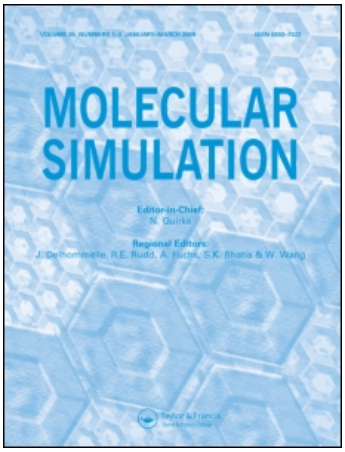

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Hydration structure, thermodynamics, and functions of protein studied by the 3D-RISM theory

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Hydration structure, thermodynamics, and functions of protein studied by the 3D-RISM theory

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The three-dimensional reference interaction site model (3D-RISM) theory is a molecular theory of solvation, which has been developed recently. Unlike the conventional statistical-mechanical theories of liquids, the applications of which are limited to rather simple systems, the new theory has been demonstrating great success in predicting the hydration structure and thermodynamic quantities of protein. Here, we review the recent applications of the 3D-RISM theory to some subjects concerning protein hydration: detecting water molecules in protein cavities, the pressure effects on protein conformation in terms of the partial molar volume, and the pressure reversal of anesthesia. These results demonstrate the outstanding capability of the theory for investigating various issues in biochemistry and biophysics.

Keywords: Three-dimensional reference interaction site model (3D-RISM) theory; Protein; Hydration; Pressure; Partial molar volume; Anesthesia

1. Introduction

Hydration structure and thermodynamics are keys for understanding the conformational stability and function of a protein. Molecular simulations have been prevalently employed for elucidating molecular detail of such processes. Although the methods are useful to investigate the short time dynamics of protein as well as small changes in its structure such as a point mutation, it is still difficult to treat a global change, which requires a sampling of the huge configuration space including solvent. The most popular alternative to bypass the difficulty is to use the continuum dielectric model complemented with an empirical method based on the accessible surface area to account for the hydrophobicity of the macromolecule. The obvious drawback of the method is its inability to account for microscopic nature of hydration including the hydrogen-bonding. The drawback has been overlooked or underestimated for long time. However, recent developments in experiments and theories have revealed a number of phenomena which demonstrate the importance of microscopic nature of water in understanding stability, dynamics, and functions of protein.

Especially important are the water molecules trapped inside a cavity or an active site of protein. When one considers the substrate binding pocket of a protein, the pocket would have been filled by some water molecules depending on the size of the cavity before it accommodates a substrate molecule. Whether or not the substrate can be accommodated into the pocket is determined by the relative affinity of the molecule to the binding site compared to that of water molecules. The substitution of water molecules by a substrate may change the thermodynamics or stability of protein entirely, depending on how the substitution takes place. In such cases, it is crucial to describe those water molecules in microscopic detail in order to understand the function of protein. The continuum theory is obviously hopeless in this regard. The molecular simulations do not have too much hope either, because the process usually involves slow and large-scale dynamics of protein and water. Then, a natural question to be asked is "Are there any other options?" The answer to the question is "yes." From our recent studies, it has been

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found that a statistical-mechanical theory of molecular liquids is a promising candidate to investigate the hydration structure and thermodynamics of proteins including water molecules trapped in a cavity.

The statistical mechanics of liquids has a rather long history [1,2], but it was quite recent that the theory could be applied to "real" biomolecular systems. Such applications had been impracticable due to the problems in the theory itself as well as the calculation algorithm and computer capability. In 2004 [3], we finally performed the first successful calculation of solvation structure and thermodynamic quantities of proteins in aqueous solution by using the three-dimensional reference interaction site model (3D-RISM) theory, an integral equation theory of molecular liquids developed recently.

It may be said that the history of integral equation theories of liquids started with the Ornstein-Zernike (OZ) equation proposed in 1914 [4]. The OZ equation was complemented with several closure relations such as the Percus-Yevick (PY) and hypernetted chain (HNC) approximations [1]. The theory was successful for describing the structure of liquids in terms of the density pair correlation functions such as the radial distribution function (RDF), which serve also as a weighting factor for a statistical average of mechanical quantities to produce thermodynamics. The application of the theory, however, had been largely limited to so-called simple liquids, systems consisting typically of hard-sphere or Lennard-Jones particles that do not form associating structures, such as hydrogen bonding. Due to the limitation, the theory could not be applied to molecular systems such as chemical reactions and protein stability which chemists and biochemists are interested in.

