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Redox-dependant Association/dissociation between terminal oxyeganse and ferredoxin componets of carbazole 1,9a-dioxygenase

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Introduction

Carbazole 1,9a-dioxygenase (CARDO) catalyzes a regio- and stereoselective dihydroxylation of various aromatic compounds. CARDO consists of three components: terminal oxygenase (Oxy), ferredoxin (Fd) and ferredoxin reductase (Red) and is a member of Rieske nonheme iron oxygenase (RO) involved in the initial step of aerobic degradation pathways for various aromatic compounds. The substrate recognition and its specificity have been well investigated in various ROs. However, component interaction followed by electron transfer has been less investigated, although these are essential for enzyme activity.

In electron transfer cycle, reduced-state of Fd interacts with oxidised-state Oxy, followed by electron transfer. Then, dissociation of oxidised-state of Fd from reduced-state of Oxy occurrs. We have already determined the crystal structure of the Oxy:Fd electron transfer complexes, $Oxy_{oxidised}$:$Fd_{oxidised}$ and $Oxy_{reduced}$:$Fd_{reduced}$[1]. However, these structures do not appear in catalytic cycle because redox states of each component are the identical.

To elucidate the association and dissociation mechanisms between Oxy and Fd components, we determined the crystal structure of $Oxy_{reduced}$:$Fd_{oxidesed}$ electron transfer complex under anaerobic conditions.

Experiment

Crystallization trials were performed in anaerobic chamber. $Oxy_{reduced}$ and $Fd_{oxidised}$ were mixed in a 1:1 molar (as a monomer) ratio and protein concentration of this solution was adjusted to 10-30 mg/mL. Hanging drops, each consisting 3 µL resultant solution and 3 µL reservoir solution (0.1 M sodium cacodylate, 14% PEG3350) were equilibrated against 600 µL reservoir solution. $Oxy_{reduced}$:$Fd_{oxidised}$ crystal was appeared after 2 days incubation at 293 K. Before X-ray diffraction data collection, redox state of the crystal was confirmed by microspectroscope. The diffraction data were collected at BL5A, NW12A and NE3A at the photon factory. The structure of $Oxy_{oxidised}$:$Fd_{oxidised}$ was used as molecular replacement model with the program MOLREP. Model building of the electron density map was carried out with COOT and refinement was performed using the program Refmac5 in CCP4.

Result

The crystals belonged to space group $P2_1$ with unit cell parameters a = 97.4, b = 81.6, c = 116.2 Å, $\alpha = \beta = 90°$, χ = 100°. This would result in solvent content of 57.8% and favourable V_M of 2.91 Å/Da. The datasets were integrated and scaled to 2.2 Å resolution.

Biological unit of Oxy is trimer and there are three binding sites for Fd in each Oxy subunit interface of trimeric Oxy molecule. In $Oxy_{reduced}$:$Fd_{oxidised}$ complex, two of three Fds were bound to Oxy, although all binding sites were dominated by Fds in $Oxy_{oxidised}$:$Fd_{oxidised}$ complex.

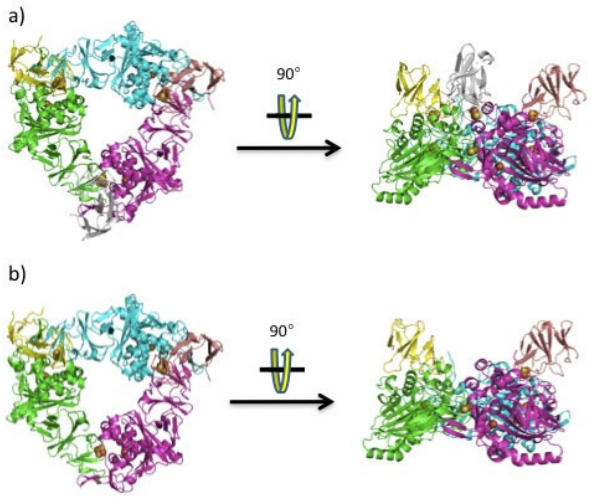

Fig. 1. Over all structures of $Oxy_{oxidised}$:$Fd_{oxidised}$ complex (a) and $Oxy_{reduced}$:$Fd_{oxidised}$ complex (b). Oxy subunits are represented by green, cyan and pink and three Fd molecules are represented by yellow, orange, grey.

Comparison of these two structures suggested reduction-specific movements of amino acid residues in Oxy molecules. Detailed structural comparison and postulated association and dissociation mechanisms will be published elsewhere.

Reference

[1] Ashikawa et al., Structure. 14, 436 (2006)

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