Compressibility of Cavities and Biological Water from Voronoi Volumes in Hydrated Proteins

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This paper is concerned with the calculation of isothermal compressibilities of hydrated proteins via molecular dynamics simulation. New fluctuation formulas are derived to compute from one molecular dynamics simulation at a given pressure the protein intrinsic compressibility, β_p, and the compressibility of surface, or biological, water, β_{pw}. Changes in the Voronoi volumes of the protein and its residues are shown to be a direct measure of changes in the volume of the protein cavities. Compressions, i.e., relative volume changes, of the atomic core of the proteins studied here are sufficiently small, less than 3% of the total compression, to be neglected in the calculation of compressibility. Although this study finds that the averaged density of water near a protein surface is similar to that of the bulk, the compressibility of biological water, which is computed here for the first time, is 20−24% smaller than the compressibility of bulk water. The results of β_p and β_{pw} can be combined with the calculations of the protein and biological water volume fractions to estimate, with a small uncertainty, the apparent isothermal compressibility.

Introduction

It has been long recognized that the compressibility of a solvated protein can offer clues on the forces involved in the denaturation of proteins occurring at high pressure. Indeed, pressure denaturation can be explained by differences in volume between the native and the denatured states.[1] The latter is favored at high pressure by a smaller volume than that of the native state.

The adiabatic compressibility of protein solutions, or β_S, obtained readily from densimetry and sound velocity measurements, contains contributions from protein, water, and protein−water interactions. Past experimental investigations have associated the former contribution to the compressibility of the protein core. This so-called *intrinsic* compressibility, or β_p, is due to the response of the protein cavities to pressure changes. In the past few years, evidence has increased that β_p is alike in all proteins and is from 2 to 3 times smaller than that of water.[2−5] β_p is not directly accessible from experiment and has been extrapolated from a series of measures of the partial molar compressibility of globular proteins, ϕ_K, for increasing protein solvent accessible surfaces.

From its definition, ϕ_K is only affected by the volumetric properties of the protein and of its hydration water. Thus, because modifications of the protein−water interaction will affect ϕ_K, protein conformational changes and mutations can be readily monitored by volumetric techniques, today increasingly precise and accessible.[3,4,6,7]

If the volumetric properties of hydration water near the protein functional groups are additive, modeling the protein ϕ_K by adding independent contributions becomes possible. Indeed, experiments have shown that the volumetric properties of molecules composed of repeating subunits vary linearly with the number of subunits.[3] These findings support the picture that only the volumetric properties of the first hydration shell around the subunits are different from those of bulk water. However, recent theoretical investigations seem to suggest that the volume of water near a functional group is different from bulk, well beyond the first neighbors.[8,9]

A few years ago, the protein volume compression computed with the Voronoi technique was shown to be remarkably related to protein intrinsic compressibility.[10] In that investigation, β_p was computed from a finite difference approach by running two molecular dynamics simulations at different pressures and the hydration contribution from estimates of the apparent compressibility were obtained. This paper develops a theoretical approach to compressibility which, in perspective, will be used to interpret the volumetric experiments on proteins. Indeed, to further the theoretical understanding of protein compressibility, this study demonstrates that fluctuations and changes in the volumes of protein cavities are related directly to the protein Voronoi volume. Following this result, fluctuation formulas involving Voronoi volumes are derived to compute, directly from one simulation, the *intrinsic* compressibility of a protein and the contribution to compressibility due to hydration and bound water. This study validates the method by running molecular dynamics (MD) simulations in the NPT ensemble for three globular proteins in water with different force fields.

Technical Details

Molecular Dynamics. The volumetric results discussed in this study were obtained from simulation of bovine pancreatic trypsin inhibitor (BPTI), lysozyme, and T4 lysozyme in water solution. The three systems were prepared with an identical procedure. The X-ray structure of each of the proteins was first minimized with 1000 conjugate gradient steps, and then the final configuration was inserted in a truncated octahedron box of TIP3P water molecules. The hydrated BPTI (Protein Data Bank ID 4pti), lysozyme (PDB ID 3lym), and T4 lysozyme (PDB ID 3lzm) systems contained 2350, 4188, and 5005 water molecules,

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respectively. Subsequently, the systems were equilibrated first for 100 ps in the NVE ensemble and then for 200 ps at constant pressure and temperature ($P = 0.1$ MPa and $T = 300$ K). While 1 ns trajectories in the NPT ensemble were run for BPTI and lysozyme, the simulation of T4 lysozyme lasted for 3 ns. The latter system was then compressed at $P = 200$ MPa, and following 200 ps of equilibration, a new trajectory of 3 ns was accumulated. BPTI and lysozyme instead were compressed at 500 MPa and, following 200 ps of equilibration, further run for 1 ns each.