There are two ingredients to be considered for a successful theory in chemistry: molecular geometry and charge distribution. In 1972, Chandler and Andersen [5] made a crucial step toward the theory of molecular liquids presenting the reference interaction site model (RISM) theory, which uses the correlation functions between the atomic sites in molecules. The original RISM theory succeeded in the incorporation of molecular geometry, but it failed to deal with polar molecules such as water. Nearly a decade later, the theory was extended by Hirata and Rossky [6] to account for the charge distribution of molecules.

The extended RISM theory has been applied to a variety of chemical processes in solution, including chemical reactions, biomolecular thermodynamics, liquid dynamics, and so on [2,7]. Among the applications of the theory, most challenging without doubt was the solvation thermodynamics of protein. Protein has a large number of interaction atomic sites, which strongly interact with solvent water molecules. The RISM theory coupled with the standard closure relations, HNC or PY, often breaks down in describing the strong correlation, especially for the globular proteins having core regions where water molecules are not accessible. The most conspicuous failure was inspected in the partial molar volume (PMV), one of the fundamental thermodynamic quantities. We observed that the PMV of peptides calculated by the RISM/HNC theory started to decrease with increasing the number of amino acids at some size and even became negative in some cases [8]. The observation is unphysical since it is well-known experimentally that the PMV of proteins increases almost linearly with the molecular weight. The problem would arise from a superposition approximation with respect to the site–site direct correlation function employed in the RISM theory.

In the last decade, the RISM theory as well as the OZ theory has been generalized to describe the three-dimensional (3D) distribution of the solvent rather than the radial distribution [9–13]. The 3D generalization made possible the perfect account of the excluded effect of solvent by the solute molecule of arbitrary shape. Recently, we have shown that the 3D-RISM theory provides a dramatic improvement in the quantitative estimation of the PMV of amino acids and peptides [8]. We have further applied the theory to globular proteins and demonstrated that the calculated PMV was in quantitative agreement with the experimental data [3]. It has been found that the 3D-RISM theory provides reasonable results not only for such thermodynamic quantities but also for the hydration structure around and inside proteins [14]. Now, we can say the 3D-RISM theory is the most promising candidate to investigate the hydration structure and thermodynamics of proteins.

Here, we review some recent applications of the 3D-RISM theory to the hydration and thermodynamics of proteins. First, we demonstrate how the theory is useful in detecting the water molecules in protein cavities, which is almost impossible by the ordinary molecular simulation. Second, the application to the PMV of protein is presented. We show the PMV calculated by the 3D-RISM theory is in quantitative agreement with the experimental data. The final application concerns the pressure reversal of anesthesia, which should be explained by the PMV change accompanying the anesthetic-protein binding under the framework of thermodynamics. Before we show those results, we will briefly outline the 3D-RISM theory in the following section.

2. 3D-RISM theory

For a solute–solvent system at infinite dilution, the 3D-RISM equation is written as [2,12,13]

$$h_\gamma(\mathbf{r}) = \sum_{\gamma'} c_{\gamma'}(\mathbf{r}) * \left(w^{vv}_{\gamma'\gamma}(r) + \rho h^{vv}_{\gamma'\gamma}(r) \right) \qquad (1)$$

where $h_\gamma(\mathbf{r})$ and $c_\gamma(\mathbf{r})$ are, respectively, the 3D total and direct correlation functions of solvent site γ around the solute, the asterisk denotes a convolution integral in direct space, $w^{vv}_{\gamma'\gamma}(r)$ is the site–site intramolecular correlation function describing the molecular geometry of solvent, ρ is the number density of solvent, and $h^{vv}_{\gamma'\gamma}(r)$ is the site–site total correlation functions of solvent, which can be

obtained independently from a single-component RISM theory. The 3D distribution function $g_\gamma(\mathbf{r})$ is defined by $g_\gamma(\mathbf{r}) = h_\gamma(\mathbf{r}) + 1$.

