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Selective Ion-Binding by Protein Probed with the 3D-RISM Theory

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The molecular recognition by protein is the most fundamental process in a living system to maintain its life. Especially, the ion binding is essential for a variety of physiological processes including information transmission through ion channels.[1] Such processes are characterized by highly selective ion recognition by a protein. It is of great importance, therefore, for the life science to clarify the origin of the ion selectivity in molecular detail.[2]

The process is among the most difficult problems to be studied by theoretical tools. Just imagine a process of how a Ca^{2+} ion is recognized by a binding-site of protein, which usually consists of negatively charged groups of amino acid residues. When a Ca^{2+} ion is in the bulk solution, it is solvated usually by several water molecules, and makes a stable "molecular complex" because of the strong electrostatic attraction. When the ion is in protein, it is likely to be coordinated by the amino acid residues without solvation shell. So, in order for the ion to be recognized by protein, it should overcome the free energy barrier concerning the dehydration process, or take the hydrating water off. There is even more serious difficulty for theories. For an ion to be recognized by the protein, it should find the recognition-site of the protein, which is usually located inside a cleft or a cavity. The process is controlled by the diffusion, and the ion should overcome the entropic penalty to enter the limited space.

Recently, we have proposed a novel method to treat a molecular recognition or ligand binding by protein on the basis of the three-dimensional (3D) RISM theory.[3] Using the method, we have calculated the 3D distribution of water molecules around, as well as inside, a protein, hen-eggwhite lysozyme.[4,5] (The structural data of the protein was prepared from PDB by removing all the coordinates concerning water molecules.) We have observed four peaks in the distribution corresponding to water oxygen in a cavity composed of the amino acid residues spanned from Y53 to I58 and from A82 to S91. Thus, water molecules inside the cavity of protein have been probed. The reason the 3D-RISM theory could have detected water molecules confined inside a cavity of protein is because the method samples essentially the entire configuration space of the solvent, and therefore it does not depend on the initial configuration of the molecules. This is a great advantage of the method compared to the molecular simulation.

In this communication, we present theoretical results for the ion binding by human lysozyme obtained through basically the same procedure with that described above, but extending the solvent from pure water to ionic solutions. The calculation was carried out for aqueous solutions of three different electrolytes $CaCl_2$, NaCl, and KCl, and for four different mutants of the protein, wild type, Q86D, A92D, Q86D/A92D, that have been studied experimentally by Kuroki and Yutani.[6] The aqueous solutions of the electrolytes are treated by the (1D)-RISM theory taking the microscopic ion−water interactions into account explicitly. The thermodynamic consistency was verified in terms of the mean activity coefficients of the electrolytes. Thermodynamic conditions of solutions, potential parameters, and the structural data of proteins are listed in Table 1. In this communication, we employ the KH closure to complement the 3D-RISM equation.[7] (see Supporting Information for computational details).

Table 1. Thermodynamic Conditions, Potential Parameter, and PDB Files[a]

temperature	298 K
concentration	0.01 mol/kg
dielectric constant	78.4973
potential parameter	
water	SPC[8,b]
Cl^-, K^+, Na^+, Ca^{2+}	OPLS[9,10]
protein	Amber99[11]
protein structure	
wild type	1LZ1[12]
Q86D-lysozyme	1I1Z[6]
A92D-lysozyme	1I20[6]
holo-Q86D/A92D-lysozyme	3LHM[13]

[a] Details of the potential parameter of solvent molecules are in Supporting Information. [b] Lennard-Jones parameters of water hydrogen, $\sigma = 0.4$ Å and $\epsilon = 192.5$ J/mol, were added.

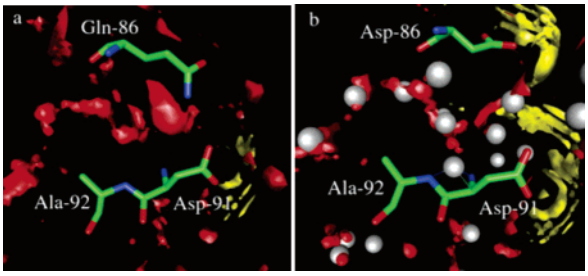

Figure 1. The distributions of water molecules and sodium cation inside and around the cleft under concern, which consists of amino acid residues of (a) wild type and (b) Q86D mutant. Gray spheres in panel b mean a water molecule was detected experimentally. All figures with 3D-DFs in the present paper were described by graphical package gOpenMol.[14]

In the figures, shown are the distributions of water molecules and the cations inside and around the cleft under concern, which consists of amino acid residues from Q86 to A92. The area where the distribution function, $g(r)$, is greater than 5 is painted with a color for each species: oxygen of water, red; Na^+ ion, yellow; Ca^{2+} ion, orange; K^+ ion, purple. A $g(r) > 5$ implies that the probability of finding water molecules at the position is 5 times greater than that of in the bulk. For the wild type of protein in the aqueous solutions of all the electrolytes studied, $CaCl_2$, NaCl, and KCl, there are no distributions ($g(r) > 5$) observed for the ions inside the cleft, as is seen in Figure 1a. The Q86D mutant exhibits essentially the same behavior with that of the wild type, but with the water

