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Low-dose X-ray structure analysis of cytochrome oxidase utilizing high-energy X-rays

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Radiation damage on macromolecular crystallography (MX) appears as degradation of crystal quality (global damage) and local structural change (specific-damage) depending on accumulated dose. Although X-ray absorption by macromolecular crystals can be reduced with utilization of high-energy X-rays, reduction of diffraction intensity and lower efficiency of area detectors will be problematic in practice [1]. However, it is expected to mitigate radiation damage by utilizing a sufficiently high-efficient area detector for high-energy photons.

Bovine hart cytochrome c oxidase (CcO) is an enzyme which functions in the cellular respiratory electron transport chain at inner mitochondrial membrane. It has been confirmed to bind a ligand peroxide at the reduction center consisting of a copper ion and a heme iron, by a damage-less structure analysis with femtosecond crystallography at SACLA [2]. However, on synchrotron based MX, the peroxide ion is readily reduced to waters by X-ray irradiation, and bond length between two oxygen atoms of peroxide is observed elongated according to the accumulated dose [3].

To verify the effect of radiation-damage suppression with high-energy X-rays for radiation sensitive proteins, a low-dose diffraction data collection for CcO crystals with 30 keV X-ray has been conducted at BL41XU, SPring-8. A high-sensitive pixel array detector with CdTe sensor (Pilatus 3 X CdTe, DECTRIS, Ltd.) was utilized for data collection by shutter-less helical scanning method. Controlling the average dose to be 55 kGy by translation-speed and frame-rate settings, a diffraction data set of maximum resolution up to 1.9 Å was collected (dose estimation with RADDOSE version 2). The result of structure analysis showed the ligand structure of 1.55 Å bond length, which provides the evidence of the largely suppressed radiation damage.

Currently, further investigation of possibility for X-ray energy dependency of site specific damage on the reduction center, utilizing microspectroscopy is in progress.

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