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Calculation of volume fluctuation for globular protein models

(protein structure/breathing motion/compressibility/solvent cage/hydrophobic force)

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ABSTRACT **The extent of volume fluctuation is calculated for two simple geometrical models of globular protein molecules subjected to a potential that is proportional to the surface area freshly generated by the thermal breathing motion. The proportionality constant, γ, has the unit of surface tension. The calculated values are compared with estimates made from the compressibility measurements. After an approximate correction for the hydration effect, the experimental values are found to be between those calculated by using γ values of 25 and 46 cal/mol/Å^2 (1 cal = 4.184 J). These values bracket previously reported independent estimates of interfacial tension that presumably operates at the interface between a nonpolar molecule and water. This result appears to indicate that the solvent water plays a significant role in determining the extent of volume fluctuation of globular proteins and that the concept, and the actual value of the estimate, of the interfacial tension around a nonpolar molecule in water may, in fact, be useful in some applications.**

1. Introduction

It is well recognized that the folded structure of a globular protein molecule is the result of and subject to many strong forces that are delicately balanced (1). These forces fall into two broad types. There are the intramolecular forces that include, among others, those that are involved in the intramolecular hydrogen bond formation, the electrostatic interactions, and the van der Waals interactions. The other represents the effect of solvent. Solvent can influence the structure and dynamics of a protein molecule by specific interaction such as forming hydrogen bonds and hydrating charged groups but also can nonspecifically influence by acting as a kind of cage around the molecule.

The thermodynamic properties of a protein molecule that are most commonly used in studying these forces are the Gibbs free energy, enthalpy, and entropy, which are all mean properties. On the other hand, the heat capacity and compressibility are the thermodynamic quantities that relate to the extent of dynamical fluctuation of some variable of the molecule. Aside from their intrinsic importance, the dynamical properties quite independently contain information on the nature of forces that govern the structure and dynamics of the molecule. This is not the case with the mean properties, the use of which must always involve study of more than one state of the molecule—for example, the native and denatured states.

In this article the compressibility and volume fluctuation of the protein have been analyzed against a simplified model, wherein the only force governing the dynamics of the molecule is assumed given by a potential that is proportional to the surface area of the molecule. Therefore, this is a study of the effect of solvent, acting nonspecifically as a cage, on the dynamics of the protein molecule. Because "hydrophobic force" often is believed to be proportional to the protein–water interfacial area, this is a study of the relevance or importance of this simple formulation of the hydrophobic effect on the protein dynamics.

2. The model

Suppose we consider a protein molecule as a sphere that is isotropically compressible up to a certain minimum size r_0. To focus on the solvent effect, let us further assume that the force responsible for the compression is given solely by an effective potential energy that is proportional to the increase in the interfacial area between the protein and the solvent. Specifically, we assume that the average potential energy, w, as a function of the radius r of the molecule is given by

$$\begin{aligned} w(r) &= \gamma\Delta A \qquad \text{when } r > r_0 \\ &= \infty \qquad \text{when } r < r_0, \end{aligned} \tag{1}$$

in which $\Delta A = 4\pi r^2 - 4\pi r_0^2$ and γ is the proportionality constant and formally represents the interfacial tension between the protein and the solvent. The probability that the molecule will have the radius between r and $r + dr$ then is assumed given by

$$P(r)dr = \frac{e^{-w(r)/kT}dr}{\int e^{-w(r)/kT}dr}, \tag{2}$$

in which k and T are the Boltzmann constant and the absolute temperature, respectively.

The potential given above is different from the usual harmonic potential in that it is wedge-shaped near the minimum. (This difference arises from the assumption that the "solvent cage force" is unidirectional—i.e., it only compresses—coupled with the assumption that there are no other forces except the infinitely large intramolecular repulsion at r_0. In contrast, all forces that give rise to a harmonic potential must be bidirectional like that on a spring, which works both to stretch and to compress the spring, depending on its state.) For small fluctuations, it can be shown that the rms fluctuations in the radius and in the volume of the molecule from their respective means are given by

$$\text{rms}(\delta r) = \frac{kT}{8\pi\gamma}\frac{1}{r_0} \tag{3}$$

and

$$\text{rms}(\delta v) = \frac{kT}{2\gamma}r_0. \tag{4}$$

These results can be derived by using different mathematical

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techniques. A particularly simple procedure is outlined in the *Appendix*.†

Note that, actually, the whole surface area of the protein need not be considered because the portion of the surface area that remains constant in size and character will not contribute to the potential. This point is important in view of the fact that the surface of a protein molecule is highly folded at atomic scale and its total area is much larger than that of the smooth sphere of equal volume. Eqs. 3 and 4 will still apply if it is assumed that the patches of surface area freshly generated by the breathing motion lie on the surface of a sphere.