The 3D-RISM equation is complemented by a 3D closure relation. Conventionally, we choose the 3D-HNC closure,

$$h_\gamma(\mathbf{r}) = \exp(-\beta u_\gamma(\mathbf{r}) + h_\gamma(\mathbf{r}) - c_\gamma(\mathbf{r})) - 1 \qquad (2)$$

where $\beta = 1/(k_B T)$, the inverse of the Boltzmann's constant times temperature, and $u_\gamma(\mathbf{r})$ is the interaction potential between solvent site γ and the whole solute. Another choice is to use the partially linearized 3D-HNC closure proposed by Kovalenko and Hirata [2,15], so-called the 3D-KH closure,

$$h_\gamma(\mathbf{r}) = \begin{cases} \exp(d_\gamma(\mathbf{r})) - 1 & \text{for} \quad d_\gamma(\mathbf{r}) \le 0 \\ d_\gamma(\mathbf{r}) & \text{for} \quad d_\gamma(\mathbf{r}) > 0 \end{cases} \qquad (3)$$
$$d_\gamma(\mathbf{r}) = -\beta u_\gamma(\mathbf{r}) + h_\gamma(\mathbf{r}) - c_\gamma(\mathbf{r})$$

It combines the HNC approximation in the spatial regions of density depletion where $g_\gamma(\mathbf{r}) < 1$, and the mean spherical approximation (MSA) [1] in the enrichment regions of $g_\gamma(\mathbf{r}) > 1$. The MSA-type linearization properly describes peaks of strong association and prevents the artifact of the HNC divergent in the regions with a large interaction potential, whereas the HNC ensures a proper physical behavior in the repulsive core region. Thus, the 3D-KH closure (3) is appropriate for species with strongly attractive chemical functionalities, which frequently occurs for systems with mixed solvents. We employed the 3D-HNC closure for all the calculations in this review.

The 3D potential functions $u_\gamma(\mathbf{r})$ are constructed from standard models used in molecular simulation, consisting of the Lennard-Jones and electrostatic interaction terms: for example, AMBER or CHARMM parameter set for protein and SPC/E or TIP3P model for water. If the solute has the net charge, special electrostatic corrections for supercell finiteness are added to equation (2) or (3) [16].

3. Application to hydration of protein

Water molecules confined inside cavities in a protein are of great importance in understanding its structural stability [17,18] and function [19–21]. Such water molecules can also be an important clue to the structure-based rational drug design [22]. Considerable efforts have therefore been devoted to observe such water molecules by several experimental methods such as X-ray [23] and neutron diffraction [24], and NMR [25,26]. However, it is still a nontrivial task. It is virtually impossible to "find" water molecules in a protein cavity by the ordinary molecular simulation because they are most likely trapped in the protein through a process of large conformational fluctuation. The simulation of such water molecules is as difficult as the protein folding itself. Indeed, water molecules could not penetrate into or escape from some interior sites of protein in molecular dynamics (MD) simulation of the nanosecond order [27–29]. The consequence is also expected from NMR studies [25,26], which have shown that the residence times of the water molecules buried in internal cavities are in the range from about 1 ns to 10 ms.

Recently [14], we carried out the 3D-RISM calculation for hen egg-white lysozyme (the structure was taken from PDB code 1hel [30]) immersed in water and obtained the 3D-distribution function of oxygen and hydrogen of water molecules around and inside the protein. The protein is known to have a cavity composed of the residues from Y53 to I58 and from A82 to S91, in which four water molecules have been determined by means of the X-ray diffraction measurement [30]. Figure 1(a) gives an isosurface representation of the 3D-distribution functions $g(\mathbf{r})$ of water oxygen and hydrogen inside the cavity, calculated with the 3D-RISM/HNC equations. The green and pink surfaces or spots are the area where $g(\mathbf{r})$ is larger than 8, for oxygen and hydrogen, respectively. There were four distinct peaks of water oxygen and seven distinct peaks of water hydrogen in the cavity. From the isosurface plot, we reconstructed the most probable model of the hydration structure, which is shown in figure 1(b). The four water molecules are numbered in the order from

