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# Catalytic Cycle of NADH-cytochrome $b_5$ reductase

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## 1 Introduction

Flavins such as flavin adenine dinucleotide (FAD) and flavin mononucleotide are redox cofactors involved in several important physiological reactions. NADH-cytochrome $b_5$ reductase (b5R), a flavoprotein consisting of NADH- and FAD- domains, catalyzes electron transfer from the two-electron carrier NADH to the one-electron carrier cytochrome $b_5$ (Cb5). The reaction catalyzed by b5R plays a role in fatty acid synthesis, cholesterol synthesis, and xenobiotic oxidation as a member of the electron transport chain on the endoplasmic reticulum. To understand the b5R catalytic cycle based on structural information, we have determined the crystal structures of porcine liver b5R in several states on catalytic cycle [1].

## 2 Experiment

The b5R crystals of oxidized (OX) form were obtained under known condition, and the fully reduced (RD) form crystals were prepared in the presence of NADH under anaerobic condition. In addition, to analyze the structural change during catalytic cycle, the RD form crystals were exposed to aerobic conditions and then quenched by flash freezing at 1, 10, 20, and 60 min exposure. Diffraction data were collected at beamlines at Photon Factory and SPring-8. Data collection and refinement statistics are summarized in Table 1.

## 3 Results and Discussion

The crystal structures of both RD and OX forms of b5R were determined. In the RD structure determined at 1.68 Å resolution, the relative configuration of the two domains was slightly shifted in comparison with that of the OX form (Fig. 1). This shift resulted in an increase in the solvent-accessible surface area of FAD and created a new hydrogen-bonding interaction between the N5 atom of the isoalloxazine ring of FAD and the hydroxyl oxygen atom of Thr66, which is considered to be a key residue in the release of a proton from the N5 atom (Fig. 2). The isoalloxazine ring of FAD in the reduced form is flat, similarly to the OX form, and is stacked together with the nicotinamide ring of $NAD^+$. Both the RD and OX structures explain how backflow in this catalytic cycle is prevented and how the transfer of electrons to one-electron acceptors such as Cb5 is accelerated. Furthermore, crystallographic analysis by the cryo-trapping method suggests that reoxidation follows a two-step mechanism (Fig. 1). These results provide structural insights into the catalytic cycle of b5R.

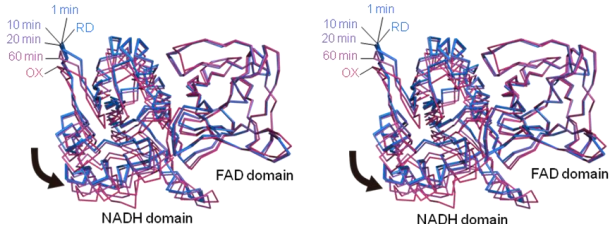


Fig. 1: Relative arrangement of the FAD and NADH domains of b5R during catalytic cycle.

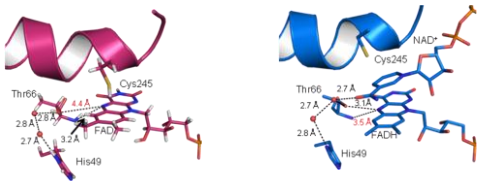


Fig. 2: Hydrogen bond network around the N5 atom of the isoalloxazine ring of FAD (left: OX, right: RD).

## References

[1] M. Yamada *et al*., *J. Mol. Biol.* **800**, 12 (2013).

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Table 1: Data collection and refinement statistics

| | OX form | RD form | 1 min | 10 min | 20 min | 60 min |
|---|---|---|---|---|---|---|
| Space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Unit cell (Å) | $a$=48.4, $b$=72.0, $c$=84.8 | $a$=58.2, $b$=72.7, $c$=86.6 | $a$=57.3, $b$=72.6, $c$=85.4 | $a$=57.5, $b$=73.0, $c$=85.6 | $a$=57.7, $b$=73.1, $c$=85.5 | $a$=57.6, $b$=72.5, $c$=83.9 |
| **Data collection** | | | | | | |
| Resolution (Å) | 0.78 (0.79-0.78) | 1.68 (1.71-1.68) | 1.75 (1.81-1.75) | 1.76 (1.82-1.76) | 2.10 (2.14-2.10) | 1.81 (1.84-1.80) |
| Completeness (%) | 95.0 (70.6) | 98.9 (98.3) | 99.6 (100.0) | 99.4 (99.8) | 98.4 (97.1) | 99.8 (98.6) |
| $R_{merge}$ (%) | 6.8 (35.7) | 5.2 (37.3) | 5.7 (40.0) | 6.3 (39.1) | 7.6 (39.3) | 7.8 (37.9) |
| $<I/\sigma(I)>$ | 16.9 (2.1) | 36.5 (4.1) | 34.2 (4.2) | 47.9 (6.2) | 41.4 (6.4) | 30.9 (4.0) |
| **Refinement** | | | | | | |
| $R_{work}$ / $R_{free}$ (%) | 12.3 / 14.4 | 15.0 / 16.9 | 16.1 / 18.3 | 16.4 / 19.2 | 17.5 / 21.3 | 16.7 / 19.7 |
| R.m.s.d. | | | | | | |
| Bond lengths (Å) | 0.025 | 0.007 | 0.006 | 0.007 | 0.007 | 0.006 |
| Bond angles | 0.039 Å | 1.31° | 1.25° | 1.25° | 1.24° | 1.15° |