Let us now consider a different model. This time, consider the protein molecule to be made of two incompressible hemispheres of radius r_0, joined at a point or hinge (Fig. 1). The breathing motion consists of opening and closing of these two hemispheres. Let θ be the half-opening angle (Fig. 1). We shall assume that this opening angle remains small so that no solvent penetrates into the opening. [In Gō's (3) language, the motion contemplated is one of the low-frequency vibrational modes of small-amplitude fluctuation.] The surface area and the volume of the protein then increase, from a minimum, by the exposed area and the volume, respectively, of the space between the two hemispheres. This then would be an idealized model for a very anisotropic breathing motion.

For the purpose of calculation, let us further assume that this opening is bounded by the circular bases of the two hemispheres and by the surface of straight lines that join each point on the periphery of one circular base to the other. Then the volume of the opening is given exactly by $2\pi r_0^3 \sin\theta\cos\theta$ and the exposed surface area is given approximately (for small θ) by $4\pi r_0^2 \sin\theta$. The potential energy of this model then is given by

$$w(\theta) = 4\pi\gamma r_0^2 \sin\theta \qquad \text{when } \theta > 0$$
$$= \infty \qquad \text{when } \theta < 0. \qquad [5]$$

Because the two hemispheres are assumed incompressible, the volume fluctuation of this molecule is the same as that of just the opening. For small fluctuations (small θ), this model gives (see *Appendix*):

$$\text{rms}(\theta) = \bar{\theta} = \frac{kT}{4\pi\gamma}\frac{1}{r_0^2} \qquad [6]$$

and

$$\text{rms}(\delta v) = \overline{\Delta v} = \frac{kT}{2\gamma} r_0. \qquad [7]$$

Thus, the volume fluctuation for this very anisotropic breathing motion is given by the same formula as that which describes the fluctuation for the isotropic breathing motion (Eq. 4). Actually, this result could have been expected because the new surface area generated by this anisotropic breathing motion lies approximately on the surface of a sphere. In fact, one of the mechanisms for the volume fluctuation considered with the isotropic model could be this sort of fissure occurring in many different directions.

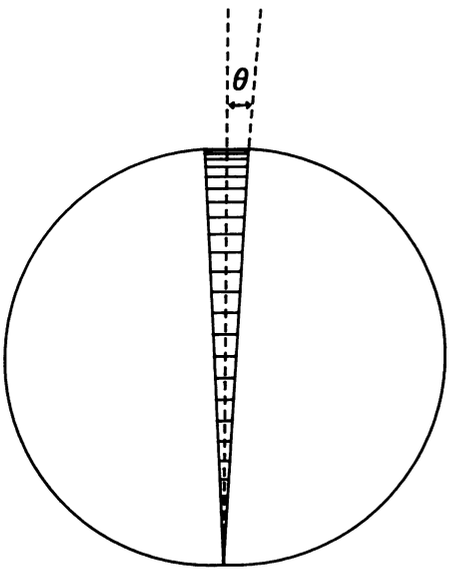

FIG. 1. Anisotropic breathing model.

For the value of r_0 in Eqs. 3, 4, 6, and 7, we shall use the following approximation,

$$r_0 \simeq \bar{r} \simeq [(3/4\pi)\bar{v}_2/N_0]^{1/3}, \qquad [8]$$

in which $\bar{v}_2$ is the experimental partial molar volume of the protein and N_0 is Avogadro's number. Note that $\bar{v}_2/N_0$ is not exactly the same as $\bar{v}$, the configurational average volume of a protein molecule, because of the effect of solvation (see section 3). Also, r_0 should perhaps be somewhat smaller than the radius of the sphere of volume $\bar{v}$ for two reasons. First, r_0 gives the minimal size and not that of the thermal average. However, because the time average extent of thermal fluctuation is typically only about 0.04 Å (see section 4), the difference between r_0 and $\bar{r}$ will be well within the experimental error of the measurement of $\bar{r}$. Second, r_0 is the radius at which new surface appears, which is likely to be at the bottom of the atomic scale folds of the protein surface. However, this feature is probably minor compared to the approximation of treating the molecule as a sphere in the first place.