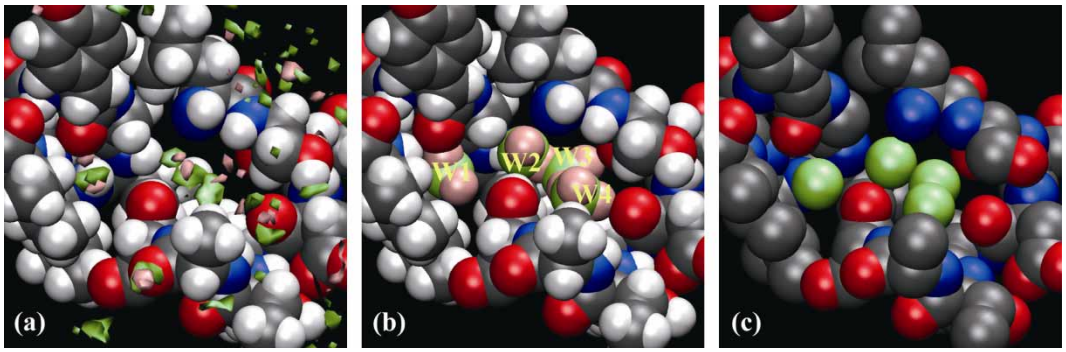

Figure 1. Water molecules in the cavity surrounded by the residues from Y53 to I58 and from A82 to S91. Only the surrounding residues are displayed, except for A82 and L83 locating in the front side; (a) the isosurfaces of $g(\mathbf{r}) > 8$ for water oxygen (green) and hydrogen (pink) obtained by the 3D-RISM theory in online version; (b) the most probable model of the hydration structure reconstructed from the isosurface plots; (c) the crystallographic water oxygen sites from X-ray analysis.

the bottom of the cavity. It is obvious that the hydration structure obtained by the 3D-RISM theory is in excellent agreement with the X-ray structure, which is given in figure 1(c). The theory also reproduced reasonable hydrogen-bonding networks among the water molecules and polar groups in the cavity. It should be emphasized here that the theory does not require any information concerning the coordinates of these water molecules in advance of the calculation. Thus, our results demonstrate strongly the capability of the 3D-RISM theory to "detect" such water molecules confined in the active site of protein.

Then we calculated the actual number of the water molecules in the cavity by integrating the distribution function within the cavity, because one peak of the distribution function does not necessarily correspond to one molecule. In fact, the number was 3.8, which is less than the number of the water-binding sites. To explain the hydration number, we carried out 5 ns MD simulation using the same parameters and under the same thermodynamic conditions as the 3D-RISM calculation. Only one exception was that the four crystallographic water molecules in the cavity were initially placed at their own sites in the MD simulation. The result of MD simulation indicated the hydration number was 3.6, which is in fair agreement with that of the 3D-RISM theory. It was found from the MD trajectory that Water 4 left the site in the beginning and then entered there again, while Water 3 got out of the cavity just for short time. That is the reason why the hydration number was less than the number of water-binding sites.

It should be noted that the hydration number of MD simulation depends much on its simulation time. In fact, the 1 ns MD simulation estimated it at 3.1, as described in our original paper [14]. That is because the time scale of such processes is at least the nanosecond order. If the coordinates of such water molecules was not known in advance, unlike in this case, the simulation could not reach the equilibrium at all. To confirm the consequence, we tested a simulation started from another initial coordinate without the water molecules in the cavity. The hydration number obtained from the simulation was 2.5. Water molecules were not able to reach the W1 site in the simulation time of 5 ns. These results indicate that the 3D-RISM theory has a distinct advantage over the MD simulation in detecting water molecules confined in protein cavities.

This unusual ability of the 3D-RISM theory will have great impact on the fields of biochemistry and biophysics, including recognition of a drug molecule by a receptor protein, accommodation of ions by an ion channel, enzymatic reaction, etc. We can replace water solvent in the present calculation by aqueous solution of ligand molecules and analyze the 3D distribution of cosolvent (ligand) and water molecules in protein cavities. Such extensions are straightforward in this theory. Then we may find peaks of the ligand as well as water. The consequence is nothing but the molecular recognition by protein. If we analyze the dependence of the peak intensity or the binding number on the concentration of the ligand, we obtain the binding affinity or the dissociation constant K_d of the ligand to the protein sites.

4. Application to partial molar volume of protein

The PMV has principal importance in the analysis of the pressure effects on protein structure and function, such as pressure-induced protein denaturation [31–33] and pressure control of enzyme reaction [34]. That is known as LeChatelier's law and is expressed by the following equation,

$$-k_B T\left(\frac{\partial \ln K}{\partial p}\right)_T = \Delta\bar{V} \tag{4}$$

where K is the equiliblium constant, p is the pressure, and $\Delta\bar{V}$ is the difference in PMV between reactants and products: in the case of protein denaturation, it is the PMV difference between native and denatured states. In a similar expression, the pressure dependence on the reaction constant k is given by the PMV difference $\Delta V^{\ddagger}$ between reactant and transition states. A large amount of experimental work has been devoted to measure the PMV [35,36] and its difference associated with conformational changes [32]. However, it is almost impossible to determine the PMV of each state or conformation by experiment unless employing drastically simplified models or assumptions.

