

Crystal Structure of Biliverdin Reductase

A heme (Fe-porphyrin complex) is a prosthetic group of hemoproteins such as hemoglobin, myoglobin, cytochromes and so on. In biological systems, the heme is decomposed by two enzymes (hemeoxygenase and biliverdin reductase) and excreted (Fig. 1). Biliverdin reductase (BVR) catalyzes the last step of the heme catabolism, in which the biliverdin is reduced into bilirubin with two electrons from NAD(P)H. Reduction of biliverdin (formation of bilirubin) is important for the disposal of the heme catabolite formed in the fetus since the placenta is permeable to bilirubin but not to biliverdin. Bilirubin is the most abundant biological anti-oxidant in mammalian tissues, and is highly related to neuroprotection, but also to cytotoxicity (kernicterus). Clearly, BVR is a key enzyme and logical pharmacological target for controlling bilirubin level *in situ*.

We have analyzed the crystal structure of rat BVR at 1.4 Å resolution (Fig. 2), whose diffraction data was collected at beamline **BL44B2** [1]. This enzyme consists of two domains that are packed tightly together. The N-terminal domain (123 amino acids) is a characteristic of a dinucleotide binding fold, the so-called Rossmann fold, while the C-terminal domain (169 amino acids) contains six β-strands and eight helices, and is dominated by a

large, and predominantly antiparallel six-strand sheet.

NAD(P)H Binding to BVR: The Rossmann fold in the N-terminal domain is the most likely NAD(P)H binding site. The 'fingerprint' region (Gly15-Val16-Gly17-Arg18-Ala19-Gly20) and a 'hydrophobic core' (Val11, Val13, Leu24, Leu27, Val42) are found in this region. Glu96 is apparently capable to interact with the nicotinamide ring of NAD(P)H through the hydrogen bond (Fig. 3). Indeed, Glu96Ala mutant of BVR, in which Glu96 was replaced with Ala, did not exhibit the enzymatic activity. A unique property of BVR is its ability to use either NADH or NADPH at different pH optima; NADH is used in the lower pH range of 6.7 - 6.9, whereas NADPH is used at the higher pH of 8.7 [2]. In a model study, in which NADH or NADPH was put on the Rossmann fold of BVR, we found that Arg44-Arg45-Glu46 is located very close to the 2'-position of the adenosine ribose (Fig. 3). In combination with the mutational studies, we proposed that Arg44 and Arg45 play crucial roles in NADPH binding, while Glu46 modulates the NADH binding.

Biliverdin Binding to BVR: The putative NAD(P)H binding site is on the lower side of the pocket that is constructed between the N- and C-terminal domains. On the other hand, there are located four basic residues, Arg171, Lys218, Arg224 and Arg226, on the upper side of this pocket (Fig. 3). Since the biliverdin recognition by BVR has been proposed to be achieved through an

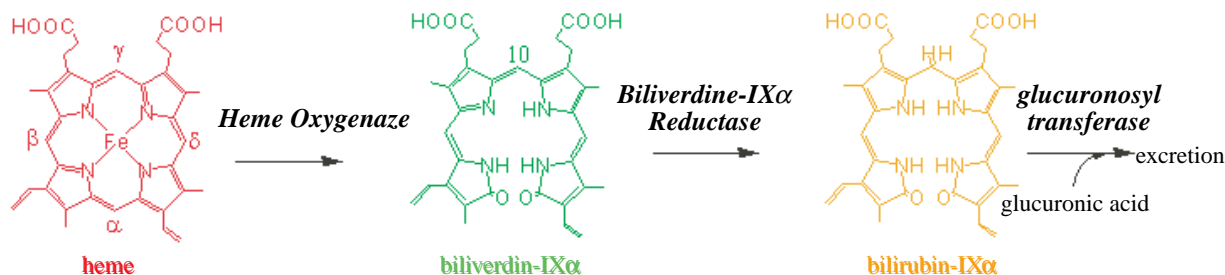


Fig. 1. Heme degradation pathway in mammals.

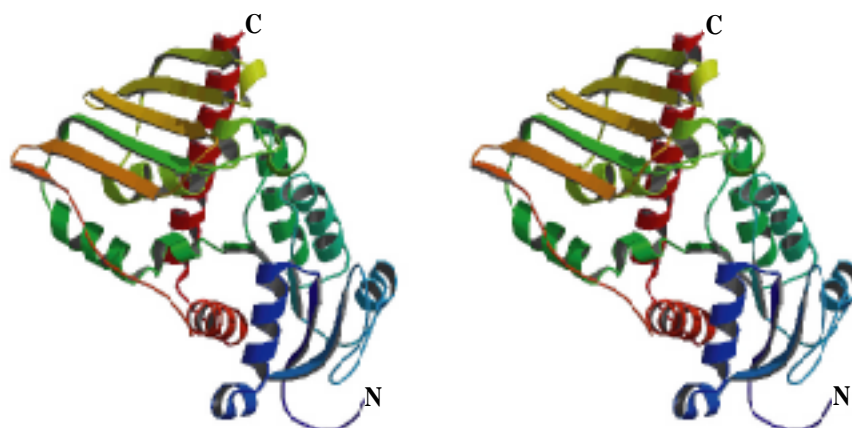


Fig. 2. Stereo view of the overall structure of rat BVR.

electrostatic interaction between the negatively charged propionate side chains of biliverdin and positively charged residues of BVR [3], we proposed that cluster of the four basic residues is a possible biliverdin binding site. The pocket is wide enough to accommodate both biliverdin and NAD(P)H. When biliverdin would bind to this site, the distance between the reduction site (C10 *meso* position) of biliverdin and the NAD(P)H nicotinamide is estimated to be ~ 10 Å. In this model, Tyr97 is located between the nicotinamide and C10 *meso* position, and therefore this residue may mediate a hydride (H^-) transfer from NAD(P)H to biliverdin in the enzymatic reaction.

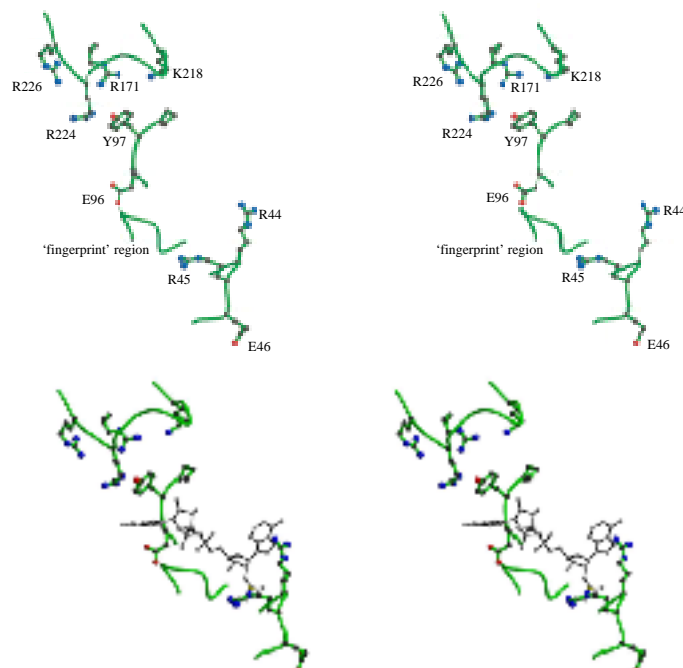


Fig. 3. The proposed binding sites for NAD(P)H (electron donor) and substrate (biliverdin) in rat BVR.

References

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