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Theoretical Study of CO Escaping Pathway in Myoglobin with the 3D-RISM Theory

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Myoglobin (Mb) is a globular protein which has an important biological function, oxygen storage.[1] Due to its biochemical function, many researchers have made intensive efforts to identify the escaping pathway of the ligand, experimentally and theoretically.[2−8] Thirty years ago, Perutz et al. proposed that the entry and exit pathway of ligand into the active site of Mb involves rotation of the distal histidine to form a short and direct channel between the heme pocket and solvent.[2] Recent studies by time-resolved X-ray crystallography have shown consistent results with the earlier studies.[3] Unfortunately, their reports were limited to conditions of low temperature and crystal structures. Several groups have argued that the detaching scheme could be different under physiological conditions due to the conformational fluctuation of the protein.[4]

Recently, Terazima and his co-workers have proposed a new method to observe the time-resolved thermodynamics in solutions based on transient-grating (TG) spectroscopy. The authors applied the method to the photodissociation process of carbon monoxide (CO) from Mb in solutions to identify not only the escaping pathway but also the thermodynamics at each step along the pathway. The study has revealed that the ligand migrates into internal cavities of Mb upon photodissociation and that the population of the two Xe sites is associated with protein relaxation that occurs after the photolysis.[5,6]

It has been well-recognized that there are several intermediate states separated by activation barriers along the escaping pathway, which are referred to as "Xe-sites."[6] The experimental results indicate that CO spends some time at the Xe sites before escaping to solvent at room temperature.[5,6] In the mixed Xe and CO solutions, the difference in affinity between Xe and CO to each Xe trapping site makes the CO escaping pathway different depending on Xe concentration. It is believed that the dissociated CO escapes to the solvent through the Xe1 trapping site predominantly under Xe-free conditions. On the other hand, CO escapes through the Xe4 site in a Xe-rich solution. Terazima and co-workers have measured the partial molar volume (PMV) change of the system along the pathway of CO escape in a Xe solution based on the TG method. They hypothesized that the intermediate of the pathway is through the Xe4 site, since the experiments were carried out under Xe-rich conditions.

The most popular computational approach to the problem is molecular dynamics (MD) simulation.[7,8] Although those studies show good agreement with experiments concerning the CO escaping pathway, it is rather difficult to evaluate the thermodynamic properties such as PMV due mainly to the computational demand.

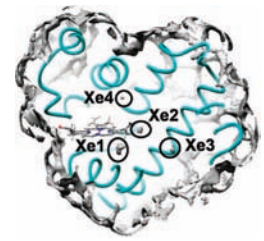

Figure 1. Distribution of Xe inside and around the Mb. Threshold of 3D-DF is 3.0 of Xe. Circles in the figure denote Xe trapping cavities.

A few years ago, a statistical mechanics theory of liquids, referred to as the "3D-RISM" theory, has succeeded for the first time to reproduce the solvation thermodynamics of a "realistic" protein. However, it was not surprising to us, because the theory had been applied more or less successfully to the solvation thermodynamics of small molecules. The real surprise was that the theory is capable of "detecting" or "probing" small ligands trapped in a cavity of protein in terms of the distribution functions just as X-ray crystallography does.[9] It had been commonly believed that such a liquid in an extremely inhomogeneous environment was far beyond the scope of the statistical mechanics.

Here, we apply the 3D-RISM theory to investigate the CO escaping pathway of Mb. As mentioned above, the ligand dissociating process of Mb occurs from heme to solvent through some specific cavities. We discuss the CO escaping pathway from Mb in terms of 3D distribution functions of the ligand and the PMV change along the pathway. The 3D-RISM calculation to evaluate the 3D distribution functions is performed with the geometry optimization of the Mb structure. The theoretical detail and the computational procedure are described in the Supporting Information.

To examine the distribution of a Xe ligand in the Xe trapping sites, we performed geometry optimization of Mb with 3D-RISM theory to obtain the relaxed structure in solution. In this calculation, we assumed that an Mb molecule is immersed in a mixture of water and Xe. Therefore, the probability of existence of Xe atoms is discussed in terms of the 3D distribution function of solvent Xe around solute Mb. The geometry search was started from the 1MBC[10] structure taken from PDB. The 3D distribution of Xe evaluated by 3D-RISM is shown in Figure 1. At a glance, four conspicuous peaks of the Xe distribution can be found. These positions correspond to the Xe trapping cavities which are reported by the experiments.[5,6]

Shown in Figure 2 are the coordination numbers (CNs) of CO and Xe in each Xe site of the RISM optimized structure, which

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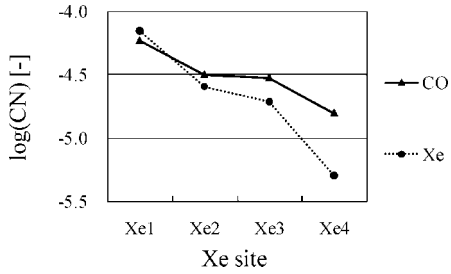

Figure 2. Coordination number of CO and Xe molecules in each Xe site, which are calculated from the radial distribution function at each Xe cavity. Vertical axis is made log scale.

