Visualizing the Breathing Motion of Internal Cavities in Concert with Ligand Migration in Myoglobin

Myoglobin (Mb) is an oxygen storage protein in muscle, which reversibly binds gas ligands (O_2, CO, NO etc.) at the heme iron site. Dissociation of these ligands can be easily triggered by photo-irradiation, and the photolysis of Mb has been studied using various techniques. However, the ligand binding site is buried deeply inside the protein matrix, and there exists no direct evidence to show the consecutive migration pathways in Mb. Our novel method of continuous illumination of protein crystals with pulsed laser light at cryogenic temperatures has led to the production of a slow dynamics movie of ligand migration in Mb for the first time.

In general, the structural analysis of protein molecules only reveals their static structures. However, in order to explore the mechanism of protein function, it is also important to unveil their dynamic structures. Myoglobin (Mb) is a small oxygen storage protein present in muscle. Mb reversibly binds gas ligands (O_2, CO, NO etc.) at the heme iron site deep inside the protein matrix. Dissociation of these ligands can be easily triggered by photo- irradiation [1], and the ligand binding reaction in Mb has been studied using various techniques. In particular, the photolysis of carbonmonoxy myoglobin (MbCO) in crystal has been extensively studied by X-ray diffraction experiments (e.g. Laue diffraction at room temperature and monochromatic X-ray diffraction at cryogenic temperatures [2, 3]). Although many details are known regarding gas ligand molecules trapped in internal hydrophobic cavities in Mb, there exists no direct evidence to show the migration pathways connecting these cavities. In order to explore these pathways in Mb, we have carried out cryogenic X-ray crystallographic investigations of native sperm whale MbCO under the novel condition of photo-irradiation. Our method of continuous illumination of MbCO crystals with pulsed laser light at cryogenic temperatures has allowed the production of a movie of ligand migration in Mb on the time scale of several hours [4].

The diffraction data were collected at AR-NW14A using the undulator U20 and marDTB stage with the marCCD165 detector [5]. The crystals were cooled to 40-140 K by cold helium or nitrogen gas. Photolysis was carried out using the second harmonic of an Nd-YAG pulsed laser at a photon wavelength of 532 nm with a 15 kHz repetition rate and an average power of 4.6 mW/mm^2.

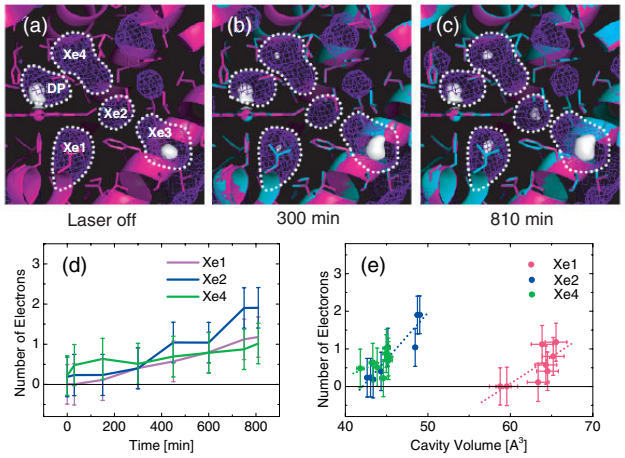

Figure 1
Ligand migration dynamics in Myoglobin at 120 K.
(a)-(c) The superimposed structures before and after laser irradiation are shown in magenta and cyan. The internal cavities are shown by the purple meshes, and the DP, Xe1, Xe2, Xe3, and Xe4 cavities are also indicated by dotted white lines. The contour surface of the CO molecule electron density in the cavities is shown in white (contoured at 0.3 electron / Å^3 of the 2Fo-Fc map). (d) The integrated number of electrons in cavities Xe1, Xe2 and Xe4 during irradiation. (e) The correlation between the number of electrons and the volume of cavities Xe1, Xe2, and Xe4.

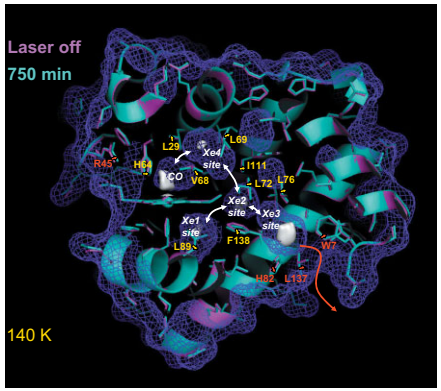

Figure 2
The migration pathway in Myoglobin at low temperature.
The superimposed structures at 140 K before and following 750 minutes of laser irradiation are drawn in magenta and cyan. The contour surface of the electron density of the photodissosiated CO molecules in the cavities is shown in white (contoured at 0.3 electron / Å^3 of the 2Fo-Fc map). The molecular surfaces and the internal cavities are shown by the mesh. The movements of amino acid residues between the cavities are shown by yellow arrows, and those between the Xe3 cavity and the solvent area are shown by red arrows. The white arrows represent the ligand migration pathway between the cavities.

We successfully observed the movement of CO molecule from the heme iron site in the distal pocket (DP) to the internal cavities in Mb, and also the associated structural changes of the amino acid residues around the cavities. Figure 1 (a)-(c) show the ligand migration at 120 K. After 300 minutes of laser irradiation, CO migrates into the cavities in the lower part of the heme plane (Xe1 cavity) and at the rear of the distal pocket (Xe4 cavity). After 810 minutes of laser irradiation CO was also observed in the Xe2 cavity. This study provides the first direct evidence of CO molecule electron density in this cavity. The Xe3 cavity is originally occupied by a water molecule, and it is difficult to estimate precisely whether the increased electron density in Xe3 corresponds to a photo-dissociated CO molecule or to a water molecule from the external solvent. Figure 1 (d) shows the integrated number of electrons during irradiation in cavities Xe1, Xe2 and Xe4 at 120 K. Figure 1 (e) shows the correlation between the number of electrons and the volume of cavities Xe1, Xe2, and Xe4 at 120 K. These results indicate that the internal cavities expand in accordance with the CO migration.

We also determined the temperature dependence of the time course of the integrated number of electrons in cavities Xe1, Xe2 and Xe4 at 100 K, 120 K and 140 K [4]. The results suggest that the route from the distal pocket to the Xe3 cavity involving the Xe4, Xe2, and Xe1 cavities is the major ligand migration pathway in Mb at cryogenic temperatures. This pathway is shown in Fig. 2. The superimposed structures before and after laser irradiation at 140 K are shown in magenta and cyan, respectively. The structural changes of the amino acid side chains around the ligand migration pathway clearly indicate expansion of the cavities and putative gating motions of the surrounding amino acid residues.

Our novel method of continuous illumination of protein crystals with pulsed laser light at cryogenic temperatures has been successful in generating a slow dynamics movie of ligand migration in a protein molecule. The method will be applied to other photo-sensitive proteins in the near future.

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A. Tomita[1, 2], T. Sato[2, 6], K. Ichiyanagi[2], S. Nozawa[2, 6], H. Ichikawa[2], M. Chollet[1], F. Kawai[3], S.-Y. Park[3], T. Tsuduki[4], T. Yamato[4, 5], S. Koshihara[1, 2] and S. Adachi[2, 6] ([1]Tokyo Tech, [2]ERATO JST, [3]Yokohama City Univ., [4]Nagoya Univ., [5]CREST JST, [6]KEK-PF)