3. Treatment of the experimental data

Considering a protein molecule as a system by itself (4), the extent of volume fluctuation can, in principle, be obtained from the compressibility measurement through the relation (5):

$$\text{rms}(\delta v) = (kT\bar{v}\,\beta_T^P)^{1/2}, \qquad [9]$$

in which β_T^P is the isothermal compressibility of the protein. However, this quantity is not directly measurable. What can be measured is the compressibility of the whole solution. Extracting the contribution made by the protein is complicated because of the difficulty of treating the effect of hydration of the protein. There is a further complication in that the compressibility can be measured most conveniently by the sound velocity

† In passing, we note the results of a harmonic potential—i.e.,

$$w_H(r) = 4\pi\gamma(r - \bar{r})^2$$

for all values of r, where $\bar{r}$ is the mean value of r and the subscript H indicates the harmonic potential. In this case, a straightforward application of the usual fluctuation theory (2) gives

$$\text{rms}(\delta r)_H = \left(\frac{kT}{8\pi\gamma}\right)^{1/2}$$

and

$$\text{rms}(\delta v)_H = \left(\frac{2\pi kT}{\gamma}\right)^{1/2} \bar{r}^2.$$

For reasonable values of γ and $\bar{r}$ (see below), these give fluctuations that are an order of magnitude larger than those given by the wedge-shaped potential.

technique, which yields the adiabatic rather than the isothermal compressibility. To convert the former to the latter, one needs information on C_V and C_P, the constant volume and constant pressure heat capacities, respectively, or C_P and α, the thermal expansivity. These data are not always available.

The effect of hydration may be handled in the following manner. Suppose that, in a solution made of n_1 moles of water and n_2 moles of protein, some of the solvent has a molar volume or the compressibility (or both) different from the remaining bulk solvent on account of the presence of the protein. Call this differently behaving solvent the water of hydration. The total volume of the solution then is given by

$$V = n_1 v_1^0 + n_2 \phi_2 \quad [10A]$$

$$= n_1 \bar{v}_1 + n_2 \bar{v}_2 \quad [10B]$$

$$= n_1^H v_1^H + n_1^B v_1^B + n_2 v_2^P, \quad [10C]$$

in which v_1^0 is the molar volume of pure water, ϕ_2 is the apparent molar volume of the protein, $\bar{v}_1$ and $\bar{v}_2$ are the partial molar volumes of the water and protein, respectively, n_1^H and n_1^B are the number of moles of hydration and bulk water, respectively, and v_1^H and v_1^B are the molar volumes of hydration and bulk water, respectively. Note that $n_1^H + n_1^B = n_1$. If the solution is dilute, $\bar{v}_1$ and v_1^B are both equal to v_1^0 and $\phi_2 = \bar{v}_2$. The volume v_2^P is defined actually by Eq. **10C** and represents the volume of what might be called the "naked" protein. Note that it is not equal to $\bar{v}_2$ unless $v_1^H = v_1^B$. We now adopt the interpretation that v_2^P/N_0 is the same as $\bar{v}$, the configurational average volume of a protein molecule, and that $\beta^P = -\frac{1}{v_2^P}\left(\frac{\partial v_2^P}{\partial P}\right)$. Differentiating Eq. **10C** with respect to pressure, we have

$$-\left(\frac{\partial V}{\partial P}\right) = n_1^H v_1^H \beta_1^H + n_1^B v_1^B \beta_1^B + n_2 v_2^P \beta^P + (v_1^B - v_1^H)\left(\frac{\partial n_1^H}{\partial P}\right), \quad [11]$$

in which the differentiation is under either isothermal or adiabatic conditions. Thus, to obtain β^P from the compressibility of the solution, one needs information on the amount, molar volume, the compressibility, and the rate of change in the amount, as a function of pressure, of the water in the state of hydration. None of these quantities is known with high reliability. Probably some of these are not precisely definable. Therefore, we shall limit ourselves to the use of the simple approximations of Eden *et al.* (6)—i.e., we assume that the molar volume of the hydration water is the same as that of the bulk, its compressibility is equal to that of ice, and its amount does not change with pressure. The first of these approximations makes v_2^P (and hence, $N_0\bar{v}$) equal to the partial molar volume $\bar{v}_2$. It then can be shown that, for a dilute solution,

