proteins. The numbers of the points correspond to the sample numbers of the respective proteins listed in Table I and Figure 1. The foaming capacity data at pH 7 were taken from a paper of Townsend and Nakai (1983).

or emulsifying activities (Kato et al., 1985), demonstrating that the flexibility of protein structure would affect these properties through the enhanced exposure of the nonpolar groups in a protein molecule to solvent. In this meaning, the internal or total hydrophobicity of proteins, a compressibility-increasing factor, would be also important for such properties as well as the surface hydrophobicity (Nakai, 1983; Townsend and Nakai, 1983). Interestingly, there seems to be a critical point around $\bar{\beta}_s$ of 4×10^{-12} cm^2/dyn in Figure 3, suggesting that a critical flexibility of the structure is necessary for a protein molecule to get a useful surface activity through denaturation at the oil–water and air–water interfaces.

Although there is no general principle for the flexibility–stability relationship of protein structure, a rigid protein might be expected to be more stable than a flexible one. This idea was examined by two types of correlation

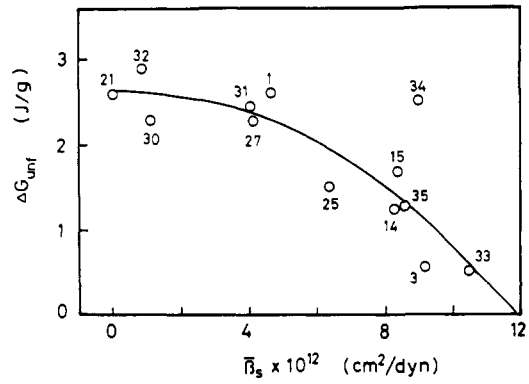

Figure 4. Plots of the free energy of unfolding, ΔG_{unf}, against the adiabatic compressibility, $\bar{\beta}_s$, of proteins. The ΔG_{unf} data around 25 °C and neutral pH were taken from the table compiled by Pfeil (1986). The numbers of the points correspond to the sample numbers of the respective proteins listed in Table I and Figure 1. The solid line represents the least-squares quadratic regression for all the points except for point 34 (myoglobin) (correlation coefficient of 0.89).

analyses. Figure 4 shows the plots of $\bar{\beta}_s$ against the free energy of denaturation (unfolding), ΔG_{unf} (J/g), in water around 25 °C and neutral pH, which was taken from the table compiled by Pfeil (1986). Despite limited data at the same condition, it can be seen that the flexible proteins, except for myoglobin ($\bar{\beta}_s > 4 \times 10^{-12}$ cm²/dyn), are easily denatured compared with rigid ones ($\bar{\beta}_s < 4 \times 10^{-12}$ cm²/dyn). This is evidence that justifies the $\bar{\beta}_s$ dependence of protease susceptibility and foaming capacity (Figures 2 and 3): these properties closely depend on the conformational stability of protein via its flexibility. In fact, Kato and Yutani (1988) recently demonstrated that the foaming and emulsifying activities increase with decreasing ΔG_{unf} by using some mutants of tryptophan synthase α-subunit. The relationship between thermal stability and protein structure has been investigated from many static viewpoints, the amino acid composition, the aliphatic or hydrophobic index, the average residue volume, the accessible surface area, and their complex roles (Stellwagen and Wilgus, 1978; Argos et al., 1979; Ponnuswarmy et al., 1982). Although there is no systematic investigation from the dynamic aspects, we found that the denaturation temperature slightly increases with increasing $\bar{\beta}_s$ for 14 proteins (Gekko and Hasegawa, 1986). This correlation suggests that a rigid protein, which is more stable relative to a flexible one at room or physiological temperature, is not necessarily thermostable and that a flexible protein may be proof against large thermal fluctuation through some buffer action of the internal cavities. It is an unknown problem whether or not the highly compressible proteins are labile under low temperature or high pressure.

In this paper, we presented compressibility data as a new measure for the flexibility of food proteins. Although this type of fluctuation is a thermodynamic and macroscopic one, it appears to be rather closely related to the structural characteristics of the proteins. Thus, it would be possible to predict the compressibility or flexibility of an unknown protein from its $\bar{v}°$ value or amino acid composition by using some empirical equations derived. The correlation observed between $\bar{\beta}_s$ and some functional properties of proteins indicates that the flexibility of the structure has an essential role in the functions of proteins and that dynamic factors as well as static ones should be taken into consideration to design the new function of proteins by chemical modification or protein engineering. Further advanced studies on the compressibility–structure–function relationships of proteins promise to give an insight into the real image of protein dynamics and into the high-pressure processing of foods, a new technique recently becoming utilizable in the Japanese food industry.

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