Protein Crystallography by MAD Method Using Xe or Cs

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Xenon and cesium are recently used as heavy atoms for phasing in protein crystallography. However, *K*-absorption edges of these atoms are located at so short wavelength region (~0.35Å) that they had not been used as heavy atoms for the MAD phasing. BL41XU is only beamline for macromolecular crystallography in SPring-8 where public users can perform MAD experiments using such short wavelength X-rays.

In the previous investigation (2001A0518-UL–np), we have evaluated beam strength and detection ability of the detector (marCCD 165) at such short wavelengths. In this study, we have tested the possibility of the MAD phasing in crystals of extracellular chaperonin LolA (space group *I*222). A Xe derivative crystal was prepared using xenon chamber with 30 atm. The crystal was frozen in liquid ethane. The wavelength of *K*-absorption edge was determined from XAFS measurement. Three data sets from the Xe derivative were collected for the MAD phasing at 0.3584Å, 0.3585Å and 0.540Å, respectively. Data statistics are listed on Table1.

A Cs derivative crystal was prepared by soaking in the harvesting solution containing 1.0M CsCl for one week. The crystal was also frozen in liquid ethane. The wavelength of *K*-absorption edge was determined from XAFS measurement. Three data sets from the Cs derivative were collected at 0.3734Å, 0.3737Å and 0.540Å, respectively. Data statistics are listed on Table2.

Father data analysis is now in progress.

Table1. Data statistics of Xe derivative

	Peak	Edge	Remote
λ (Å)	0.3584	0.3585	0.540
Resolution(Å)	25.0 – 1.80		
(last shell)	(1.86 – 1.80)		
Compl. (%)	99.1	98.9	99.7
(last shell)	(93.1)	(92.4)	(98.5)
Rsym	0.053	0.053	0.068
(last shell)	(0.289)	(0.299)	(0.220)

Table2. Data statistics of Cs derivative

	Peak	Edge	Remote
λ (Å)	0.3734	0.3737	0.540
Resolution(Å)	25.0 – 2.30		
(last shell)	(2.38 – 2.30)		
Compl. (%)	95.8	96.0	95.9
(last shell)	(75.5)	(77.6)	(79.5)
Rsym	0.066	0.064	0.057
(last shell)	(0.204)	(0.229)	(0.166)

Replacement of the Mn-cluster in the Photosystem II Complex by Os

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We have been analyzing the crystal structure of Photosystem II (PSII), a membrane protein complex consisting of 14 membrane-spanning proteins and 3 hydrophilic, peripheral proteins with a total molecular mass of 320,000 Da. In the previous studies, we collected and analyzed data sets from multiple heavy atom derivatives, in order to improve the phase we obtained by SIRAS of a Ta_6Br_{14} derivative. During the course of these studies, we used several Os compounds to prepare derivatives and the diffraction data collected from one of the Os derivatives, a $(NH4)_2OsBr_6$ derivative, showed heavy atom peaks in the difference Fourier-map close to the Mn site of PSII. Mn is the site of the water-splitting reaction in PSII in which, four Mn atoms are present to form a unique catalytic cluster. Attempts have been made to use other transient metal cations to substitute the Mn atoms in order to modulate the water-splitting reaction of PSII; however, these efforts have been unsuccessful so far. Since our PSII structural analysis is at a resolution level of 3.7-4.0 Å, we were unable to determine whether Os indeed replaced Mn or was located near the Mn-cluster in the PSII crystal. The purpose of the present study is to clarify this question by measuring XAFS spectra of Mn with Os-derivative crystals. We prepared Os-derivatives at several different $(NH4)_2OsBr_6$ concentrations, and measured their XAFS spectra around the Mn-edge and Os-edge. The relative fluorescence yields obtained were summarized in Table 1. It was shown that, while XAFS spectra at the Os-absorption edge confirmed the presence of Os in the derivative crystals, the relative fluorescence yield of Mn virtually did not change in the Os-derivatives as compared with the native PSII crystals. We also measured the Mn-XAFS spectrum of a derivative made with 0.5 mM $(NH4)_2OsBr_6$ and observed no clear decrease in the Mn-fluorescence yield. These results suggested that Mn of PSII was not replaced by Os; the Os peaks observed in the difference Fourier-map must be attributed to a site close to the Mn-cluster.

Table 1. Relative fluorescence yield of Os and Mn in PSII native and Os-derived crystals

$(NH4)_2OsBr_6$ (mM)		XAFS Yield of Os and Mn Peak (P)	Edge (E)	P-E
0	Os	0.387	0.386	0.001
	Mn	0.788	0.568	0.220
0.1	Os	0.573	0.380	0.193
	Mn	0.839	0.597	0.242
0.25	Os	0.778	0.578	0.200
	Mn	0.739	0.519	0.220