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Report:

We performed an experiment entitled "Probing structural dynamics of myoglobin using site-specific iodine labeling scheme" at the ID09B beamline. We labeled iodine atoms on the surface cysteines in sperm whale myoglobin (swMb) mutants (G5C-D60C, D60C-K102C, and D60C-K147C). Iodine-labeled swMb mutants were successfully prepared, dialyzed against 100 mM sodium phosphate buffer with pH 7.0, and concentrated to 8 mM. In order to extract the principal components (PCs) of the data at late time delays, we collected scattering data at the following time points; -10 ns, 1 us, 3.16 us, 5.62 us, 10 us, 17.8 us, 31.6 us, 56.2 us, 100 us, 178 us, 316 us, 562 us, 1 ms, 1.78 ms, and 3.16 ms.

We used a typical pump-probe setup installed at ID09B for the experiment. The sample was sealed in a capillary and loaded onto the goniometer. The reaction was initiated by 532 nm-wavelength laser pulses (150uJ power, 5mJ/mm2 energy density). Then, the reaction was probed by hard X-ray pulses (E_{photon} = 18.0 keV). Scattering signals were collected using FReLoN CCD at 10 Hz. After collecting the data from the full time series, radial integration of 2D images for each time delay was performed to convert 2D images to 1D curves. Subsequently, difference curves were obtained by subtracting the scattering signal at a negative time delay, -10us, from each curve at positive time delays. Figure 1 shows the data from the experimentally obtained scattering curves from three different mutants.

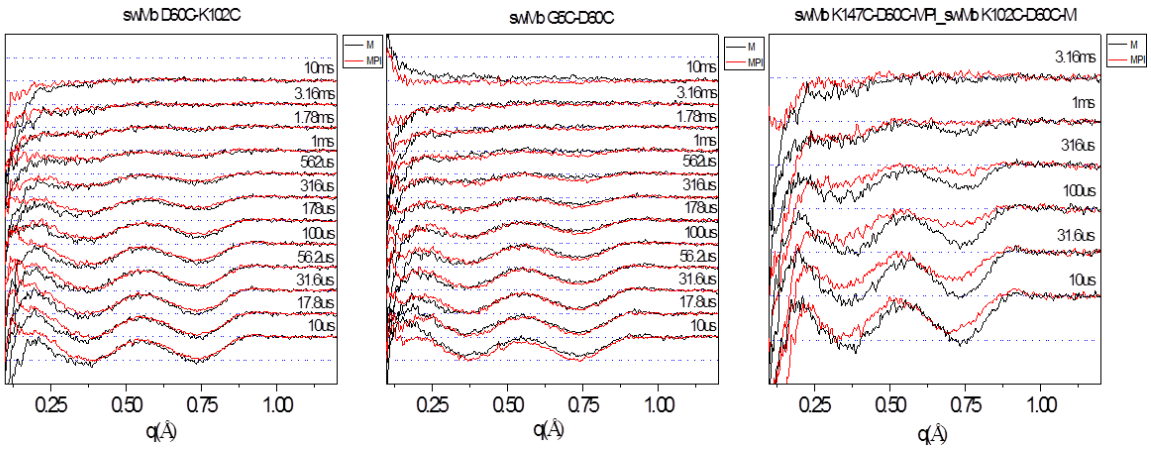

Figure 1 Comparison between myoglobin mutant prior to iodine labeling (black) and iodine labelled myoglobin mutant (red) at several time delays (swMb D60C-K102C, G5C-D60C, D60C-K147C). MPI means iodine labeled mutant. M means unlabeled mutant. We confirmed that the signals between the Mb mutants and the iodine-labelled Mb mutants at several time delays are different.

The difference curves show a distinctive feature between the iodine-labeled swMb mutant and the unlabeled swMb mutant. We presume that this variation is due to the scattering contribution from the two iodine atoms labeled on the two surface cysteine residue.