The force field due to MacKerell et al.[11] was used to model the atomic interactions within the systems. Simulations and minimizations were performed with the parallel version of the program ORAC.[12,13] The code implements highly efficient MD techniques for the sampling of biomolecular systems based on a multiple time scale approach. The simulations in the NPT ensemble allowed only for isotropic cell volume fluctuations and used a technique, described in ref 14, combining an r-RESPA algorithm with smooth particle mesh Ewald.[15] The barostat and thermostat parameters were those used in ref 14. Finally, bond stretching involving hydrogen atoms were frozen by using standard constraint techniques.

To check for the effect of the force field on the protein volumetric properties, an additional simulation of BPTI was run with a different protein potential under the same conditions as those described above.[16]

All the simulations at different pressures discussed in this paper reached stable conformational states after equilibration. In all cases, the deviation from the corresponding X-ray structure never exceeded 1.4 Å. The interested reader is referred to refs 13, 14, and 17 for a full discussion of the simulation techniques, parameters, and equilibration of molecular volumes used in this article.

Voronoi Volume and Cavities. In this study, the MD-generated trajectories of the solvated proteins have been used to compute directly the Voronoi volumes of all the atoms of the system except hydrogen for each saved configuration. The Voronoi volume of any parts of the system (protein, functional group, water, etc.) can be readily obtained by adding contributions from its constituent atoms. The Voronoi construction for each saved configuration was carried out using the recursive algorithm described in ref 18 and implemented in the program ORAC.

The Voronoi polyhedron construction is very useful to identify neighbors around a protein. Indeed, since all neighbors of any given atoms share a different Voronoi face, it is possible to count the hydration waters in the first shell as those sharing at least a face with one protein atom.

Calculation of the cavity volume or voids was performed after the Voronoi polyhedrons were obtained for each atom. It proceeded as follows. First, for each configuration studied, a three-dimensional grid was constructed with points equally spaced $\simeq$0.3 Å from each other. Second, each grid point was assigned to the Voronoi polyhedron containing it. Third, grid points were assigned to voids if their distance from the neighboring atoms was closer than a hard-sphere contact. A hard-sphere diameter equal to its Lennard-Jones σ parameter was assigned to each atom of the system.

The volume of cavities for each atom was then evaluated by multiplying its Voronoi volume by the fraction of grid points assigned to voids in the corresponding Voronoi polyhedron. Although this procedure is not exact, it was verified that with this fine-grid errors on the cavity volume per residue was $<0.1\%$.

Results and Discussion

Volumes and Compressibilities. The apparent protein volume, V_a, and compressibility, β_a, can be derived experimentally by combined densimetry and sound velocity measurements. From experiments at different protein concentrations, the protein partial molar volume, $\bar{V}_0$, and compressibilities, $\bar{K}_0$, can be derived. In a constant pressure, P, and temperature, T, simulation of one protein, V_a, can be obtained by subtracting the averaged volume of simulation water in standard bulk conditions from the averaged system volume, or

$$V_a = V - n_w v_w^0 \tag{1}$$

where n_w is the number of waters contained in the simulation box and v_w^0 is the volume of a bulk water molecule at the same P and T conditions.

The apparent isothermal compressibility (sometimes called the coefficient of isothermal compressibility in experimental literature) is then obtained as

$$\beta_a = -\frac{1}{V_a}\left(\frac{\partial V_a}{\partial P}\right)_{NPT} \tag{2}$$

$$= \frac{1}{1 - x_w^0}\beta - \frac{x_w^0}{1 - x_w^0}\beta_w \tag{3}$$

with β and β_w the isothermal compressibilities of the simulated system and of bulk water, respectively, and $x_w^0 = n_w v_w^0/V$ the volume fraction of bulk water.

Volumetric analysis based on data from X-ray crystallography of water molecules in contact with the protein has shown that, on average, the volume of surface water is not far from that of the bulk water. Nevertheless, the compressibility of water at the interface is thought to be significantly different than in the bulk water. Thus, the system volume V is separated initially into three components:

$$V = V_p + V_{pw} + V_w \tag{4}$$

where V_p is the protein volume, V_{pw} is the volume of the water near the protein surface, or biological water,[19,20] and V_w is the volume of the bulk water of the system.