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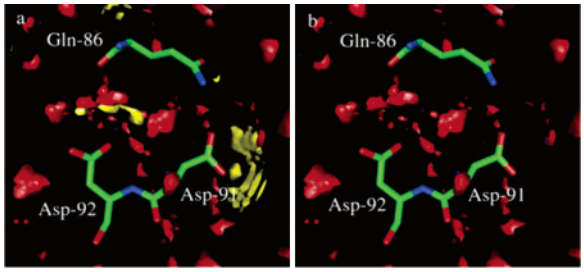

Figure 2. The distributions of water molecules and the cations inside and around the cleft under concern, which consists of amino acid residues of A92D-lysozyme in (a) NaCl and (b) KCl electrolyte solution.

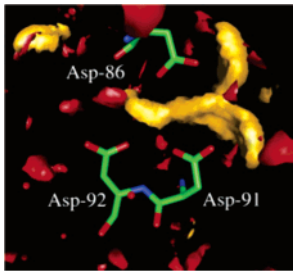

Figure 3. The distributions of water molecules and the calcium cation inside and around the cleft under concern, which consists of amino acid residues of holo-Q86D/A92D mutant in $CaCl_2$ electrolyte solution.

distribution changed slightly. (There is a trace of yellow spot that indicates a slight possibility of finding a Na^+ ion in the middle of the binding site, but it would be too small to make a significant contribution to the distribution.) Instead, the distribution corresponding to water oxygen is observed as is shown in the red color in Figure 1b. The distribution covers faithfully the region where the crystallographic water molecules have been detected, which are shown with the spheres colored gray. There is a small difference between the theory and the experiment concerning the crystallographic water bound to the backbone of Asp-91. The theory does not reproduce the water molecule for unknown reasons. Except for this difference, the observation is consistent with the experimental finding, especially, that the protein with the wild-type sequence binds neither Na^+ nor Ca^{2+}.

The A92D mutant in the NaCl solution shows conspicuous distribution of a Na^+ ion bound in the recognition site, which is in accord with the experiment. (Figure 2a) The Na^+ ion is apparently bound to the carbonyl oxygen-atoms of Asp-92 and is distributed around the moieties. There is water distribution observed in the active site, but the shape of the distribution is entirely changed from that in the wild type. The distribution indicates that the Na^+ ion bound in the active site is not naked but is accompanied by hydrating water molecules. The mutant does not show any indication of binding K^+ ion as is seen in Figure 2b. The result is just a prediction by our theory, since there has been no experimental report concerning K^+ binding by the protein to our knowledge. However, the finding clearly demonstrates the capability of the 3D-RISM theory to realize the ion selectivity by protein.

Figure 3shows the distributions of Ca^{2+} ions and of water oxygen in the ion-binding site of the holo-Q86D/A92D mutant. The mutant is known experimentally as a calcium binding protein. The protein, in fact, exhibits a strong calcium binding activity as can be seen in the figure. The calcium ion is recognized by the carboxyl groups of the three Asp residues and is distributed around the oxygen atoms. The water distribution at the center of the triangle made by the three carbonyl oxygen atoms is reduced dramatically, which indicates that the Ca^{2+} ion is coordinated by the oxygen atoms directly, not with water molecules in between. The Ca^{2+} ion, however, is not entirely naked, because the persistent water distribution is observed in at least two positions where original water molecules were located in the wild type of the protein.

In the present communication, we have presented remarkable results, which demonstrate the ability of the 3D-RISM theory to probe Na^+ and Ca^{2+} ions bound inside a cavity of protein. The ions are detected only for the mutated protein produced from the wild type by changing particular amino acids. The theory has also exhibited its capability to discriminate Na^+ from K^+ ions. This suggests that the theory can be applied to the selective transmission of ions by a channel protein, which is of paramount importance in the physiology.[15]

A question may be raised concerning the structural fluctuation of protein, since our method fixes the structure of protein. The fluctuation also plays an important role in the ion recognition process. The process can be realized by sampling the structure of the protein around its equilibrium conformation using molecular simulations and by calculating the distribution of ions and water molecules around as well as inside the macromolecule by means of 3D-RISM. If one detects ion distribution in a cleft or a cavity in any conformations of the protein, it is nothing but the ion recognition induced by structural fluctuation. In this regard, the possibility of a potassium ion being recognized by the A92D mutant stated above should not be entirely excluded, since the B-factor of the atoms comprising the cleft is 10∼15 $Å^2$. Further studies along this line are in progress in our group.

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Supporting Information Available: Calculation methods and detail of solvent potential parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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