Preliminary analysis using singular value decomposition (SVD) and principal component (PC) analysis was employed; we extracted the principal components (PCs) for the late time delay of the labeled and unlabeled Mb mutants. First, the scattering data from the unlabeled mutants and the wild type Mb were compared. Figure 2 shows a comparison of the PC results for the wild type, mutant, and iodine-labeled mutant. The PCs of the unlabeled Mb mutants (K120C-D60C, G5C-D60C) and the wild type Mb were identical within the signal-to-noise ratio, confirming that the surface mutation process and subsequent attachment of linkers do not appreciably affect the structure change (Fig 2A). On the other hand, comparison between the PCs of the iodine-labeled myoglobin mutants and the unlabeled mutants shows a noticeable difference due to the contribution of scattering from the two iodine atoms (Fig 2B). In addition, the PCs of various Mb mutants that were iodine labeled show that the samples with different labeled positions yield different curves (Figure 2C). Specifically, the iodine-labeled K147C-D60C mutant (blue) shows a greater difference.

This result strongly supports the idea that the two iodine atoms on the surface of the swMb mutant alter the scattering patterns and reveal the applicability of the site-specific iodine-labeling method. So, we can confidently state that the iodine labeling scheme will help us obtain more information about intermediate structures during structural transitions.

In conclusion, we collected and analyzed time-resolved X-ray solution scattering data for iodine labeled swMb mutants at several late time delays and investigated the applicability of the iodine labeling method. Accumulating the scattering curves, which were derived from various iodine-labeled protein sets, will greatly increase the structural information contents and allow a refining of the calculated structure. Therefore, we will be able to analyze the intermediate protein structures with high reliability due to the increased information about changes in the protein structure after through iodine labeling method.

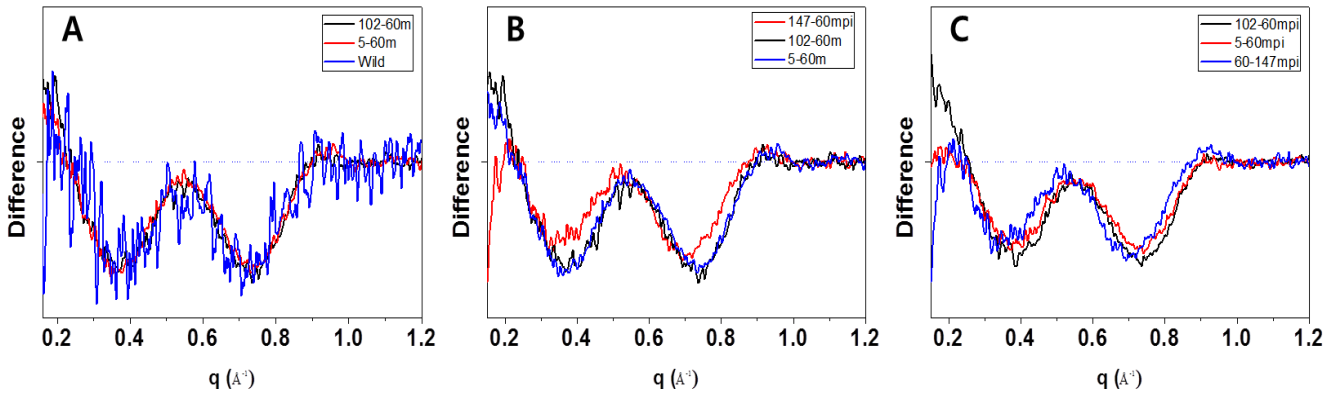

Figure 2. Comparison of the PCs from wild Mb, Mb mutants, and iodine-labelled Mb mutants: (A) Comparison of the PCs of the unlabeled Mb mutants (black: D60C-K102C and red: G5C-D60C) and the wild Mb (blue). (B) Comparison of the PCs of the mutants (black: D60C-K147C and blue: G5C-D60C) and iodine-labelled mutants (red). (C) Comparison of the PCs of iodine-labelled Mb mutants (black: D60C-K102C, red: G5C-D60C, blue: D60C-K147C).