With the breakdown of eq 4 one can write β as a sum of three contributions: from the protein compressibility β_p, the compressibility of biological water β_{pw}, and the bulk water β_w. Thus,

$$\beta = x_p\beta_p + x_{pw}\beta_{pw} + x_w\beta_w \tag{5}$$

Here, x_p, x_{pw}, and x_w are the volume fractions of the protein, biological water, and bulk water, respectively, and β_p, β_{pw}, and β_w are defined as

$$\beta_x = -\frac{1}{V_x}\left(\frac{\partial V_x}{\partial P}\right)_{NPT} \tag{6}$$

with the x standing for subscripts p, pw, and w. If the volume of the hydration water is not far from that of the bulk water $x_w^0 \simeq x_w + x_{pw}$, with elementary algebra one can rewrite β_a in eq 3 as

$$\beta_a = \beta_p + \lambda(\beta_{pw} - \beta_w) \tag{7}$$

In the above equation, $\lambda = x_{pw}/x_p$, which is the ratio between the surface water and protein volumes. The second term on the

right-hand side of eq 7, or β_{hyd}, is expected to be negative because of the electrostrictive reduction of the biological water compressibility.[2,21] As shown further in the paper, the relation $x_w^0 \simeq x_w + x_{pw}$ is satisfied by the model of water used in this study.

It must be noticed that results in eqs 5 and 7 derive directly from the additivity of the subsystem volumes defined in eq 4. This property is crucial to the calculation of compressibility from computer simulations. Indeed, the volume of the simulation box is a well-defined observable in simulations at constant pressure, whereas the volume of any given subsystem is not.

As shown by various applications found in the literature, V_p can be readily obtained from structural data as the van der Waals or molecular volume of the protein.[22] Unfortunately, this approach does not easily allow for the computation of V_{pw} and V_w consistent with the additivity of eq 4. In contrast, the Voronoi polyhedrons associated with a given distribution of atoms for a periodic system, such as a simulation box, are additive by definition because the sum of their volumes equals the total system volume. Thus, for each point of an MD trajectory the use of the Voronoi construction allows the computation of the volume of each subpart of the total system by straightforwardly adding up the contributions of all of the atoms of the subpart.

Once the three components of the system volume V are calculated consistently for each point of the MD trajectory, β_p, β_{pw}, and β_w can be obtained from the following fluctuation formula:

$$\beta_x = \frac{1}{k_B T} \frac{\langle \delta V_x \delta V \rangle}{\langle V_x \rangle} \tag{8}$$

where the symbol $\langle ... \rangle$ stands for the average of the statistical ensemble and the subscript x can be replaced by p, pw, and w. Equation 8 can be readily obtained from the definitions of β_p, β_{pw}, and β_w in eq 6 by taking the derivative of the statistical ensemble averages which define V_p, V_{pw}, and V_w, respectively.

Further advances can be made if one again divides the protein volume into two contributions:

$$V_p = V_c + V_{cav} \tag{9}$$

where V_c is the protein core volume, which in this paper is defined as the volume of the van der Waals envelope of the protein, and V_{cav} is the free volume (or volume of voids) of the protein not occupied by the core. As defined in the past by numerous experimental articles (see, for instance, ref 21) the protein intrinsic compressibility is:

$$\bar{\beta}_p = -\frac{1}{V_p}\left(\frac{\partial V_{cav}}{\partial P}\right) \tag{10}$$

If V_c changes little with pressure, then $\beta_p = \bar{\beta}_p$. Table 1 shows that this is at least the case for the three proteins studied here. Differences in Voronoi and cavity volume between two pressures, or $\Delta V_{P_1}^{P_0}$, are very similar to each other for the three simulations with deviations within 3% in the worst case. Identical results are also found for the relative changes of the residue volumes for lysozyme and BPTI, as shown in Figure 1.

On the basis of these findings, the volume fluctuations δV_p and δV_{pw} in eq 8 can now be replaced by the corresponding fluctuations in the Voronoi volumes of the protein and of the hydration water. To compute δV_{pw}, each water molecule with at least one face shared with a protein atom was assigned to the protein solvation shell and its volume counted in the average of bound water volume at each instant. With this definition of bound water, areas of 14, 16, and 14 Å^2 per hydration water were obtained for BPTI, lysozyme, and T4 lysozyme.[28] A value of 15 Å^2 is generally accepted in the literature (see refs 23−24).