We have developed the methodology of calculating PMV, especially for biomolecules, based on the RISM [37,38] and 3D-RISM theories [8]. The PMV of solute molecules can be calculated from the Kirkwood-Buff (KB) theory extended to the (3D-) RISM description. In the 3D description, it is given by [8]

$$\bar{V} = k_B T \chi_T \left(1 - \rho \sum_{\gamma} \int_{V_{\text{cell}}} c_{\gamma}(\mathbf{r}) \mathrm{d}\mathbf{r}\right) \tag{5}$$

where χ_T is the isothermal compressibility of pure solvent, which can be obtained from the site–site direct correlation functions of pure solvent calculated by the RISM theory [37]. The 3D direct correlation functions are calculated by the 3D-RISM theory.

Figure 2 shows the PMV of several representative proteins calculated by the 3D-RISM/HNC and KB theories, plotted against the molecular weight [39]. The selected proteins are melittin, BPTI, erabutoxin B, ubiquitin, RNase A, lysozyme, β-lactoglobulin A, and α-chymotrypsinogen A, the number of residues of which ranges widely from 26 to 245. The 3D-RISM theory successfully reproduced a nearly linear relationship between the PMV and the molecular weight, which the RISM theory fails to reproduce as described in Introduction. Figure 2 also shows that the 3D-RISM results are in quantitative agreement with the corresponding experimental data. It should be noted again here that

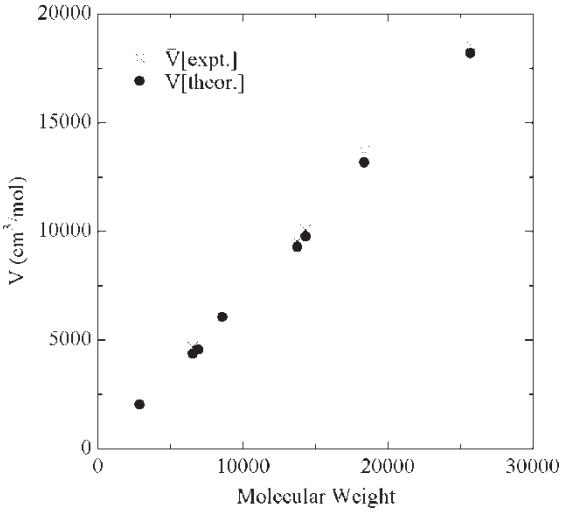

Figure 2. The PMV calculated with the 3D-RISM theory and the experimental data versus the molecular weight.

the 3D-RISM theory uses no adjustable parameters, but employed usual force field parameters for describing the intermolecular interactions, AMBER and SPC/E in this calculation. Thus, the present result strongly suggests that the 3D-RISM theory has high capability of calculating the PMV and the other thermodynamic quantities as well.

We applied the 3D-RISM theory to investigate the PMV change of a protein associated with a conformational transition induced by pressure [39]. Recently, the low (30 bar) and high (2000 bar) pressure structures of hen egg white lysozyme in aqueous solution were determined by means of NMR [40]. The experiment naturally concludes that the PMV of the high-pressure structure (HPS) is less than that of the low-pressure structure (LPS), based on equation (4). However, the experiment cannot determine the PMV value itself of each structure. In this respect, the theory has a definite advantage in determining the PMV of any given conformation, even if it is a hypothetical conformation. We calculated the PMV of both LPS and HPS of the protein at atmospheric pressure by the 3D-RISM/HNC and KB theories. The theoretical values were 9416 and 9296 cm^3/mol, respectively, for LPS and HPS. The decrease in the PMV by 120 cm^3/mol upon the transition from LPS to HPS is consistent with the experimental observation stated above. The value is also reasonable because $p\Delta\bar{V} = 24\,\mathrm{kJ/mol}$, where $p = 2000$ bar and $\Delta\bar{V} = 120\,\mathrm{cm^3/mol}$ have been used, is the same order of general values of the free energy differences between native and substable states, which are considered to be several times of k_BT, where $k_BT = 2.5$ kJ/mol at 298 K. The present result is nothing but the realization of LeChatelier's law at molecular level. This is actually the first realization of the law by a theoretical method. Further analyses of the volume decomposition to geometrical and hydration contributions indicated that the dominant component in the PMV change was the decrease in structural voids in the interior of the protein. In other words, the structural voids were compressed by pressure. This is a molecular picture of the volume and conformational change at an initial step in pressure-induced denaturation processes since the whole protein structure does not change so much in the present case. Applying higher pressure may cause global structural changes, and then the hydration contributions would be significant.