$$\beta_T^P = \beta_T + \beta_h \quad [12A]$$

$$\beta_T = -\lim_{n_2 \to 0} \frac{1}{\phi_2}\left(\frac{\partial \phi_2}{\partial P}\right)_T \quad [12B]$$

$$\beta_h = h\frac{v_1^S}{v_2^S}(\beta_1^B - \beta_1^H), \quad [12C]$$

in which h is the number of grams of hydration water per gram of protein, $v_1^S = 1$ ml/g is the specific volume of water, and v_2^S is the specific volume of the protein. Following Eden *et al.* (6), we use the compressibility of water, 45×10^{-12} cm^2/dyne, and that of ice, 18×10^{-12} cm^2/dyne, for β_1^B and β_1^H, respectively. For h, we use either the experimental value, when available, or 0.4 g/g of protein, which is the average of the values reported by Gekko and Noguchi (ref. 7; Table 2).

In addition, because β_T often is not available, we shall generally use

$$\beta_T = \beta_S + 3.5 \times 10^{-12} \text{ cm}^2/\text{dyne}, \quad [13]$$

in which β_S is the apparent adiabatic compressibility of the protein defined similarly as β_T. The constant difference between β_T and β_S is the average difference between values given by Gekko and Noguchi (ref. 7; Tables 1 and 2).

Table 1. Primary experimental data used

	M_r	v_2^S, ml/g*	β_S, cm^2/dyne $\times 10^{12}$†
Ferrocytochrome *c*	12,800	0.733	−2.56
Ferricytochrome *c*	12,800	0.731	3.20
Ribonuclease A	13,700	0.704	1.12
Lysozyme	14,300	0.712	4.67
α-Lactalbumin	14,300	0.736	8.27
Myoglobin	17,000	0.742	8.98
β-Lactoglobulin	18,400	0.751	8.45
Trypsin	23,000	0.717	0.92
α_S-Casein	23,600	0.732	5.68
α-Chymotrypsinogen A	25,700	0.733	4.05
Ovomucoid	28,000	0.696	3.38
Pepsin	35,500	0.743	8.60
Ovalbumin	46,000	0.746	9.18
Bovine serum albumin	68,000	0.735	10.5
Hemoglobin	68,000	0.754	10.9
Conalbumin	75,500	0.728	4.89

All data are from Gekko and Noguchi (7), except those for the cytochrome *c*, which are from Eden *et al.* (6).
* Specific volume.
† Apparent adiabatic compressibility.

4. Results

The experimental data used are given in Tables 1 and 2. All of the compressibility data came from the sound velocity "sing around" technique of measurements made at 2 or 3 MHz. The rms(δv) values calculated from these data and the γ values calculated, in turn, from these rms(δv) values are given in Table 3.

An unsatisfactory aspect of this study is the fact, pointed out by Gekko and Noguchi (7), that the hydration effect plays such

Table 2. Additional experimental data used

	β_T, cm^2/dyne $\times 10^{12}$*	h, g/g of protein†
Ribonuclease A	5.48	0.40‡
Lysozyme	7.73	0.34
β-Lactoglobulin	11.8	0.55
α-Chymotrypsinogen A	6.95	0.34
Ovalbumin	12.1	0.33
Bovine serum albumin	14.6	0.40

All data are from Gekko and Noguchi (7), except for the one value noted.
* Apparent isothermal compressibility.
† Amount of hydration.
‡ This entry is not given by Gekko and Noguchi (7). The number given is the average of the other five numbers in this column.

Table 3. Volume fluctuations and empirical interfacial tensions

	Radius, Å*	rms(δv), Å^3†	γ, cal/mol/Å^2‡
Ferrocytochrome *c*	15.5	100	46
Ferricytochrome *c*	15.5	117	39
Ribonuclease A	15.6	115, 117	40, 40
Lysozyme	15.9	127, 120	37, 39
α-Lactalbumin	16.1	138	35
Myoglobin	17.1	153	33
β-Lactoglobulin	17.6	158, 173	33, 30
Trypsin	18.7	148	37
α_S-Casein	19.0	168	33
α-Chymotrypsinogen A	19.5	169, 158	34, 37
Ovomucoid	19.8	173	34
Pepsin	21.9	219	30
Ovalbumin	23.9	252, 237	28, 30
Bovine serum albumin	27.1	313, 316	26, 25
Hemoglobin	27.3	317	25
Conalbumin	27.9	295	28

* Computed from the partial specific volume and M_r according to Eq. 8.