TABLE 1: Compression of Protein Cavities[a,b,c]

	0.1 MPa	500 MPa	$\Delta V_{500}^{0.1}$
	BPTI		
V_{cav}/V_p	0.283	0.230	
V_{cav}	2245	1695	550
V_p	7932	7371	561
	LYSOZYME		
V_{cav}/V_p	0.297	0.245	
V_{cav}	5266	4036	1230
V_p	17733	16475	1258
	T4LYSOZYME		
V_{cav}/V_p	0.295	0.271	
V_{cav}	6916	6145	771
V_p	23443	22649	794

[a] V_p and V_{cav} are the Voronoi volume and the volume-free space in the protein, respectively, as defined by eq 9. [b] $\Delta V_{P_2}^{P_1}$ is the difference between two volumes computed at pressures P_1 and P_2. [c] All volumes are in Å^3.

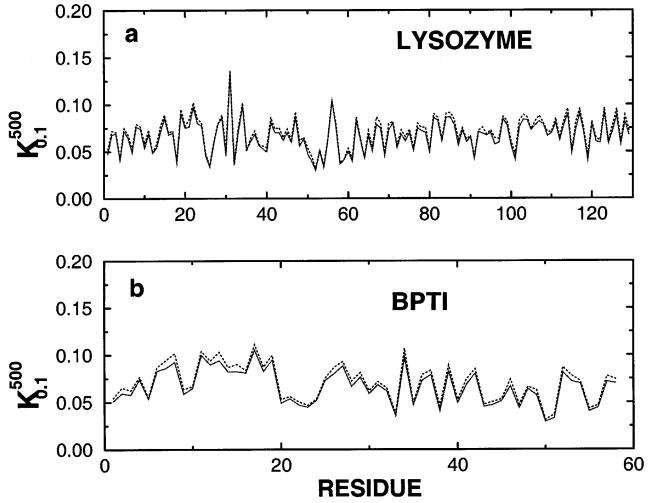

Figure 1. Comparison between the compressions of the residues. $K_{0.1}^{500}$ is defined as the relative change in Voronoi or cavity volumes of each residue between 500 and 0.1 MPa. On panels a and b, results for lysozyme and BPTI (CHARMM force field) are shown, respectively. Dotted lines are cavity compressions, whereas solid lines are results from Voronoi volumes.

In this way, β_p and β_{pw} can be computed directly from simulations at any given pressure. The compressibility of bulk water, $\beta_w = 54 \times 10^{-5}$ MPa^{-1}, was obtained from an extra simulation of 5000 tip3 waters run at $T = 300$ K and $P = 0.1$ MPa for 500 ps. This result is in fair agreement with an experimental value of β_w of 45×10^{-5} MPa^{-1}.

In Table 2, the results from 4 MD simulations are shown. In this table, the uncertainty of the Voronoi volumes of the protein and solvation water is small, less than 2 Å^3. The statistical error of the β's is larger. On compressibilities obtained from 1 ns simulations (BPTI and lysozyme), an uncertainty of $\simeq 3 \times 10^{-5}$ MPa^{-1} is estimated. For T4 lysozyme which was run 3 ns, the statistical error is smaller, around 2×10^{-5} MPa^{-1}. Larger errors around $4-5 \times 10^{-5}$ MPa^{-1} are found in β_a, computed from eq 7. It must be pointed out that errors on the order of 15 units are to be expected if eq 3 is used instead.

First, in all four cases the hydration contribution, or β_{hyd}, to β_a is found to be negative, as expected from experimental estimates. A negative value was also obtained in a previous

TABLE 2: Isothermal Volumes and Compressibilities[a]

protein	N_w	N_{pw}	V_{pw}	β	β_p	β_{pw}	β_{hyd}	β_a	λ	$\Delta\beta_w$
BPTI[b]	2346	251	7448	49	24	39	−14	10	0.945	2.4
BPTI[c]	2346	245	7385	50	21	44	−14	7	0.931	0.12
Lym	4059	441	13 102	49	22	41	−10	12	0.739	1.0
T4[d]	5005	571	17 014	48	24	44	−7	17	0.726	2.4
T4[e]	5005	610	17 022	27	15	24	−5	10	0.752	
T4[f]				35	17	32	−4	13		

[a] Results are from four simulations. N_w and N_{pw} are the number of water molecules in the simulation and in the solvation shell of the protein, respectively. All other symbols are explained in the text. The units for volume and compressibilities are Å and 10^{-5} MPa^{-1}, respectively. The hydration contribution, β_{hyd}, was computed using $\beta_w = 54 \times 10^{-5}$ MPa^{-1}, obtained from an independent simulation at $T = 300$ K and $P = 0.1$ MPa. $\Delta\beta_w$ defined in the text is given in percentage points. [b] Simulation of BPTI with the AMBER[16] force field. [c] Simulation of BPTI with the CHARMM[11] force field. [d] Results obtained from fluctuation formulas at $P = 0.1$ MPa. [e] Results obtained from fluctuation formulas at $P = 200$ MPa. The hydration contribution was obtained with $\beta_w = 31 \times 10^{-5}$ MPa^{-1} and computed from a simulation of water at $P = 200$ MPa. [f] Results obtained with finite differences.