5. Application to pressure reversal of anesthesia

Since Johnson and Flagler [41] observed that the application of hydrostatic pressure restored swimming activity of anesthetized tadpoles, the pressure reversal of anesthesia has been considered as a key to for understanding the molecular mechanism of anesthesia [42–45]. Recent studies indicated that anesthetics interact with specific sites of proteins and the anesthetic-protein binding is a physico-chemical or thermodynamic process at molecular level [46,47]. If this is the case, the pressure reversal of anesthesia should be also explained by equation (4). According to the relation, the physical origin of the pressure reversal should lie in PMV increase due to the anesthetic-protein binding. By applying pressure, the equilibrium shifts toward the state with less PMV by releasing anesthetic molecules from the binding site. Ueda *et al.* [48] reported experimental results that the binding of halothane (anesthetic) to luciferase (model lipid-free protein) with low affinity increases the PMV, whereas the binding of myristate (specific inhibitor) with high affinity decreases it.

Eyring [42] and Ueda [45] proposed a possible molecular mechanism of the volume expansion, interestingly enough, which concerns hydration of the protein. Water molecules around an ionic species becomes denser compared to the bulk due to the strong electrostatic interactions, and consequently the PMV is reduced. The phenomenon is known as the electrostriction [49]. They suggested that the binding of anesthetics releases such "electrostricted" water molecules from the vicinity of ionic or polar residues of protein, and therefore it increases the PMV. However, no experimental evidence for the electrostriction hypothesis has been presented, and the question with respect to the molecular mechanism of the pressure reversal remains unanswered.

We applied the 3D-RISM theory to reveal the molecular mechanism of the volume expansion associated with anesthetic-protein binding [50]. We chose a model system consisting of xenon, which is known as an anesthetic, and lysozyme, because the xenon–lysozyme binding model had been constructed by docking simulation [51] based on the X-ray structure of xenon–lysozyme complex [52] and the NMR structure of lysozyme in aqueous solution [40]. Lysozyme has two binding site of xenon: one is at the binding pocket of native ligands (substrate binding site), the other is located in a cavity inside the protein (internal site). We calculated the PMV of the isolated state and the two complexes by the 3D-RISM/HNC and KB theories.

Table 1. PMV $\bar{V}$(cm^3/mol) of xenon, lysozyme, and two complexes in aqueous solution at 298.15 K, and its components[†].

	$\bar{V}$	V_{id}	V_W	V_V	V_T	V_I
Xenon	28.6	1.3	13.7	0.0	13.6	0.0
Lysozyme	9413.8	1.3	6082.4	2580.4	804.4	−54.8
Xenon + Lysozyme[‡]	9442.4	2.7	6096.1	2580.4	818.1	−54.8
Complex (Substrate binding site)	9451.9	1.3	6096.1	2603.8	802.3	−51.6
$\Delta V_{binding}$[¶]	9.5	−1.3	0.0	23.4	−15.7	3.2
Complex (Internal site)	9440.6	1.3	6096.0	2600.3	789.9	−56.0
$\Delta V_{binding}$[¶]	−1.8	−1.3	0.0	19.9	−19.1	−1.2

[†] Ideal volume (V_{id}), van der Waals volume (V_W), void volume (V_V), thermal volume (V_T), and interaction volume (V_I). [‡] $V_{xenon} + V_{lysozyme}$. [¶] $V_{complex} - (V_{xenon} + V_{lysozyme})$.

Table 1 gives the theoretical values of the PMV and its change accompanying the xenon–lysozyme binding. The PMV increases by 10 cm^3/mol at the substrate binding site. This result implies the reduction of the binding constant of xenon by pressure, based on equation (4). Unlike the substrate binding site, the binding to the internal site slightly decrease or negligibly changes the PMV. It gives little pressure effect on the binding to the internal site. Therefore, the pressure reversal can happen only at the substrate binding site.