† Obtained by using the β_S values and assuming that $h = 0.4$ g/g of protein. When two numbers are given, the second number was obtained by using the β_T and hydration values given in Table 2.

‡ Obtained by using the rms(δv) values and Eq. 4 at 25°C.

a major role in determining the observed compressibility. The estimate of this effect can be made readily by means of Eq. **12C**. By using $h = 0.4$ and $v_2^S = 0.73$ ml/g, the value of β_h is 15 $\times 10^{-12}$ cm^2/dyne. This is larger than β_S or β_T of any protein used in the data set. The ranges of values obtained by Gekko and Noguchi [the $1/\bar{v}^0\ (\partial\Delta V_{sol}/\partial P)_S$ term in their notation], using much more detailed information on the hydration, also are generally centered about this value. Unfortunately, despite the large amount of effort made on the subject (8), much is still unknown about protein hydration. Because this has such a large effect on the measured compressibility, the extent of volume fluctuation calculated from the compressibility data necessarily carries a large degree of uncertainty. The magnitude of this uncertainty also is not known. For lack of other measures, we might use the ranges of values of β_h estimated by Gekko and Noguchi as an estimate of this uncertainty. They are about 8 $\times 10^{-12}$ cm^2/dyne on the average. Assuming that errors in the estimate of β_T can be ignored, this translates roughly into about ±15–20% error in the estimate of β_T^P and about ±10% error in the estimate of rms(δv). Therefore, the values of γ given in Table 3 must carry an error of at least this magnitude.

If one uses the value of 31 cal/mol/Å^2 (1 cal = 4.184 J) for γ and 20 Å for the radius of a typical protein, we obtain the following representative values for the quantities that measure the extent of fluctuation. The rms(δr) is calculated from Eq. 3 to be 0.04 Å at 25°C. The value of the half-opening angle θ in the anisotropic model is calculated by Eq. 6 to be 0.2°. The separation between the two hemispheres at the end opposite to the hinge point is 0.3 Å at this opening angle. It should be realized that these estimates of the extent of fluctuation are time averages. They do not measure the extent of occasional short-time fluctuations, which can be very large in magnitude and which must surely occur, as evidenced by the hydrogen exchange, chemical modification, and other experimental data.

5. Discussion

The compressibility may be calculated for an isolated protein molecule by using a uniform elastic solid model (9) if proper elasticity parameters are used (10). For example, by using the values used by Gō (3, 9), one obtains a value of $\beta_P^T = 30 \times 10^{-12}$ cm^2/dyne, which compares favorably with the values of 16–32 $\times 10^{-12}$ cm^2/dyne obtained from the experimental measurements and Eq. 12. The success of this model depends on the proper choice of the elasticity parameters assumed for the protein and on the validity of the harmonic approximation. Results of recent molecular dynamics calculations on an α-helix (11) indicate that harmonic approximation significantly underestimates the extent of fluctuation. The approach taken in this article is the opposite in the sense that it, at least formally, considers only the solvent.

The idea of solvent cage force and of the interfacial tension that presumably exists at the interface of a nonpolar solute and water is not new (12). However, whether the change in the Gibbs free energy associated with hydrophobic effect can be usefully approximated so simply in terms of an interfacial tension is a matter of controversy (see ref. 13 and the discussions following it). Apparently there is little theoretical basis for expecting such a simple formulation (14).

Yet, I am aware of three different but related experimental systems that yielded the magnitude of hydrophobicity in terms of what is at least formally an interfacial tension between a nonpolar solute and water.

Nozaki and Tanford (15) measured solubilities of amino acids in ethanol, dioxane, and water and reported a table of values for the change in the standard Gibbs free energy upon transferring nonpolar amino acid side chains from ethanol or dioxane to water. Chothia (16) noted that these values varied in proportion to their surface area, as calculated by Lee and Richards (17). The proportionality constant, which has the dimensions of a surface tension, was found to be about 25 cal/mol/Å^2.

Similarly, the solubility of a liquid hydrocarbon in water gives the value of the change in the standard Gibbs free energy upon transferring the hydrocarbon from its pure liquid environment to water. Hermann (18, 19) noted that these values were a linear function of their surface area with a slope of 33 cal/mol/Å^2. Reynolds *et al.* (20) obtained a value of 29 cal/mol/Å^2 from similar data.