calculation from finite differences.[10] Also noticeable is the finding that β_{pw} is 20−24% smaller than the compressibility of bulk water. This is in good agreement with experimental estimates of 35×10^{-5} MPa^{-1} for bound water in ref 2. Despite these significant differences between β_{pw} and β_w, the average volume available to bound water near the protein, i.e., to biological water, is on average close to that of water in the bulk. For T4 lysozyme, bound water volume is 29.78 which is 0.4% larger than that of tip3 bulk water. Similar results were also obtained for the other simulations, confirming that the relation $x_w^0 \simeq x_w + x_{pw}$ as the basis of eq 7 is correct.

Results for the protein intrinsic compressibility are also reported in Table 2. Here, β_p is, for all proteins, within statistical error of each other with an average value of 23×10^{-5} MPa^{-1}, which compares with an experimental estimate of 12×10^{-5} MPa^{-1}.

Also, the apparent compressibilities, β_a, reported in Table 2 are close to one another for all the systems studied; the larger value, 17×10^{-5} MPa^{-1}, having been obtained for T4 lysozyme. β_a of lysozyme is close to its experimental estimate of 8×10^{-5} MPa^{-1}, reported in ref 21.

Because of the relation

$$x_w{}^0\beta_w^T = x_{pw}\beta_{pw} + x_w\beta_w \qquad (11)$$

β_w can be computed from the protein simulation once β_{pw} and β_w^T are determined. Here, β_w^T is the compressibility of all the water of the simulation. The last column of Table 2 reports the relative differences, or $\Delta\beta_w$, between the $\Delta\beta_w$ computed from an independent simulation of the bulk and those obtained for the three proteins from eq 11. This quantity is, in all cases, less than 3%, a deviation probably due to solvation effects beyond the first neighbors.

Finally, in the last line of Table 2, finite difference compressibilities are reported for T4 lysozyme obtained from simulation at $P = 200.0$ MPa (label T4[f]). The comparison of these values with those obtained at $P = 0.1$ MPa (label T4[d]) from the fluctuation formula in eq 8 shows a pronounced nonlinear behavior with differences between compressibilities of about 24−29%. On the line labeled T4[e], compressibility results from fluctuation formulas at $P = 200$ MPa are also shown. A finite difference estimates of the Moelwyn−Hughes' nonlinearity index, $\mu = (\partial\beta_p^{-1}/\partial P)_T$, gives a value of 12.5. Experimental estimates[5] from sound velocities are larger than 10.

Conclusion

This paper has shown how isothermal compressibilities of hydrated protein solutions can be readily and simply computed from molecular dynamics simulations. The Voronoi volumes of the protein, hydration, and bulk water are used in the calculation of the respective compressibilities. Because of the little changes in the molecular core volume, variations in the Voronoi volumes are a direct measure of the changes in the volume of cavities in proteins. This paper has thus demonstrated that the compression of cavities or voids in three different proteins is within 2−3% of that of their corresponding Voronoi volumes. This is also true for the compression of the amino acid residues.

On the basis of these findings, new fluctuation formulas have been derived to compute, from only one simulation, the protein intrinsic compressibilities, β_p, and that of the hydration, or biological, water, β_{pw}. This study confirms that β_p does not vary much within different proteins, as suggested experimentally and inferred in a previous theoretical study. Although the averaged density of water near a protein surface is similar to that of the bulk water, its density fluctuations contribute to an anomalous β_{pw}, which is here computed for proteins for the first time. This finding confirms once again the peculiar character of biological water, already pointed out in previous experimental and computational papers.[19,20,23−27] This differential between surface and bulk water is at the base of the solvent negative contribution to the apparent compressibility.

Finally, eq 7 is found particularly accurate for computing the apparent compressibility β_a. It is based on the assumption, verified by this study, that only the first shell of hydration water has a compressibility different from that of the bulk water. This result is of crucial importance for studies of volumetric properties of hydrated proteins aimed at theoretically relating compressibility and structure.

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(28) This calculation was carried out by dividing the statistically averaged protein Voronoi surfaces by the hydration numbers found in Table 2. Each protein Voronoi surface was obtained by counting as protein surface all facets of the Voronoi polyhedron of each protein atom shared with a water molecule.