Then we decomposed the PMV to some contributions to find the molecular mechanism of the volume changes. According to previous studies [39,53], we decomposed the PMV as follows,

$$\bar{V} = V_{\mathrm{id}} + V_{\mathrm{W}} + V_{\mathrm{V}} + V_{\mathrm{T}} + V_{\mathrm{I}} \tag{6}$$

where the components are identified as follows. V_{id} is the ideal volume contribution from the translational degrees of freedom of a molecule. The next two components are the geometric volume contributions: V_{W} is the van der Waals volume and V_{V} is the void volume owing to structural voids within the solvent-inaccessible core. These geometric volumes can be easily obtained by the geometric volume calculation. The last two components are the solvation effects on the PMV. V_{T} is the so-called thermal volume which results from thermally induced molecular fluctuations between the solute and solvent molecules. The volume was introduced to explain the distinction between the PMV and the molecular volume ($V_{\mathrm{W}} + V_{\mathrm{V}}$) for small nonpolar molecules [53]. The thermal volume may be simply considered as the volume of "voids" formed between solute and solvent due to the imperfect packing. V_{I} is the interaction volume representing the change in the solvent volume by the intermolecular electrostatic interaction between the solute and solvent molecules. This volume component apparently concerns the electrostriction described above. The solvation terms would not be calculated by any method other than the 3D-RISM theory. We defined the thermal volume by $V_{\mathrm{T}} = \bar{V}_0 - V_{\mathrm{id}} - (V_{\mathrm{W}} + V_{\mathrm{V}})$ and the interaction volume by $V_{\mathrm{I}} = \bar{V} - \bar{V}_0$, where $\bar{V}_0$ is the PMV of a hypothetical molecule whose atomic charges are completely removed, and thus is essentially the solvent-packing contribution of PMV. This decomposition has been found to be effective in understanding the volume changes of biomolecules [39,54,55].

The volume components are also given in table 1. The PMV increase for the substrate binding site primarily consists of a large void volume increase which is partially compensated by a decrease of the thermal volume. The interaction volume contributes positively, but not so much to the volume change. It is thus concluded that the PMV increase is related to the packing effects rather than the interactions such as the electrostriction, as opposed to the electrostriction hypothesis. For the internal site, an increase in the void volume is almost completely compensated by a decrease in the thermal volume. The contribution of the interaction volume is negligible, too.

We found another interesting distinction between the xenon bindings to the substrate binding site and the internal site by analyzing the distribution function of water at these sites. Figure 3(a) shows the RDFs of water from an atomic site in the substrate binding site before and after binding of xenon (RDFs were reconstructed from the 3D distribution functions). The running coordination numbers defied by

$$N(r) = \rho \int_0^r 4\pi r^2 g(r) \mathrm{d}r \tag{7}$$

are also shown in figure 3(b), which represents the number of water molecules within the distance r from the origin. It can be seen from the figure, the hydration number in the substrate binding site was reduced from about 4 to 2 by the xenon binding. In other words, two water molecules were expelled from the site by binding one xenon atom to the site. On the other hand, it was found in a similar analysis that the xenon binding to the internal site resulted in only one-to-one interchange of xenon and water.

From these results, we draw a molecular picture of the PMV changes. When a xenon atom binds to a lysozyme site, some voids between xenon and water, which account for the thermal volume, vanish because the xenon atom gets partially dehydrated. Instead, some structural voids between xenon and lysozyme, which account for the void volume, are created. In the same way, some voids between lysozyme and water transform to the structural voids between xenon and lysozyme. If the packing between xenon and lysozyme are similar to the packing of water,

