

MS01-01 | ELUCIDATION OF NO REDUCTION MECHANISM IN SOLUBLE NO REDUCTASE BY TIME-RESOLVED CRYSTALLOGRAPHY WITH PHOTOSENSITIVE CAGED COMPOUND

Tosha, Takehiko ; Nomura, Takashi (University of Hyogo, Akoh, JPN); Sugimoto, Hiroshi ; Shiro, Yoshitsugu (University of Hyogo, Akoh, JPN); Kubo, Minoru (University of Hyogo, Akoh, JPN)

Structural determinations of reaction intermediates in metalloenzyme-catalyzed reactions are great challenge in structural biology and very useful for understanding chemistry in metalloenzymes. Recently developed technique called serial femtosecond X-ray crystallography (SFX), in which micro-crystals are continuously supplied to the X-ray irradiation spot, using an X-ray free electron laser facility, SACLA, enabled time-resolved X-ray crystallography (TR-SFX). Since the X-ray laser from SACLA is a ~ 10 fs pulse, it is possible to carry out a pump-probe type experiment. Here, we applied this method for the observation of enzymatic reaction using photosensitive caged-compound. We utilized a heme-containing enzyme, P450nor, which catalyzes a following reaction; $2\text{NO} + \text{NADH} + \text{H}^+ \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} + \text{NAD}^+$. Photosensitive caged-NO which release two equivalent of NO upon UV irradiation was utilized as a trigger and NO source for the P450nor reaction. We performed TR-SFX at SACLA, and obtained an intermediate structure, substrate NO-bound form, at 20 ms after the UV illumination¹. Current results suggest that TR-SFX with photosensitive caged compound is promising approach for the characterization of the reactions catalyzed by metalloenzymes. In the presentation, characterization of next process of the P450nor-catalyzed reaction will be shown.

[1] Capturing an initial intermediate during the P450nor enzymatic reaction using time-resolved XFEL crystallography and caged substrate. T. Tosha et al., Nat. Commun. 2017, 8, 1585.