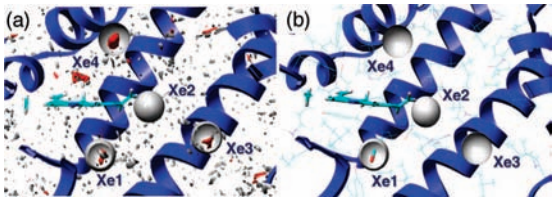

Figure 3. (a) CO molecules in Xe1, Xe3 and Xe4 sites which are in the same plane. (b) Intermediate model for which a CO molecule exists in Xe1 site reproduced by CO distribution.

are calculated from the radial distribution function at each Xe cavity. Since the structure was optimized in the Xe−water mixture, these results are regarded as the ligand affinity in *Xe-rich condition*s. As seen in the figure, CNs of both ligands show similar behavior; namely, the Xe1 site has the largest affinity, while Xe4 site has the smallest. Note that only in Xe1 site the affinity of Xe is greater than those of CO unlike others. This result indicates that CO is excluded from the Xe1 site in the *Xe-rich conditions* due to the preferential binding of Xe. On the other hand, CO is preferentially bound to the Xe4 site in the *Xe-rich condition*. These results are consistent with the experimental observations indicating that the Xe4 site is the intermediate of the escaping pathway of CO in the *Xe-rich conditions.*[6]

As is stated in the introductory remark, Terazima et al. have determined experimentally the PMV change from an intermediate state to the next along the escaping pathway. It is of great interest to verify their result theoretically, because it will be the first nontrivial theoretical result for the time-resolved solvation thermodynamics. To evaluate the PMV change, we have calculated the PMV of each intermediate structure of Mb along the escaping pathway of CO. Here, "intermediate structure" means the structure in which a CO molecule is trapped in each specific Xe site of Mb. Therefore, the CO molecule is regarded as a part of the solute species. The position of the trapped CO molecule is determined by the peak positions of the 3D distribution functions. Depicted in Figure 3a are the 3D distributions of CO in the Xe1 to Xe4 sites in the optimized Mb, calculated by the 3D-RISM method. The results show conspicuous distributions of C- and O-atoms of CO in the sites. Not only the molecular position but also their orientation can be deduced from the peak positions. From the peak positions of the distribution functions, we can reproduce the 3D-structure of Mb−CO complexes in which a CO molecule is bound in one of the Xe sites, which is depicted in Figure 3b taking the Xe1 site as an example. It was found that the orientation of a CO molecule in the Xe1 site shown in Figure 3b is consistent with the configuration of polar groups of amino acid residues surrounding the molecule, such as the hydroxyl groups. The structure of the Mb−CO complex with CO in the Xe4 site was determined in a similar manner.

The PMVs of the CO attached (MbCO), intermediate (Mb:CO), and CO dissociated (Mb+CO) states and its changes are shown in Table 1. The PMV of Mb:CO(Xe1) is smaller than that of Mb:

Table 1. PMVs of Each Model and These Changes[a]

models	PMV [cm^3/mol]
MbCO	9029.0
Mb:CO(Xe1)	9030.4
Mb:CO(Xe4)	9032.8
Mb+CO	9019.6

	Xe1	Xe4	(exptl[5])
$\Delta V_1 =$	1.4	3.8	(3 ± 1)
$\Delta V_2 =$	−10.8	−13.2	(-12.6 ± 1.0)
$\Delta V_{\text{total}} =$	−9.4	−9.4	(-10.7 ± 0.5)

[a] ΔV_1 represents partial molar volume change from MbCO to Mb:CO (Xe4), and ΔV_2 represents change from Mb:CO (Xe4) to Mb+CO. ΔV_{total} represents change from MbCO to Mb+CO.

CO(Xe4). Because the size of the cavity of Xe1 is large enough, it can accommodate water even after trapping CO to be filled up. In contrast, since the size of the Xe4 cavity is larger than the CO molecule but is too small to accommodate water simultaneously, extra void is created in the cavity. This extra void causes the increase in PMV of Mb:CO(Xe4). The changes of PMV through the Xe4 site show excellent agreement with those by the experiment.[6]

Here, we have investigated the CO escaping pathway of Mb in terms of 3D distribution functions which are calculated by the 3D-RISM theory. The geometry optimization was carried out to determine the Xe trapped structure of Mb. We also have examined the ligand selectivity of Mb, which is evaluated in terms of CN. Although both ligand species show similar tendencies, the dependence of affinity on each Xe trapping site is different. This difference indicates that CO prefers Xe4 to Xe1 sites in Xe-rich conditions. The PMVs of each CO binding state were also estimated by using the site−site Kirkwood−Buff equation.[11] The PMV changes indicated that the CO escaping pathway through Xe4 is dominant. This result supports the conjecture made by Terazima et al.[6]

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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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