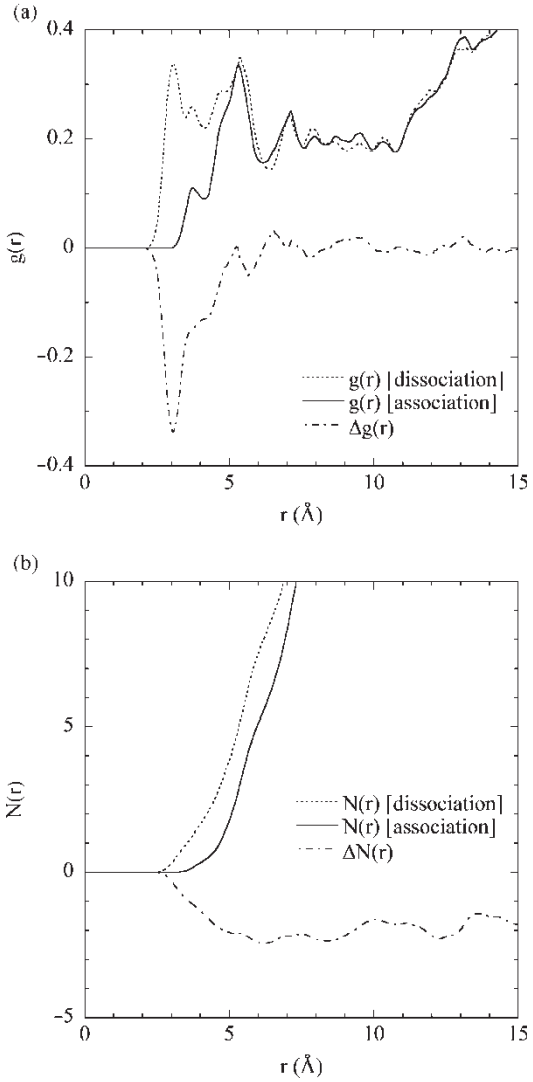

Figure 3. The RDF $g(r)$ and the running coordination function $N(r)$ from main-chain oxygen of Gln57 in substrate binding site; (a) dotted line, $g(r)$ for lysozyme; solid line, $g(r)$ for lysozyme-xenon complex; dot-dashed line, the difference $\Delta g(r) = g(r)$[complex] $- g(r)$[lysozyme]; (b) dotted line, $N(r)$ for lysozyme; solid line, $N(r)$ for lysozyme-xenon complex; dot-dashed line, the difference $\Delta N(r) = N(r)$[complex] $- N(r)$[lysozyme].

the increase in the void volume balances the decrease in the thermal volume. The binding of xenon to the internal site expelled only one water molecule so that the voids between xenon and water and between lysozyme and water entirely transform to the structural voids between xenon and lysozyme. On the other hand, at the substrate binding site two water molecules were exchanged to one xenon atom. Such an exchange makes larger voids between xenon and lysozyme. Therefore, the difference of the packing between xenon and lysozyme from the packing of hydration water, or interfacial water, creates the increase in the void volume which exceeds the decrease in the thermal volume. Based on the idea, we proposed a new hypothesis of the molecular mechanism of the pressure reversal of anesthesia: the pressure reversal of anesthesia is caused by loose packing between anesthetics and the active site.

6. Concluding remarks

We have reviewed the recent applications of the 3D-RISM theory to the hydration structure and thermodynamics of protein, which are the subjects virtually impossible to be approached with the other methods including molecular simulation and continuum models. Those are only the beginnings of the possible applications. The 3D-RISM theory can be applied to a wide variety of subjects in the fields of biochemistry and biophysics, such as the conformational stability of protein against temperature and pressure, the effects of cosolvent, such as ions, alcohols, denaturants like urea, etc. on the stability, and the molecular recognition by receptor protein. The theory also has potential to tackle medical and pharmacological issues such as structure based drag design and molecular mechanism of anesthesia.

The combination of the 3D-RISM theory with other theoretical methods extends the field where the theory can be applied. The combination with the molecular orbital theory [56] provides the electronic structure of a solute molecule in aqueous solution, so that it can be used to solve the problems involving chemical reactions, such as enzyme reactions and electron transfer and proton pumping by protein. Another combination is with molecular simulation, especially generalized-ensemble simulation methods. The combinations of some generalized-ensemble algorithms and the RISM theory have been successfully implemented [57–59], though that with the 3D-RISM theory is in progress. This combination dissolves a major weakness of the (3D-) RISM theory: in the present stage, the theory can only examine one protein conformation at a time. In the combination, the protein conformation is sampled by a generalized-ensemble algorithm adopting the hydration free energy of each conformation calculated by the (3D-) RISM theory at the same time. This approach allows us to deal with the problems concerning the conformational fluctuation of protein, such as the protein folding itself, the thermodynamics of fluctuating protein, and the induced fitting process in molecular recognition.

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