Lastly, a most interesting observation was made by Parsegian (21, 22) in connection with a study of the membrane deformability. The liquid–crystal lattice structure formed by fatty acid bilayers at high concentration is determined primarily by the electrostatic force between and among the charged head groups of the fatty acid and the counter ions present and by the effective repulsion between the hydrophobic fatty acid tail and water. Simply stated, the net electrostatic force tends to spread the charged head groups apart from one another, expose the hydrophobic tail to water, and make the bilayer thinner. The hydrophobic force tends to decrease the exposure of the tail, pull the charged polar goups together, and make the bilayer thicker. Therefore, when the electrostatic forces are properly accounted for, the equilibrium structure of such a system yields information on the magnitude of the repulsive force between water and the hydrocarbon tail. Parsegian assumed this force to be given in the form of an interfacial tension and found the value of γ to vary within a narrow range of 18.5–19.5 dyne/cm, or 27–28 cal/mol/Å^2, for a large number of different fatty acids. The same result was obtained more recently by Jonsson and Wennerstrom (23).

It is rather remarkable that the values of γ found in this study (Table 3) are quite similar to the values quoted above. Recall that the model neglects all intramolecular forces and includes only the protein–solvent interaction term. Furthermore, this term is assumed given in the form of surface tension. Therefore, there is the possibility that the similarity in the γ values obtained is produced by a cancellation of errors—one due to the

neglect of all intramolecular forces and the other due to approximation of the effect of solvent by a surface tension force. However, it seems unlikely that such cancellation of errors would occur by pure accident. Rather, it seems possible that a proper treatment of all of the forces involved could be reduced, to a certain useful degree of approximation, to an effective surface tension formulation. Clearly, this formulation is completely inadequate for certain applications—e.g., in explaining the observed difference in the compressibility of the oxidized and reduced cytochrome *c*. However, the concept appears to be useful in providing a general order of magnitude of the extent of volume fluctuation.

APPENDIX

1. Wedge potential

Suppose the potential energy of a system is given by

$$W(x) = a'x \quad \text{when } x > 0 \qquad [A1]$$
$$= \infty \quad \text{when } x < 0,$$

in which x is some dynamical variable and a' is a constant. The partition function of the system then is given by

$$Q = \int_0^\infty e^{-ax}dx, \qquad [A2]$$

in which $a = a'/kT$. Consider another dynamical variable y, which is linearly related to x—i.e.,

$$y = bx. \qquad [A3]$$

It then is simple to obtain the following configurational averages:

$$\bar{y} = b/a \qquad [A4]$$

$$\overline{y^2} = 2(b/a)^2 \qquad [A5]$$

$$\text{rms}(\delta y) = (\overline{(\delta y)^2})^{1/2} = (\overline{y^2} - \bar{y}^2)^{1/2} = \bar{y} = b/a. \qquad [A6]$$

2. Isotropic model

In the limit of small fluctuations, $\Delta A \approx 8\pi r_0^2 x$, in which $x = (r - r_0)/r_0$. Therefore, the potential given by Eq. 1 in the text is in the form of Eq. A1 and the partition function is given by Eq. A2 with $a = 8\pi r_0^2\gamma/kT$.

Writing $r = r_0 + \Delta r$, it is simple to show that the rms of r about its mean is the same as that of Δr about its mean. Because $\Delta r = r_0 x$, it is in the form of Eq. A3 with $b = r_0$. Application of Eq. A6 then immediately yields Eq. 3. Similarly, we can write $v = v_0 + \Delta v$ and $\Delta v = (4\pi/3)r_0^3[(1 + x)^3 - 1] \approx 4\pi r_0^3 x$. The last expression, obtained after dropping higher order terms, is again in the form of Eq. A3 with $b = 4\pi r_0^3$. Eq. 4 then follows from Eq. A6.

3. Anisotropic model

The potential energy given by Eq. 5 in the text is in the form of Eq. A1 if we write $\sin\theta \approx \theta$ for small θ. In this case, $a = 4\pi r_0^2\gamma/kT$. For θ, $b = 1$. The volume of the opening given in the text is in the form of Eq. A3 because $2\sin\theta\cos\theta = \sin 2\theta \approx 2\theta$ for small θ. In this case, $b = 2\pi r_0^3$. Application of Eq. A6 then yields Eqs. 6 and 7 of the text.

I thank Dr. F. M. Richards and Dr. Don Eden for stimulating discussions and for making their manuscript on the compressibility of cytochrome *c* available before publication. I am indebted to Dr. V. A. Parsegian for pointing out the essential difference between the potential used and the harmonic potential. The derivation presented in the *Appendix* was initially suggested by him.

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