Crystal structure of an associated form of NADPH-cytochrome P450 reductase with heme oxygenase

NADPH-cytochrome P450 reductase (CPR) supplies electrons to various heme proteins, including microsomal cytochrome P450s and heme oxygenase (HO). Thus, CPR is fundamentally important for metabolism of xenobiotics, steroids, and heme. In fact, some mutations of CPR impair biosynthesis of steroid hormones, which may be a possible etiology of Antley-Bixler syndrome. CPR contains two redox coenzymes (FMN and FAD). Electrons from NADPH flow first to FAD, then to FMN, and finally to heme in the redox partner. CPR has at least two distinct conformations (open and closed). In the closed conformation [1], the FMN-binding site is covered by FAD-binding domain, and as a result, FMN is not exposed to the surface. However, rapid electron transfer from NADPH to FMN could take place in this conformation because FMN and FAD are in close proximity. In the open conformation [2], FMN is exposed to the surface. Therefore, this conformation should be favorable for interactions with a redox partner. To date, structures of CPR in a complex with its redox partner have yet to be determined.

HO catalyzes heme degradation, utilizing three oxygen molecules and seven electrons to produce biliverdin, ferrous ion, and carbon monoxide (Fig. 1). The seven electrons required for the HO reaction are provided by CPR. Both CPR and HO are localized in the microsomal membrane *in vivo*, but *in vitro*, a solubilized rat CPR (sCPR), in which the membrane-bound N-terminal segment has been truncated, can support heme degradation catalyzed by solubilized rat HO-1 (sHO-1), with a truncated membrane-bound C-terminal segment. Extensive crystallographic, spectroscopic, and biochemical studies of sHO-1 have revealed the unique mechanism of the HO reaction [3,4]; however, how the reducing equivalents are transferred from CPR to HO will remain unclear until the tertiary structures of the associated forms of CPR with HO are determined.

Although the tertiary structure of the associated form of CPR with HO is interesting, it is difficult to crystallize due to its instability. To overcome this problem, we attempted to prepare a stable associated form using mutations of sCPR. A mutated sCPR, designated ΔTGEE, can keep stable open conformations [2] and associate with heme-bound sHO-1 (heme-sHO-1). ΔTGEE can support the HO reaction, although its efficiency is extremely limited.

Furthermore, we have determined the crystal structure of ΔTGEE in a complex with heme-sHO-1

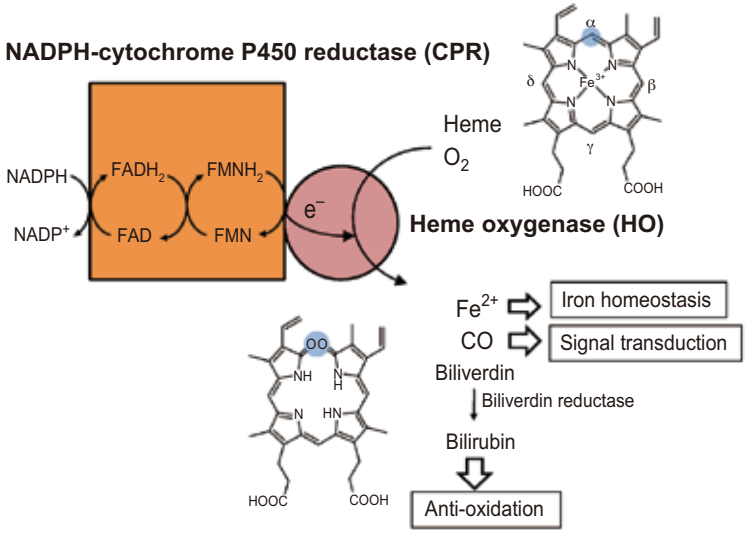

Fig. 1. Physiological functions of heme metabolism. HO catalyzes heme degradation utilizing three oxygen molecules and seven electrons to produce biliverdin, ferrous ion, and carbon monoxide. Ferrous ions produced by the HO reaction are pivotal for iron homeostasis because ~95% of the daily iron requirement is supplied by recycling the iron released by this reaction. In addition, bilirubin produced from biliverdin by biliverdin reductase plays a major role as an anti-oxidant at its physiological concentration. Carbon monoxide is suggested to be involved in several cell signaling processes, such as anti-inflammation, anti-apoptosis, and vascular constriction and dilation.

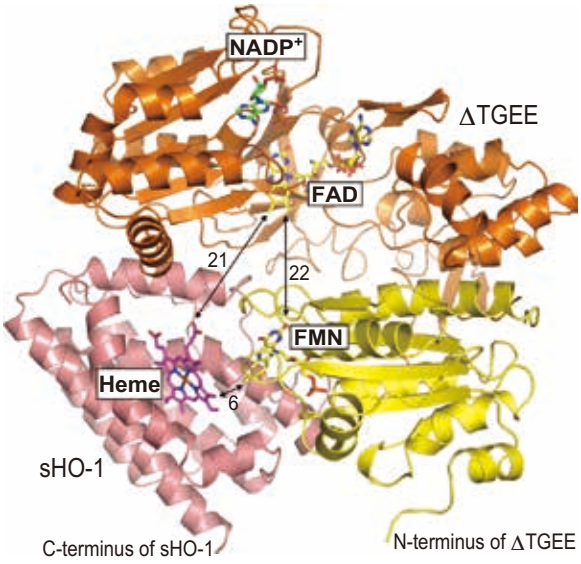

Fig. 2. Crystal structure of ΔTGEE-heme-sHO-1 complex. Orange and yellow ribbon diagrams show FAD and FMN binding domains of ΔTGEE, respectively. Pink ribbon diagram shows sHO-1. $NADP^+$, FAD, FMN, and heme are shown with stick models. Numerals stand for various distances in Angstrom.

at 4.3 Å resolution by molecular replacement using synchrotron radiation data collected at beamline **BL44XU** [5]. ΔTGEE assumes the open conformation in the complex structure. The N-terminal side of ΔTGEE and the C-terminal side of heme-sHO-1 face the same direction, indicating that the microsomal membrane into which these terminals are embedded *in vivo* does not interfere with the association of CPR and HO (Fig. 2). X-ray scattering and cross-linking analyses indicate that the structure of ΔTGEE and heme-sHO-1 complex is almost identical to that of sCPR and heme-sHO-1 complex.

The short distance (6 Å) between heme and FMN in the ΔTGEE and heme-sHO-1 complex implies that direct electron transfer from FMN to heme is plausible. On the other hand, FAD is far from FMN and heme (~20 Å), suggesting that electron transfer from FAD to FMN is difficult if CPR keeps the open conformation. This would explain the extremely limited heme degradation efficiency of the ΔTGEE and heme-sHO-1 complex. Based on the structure, we propose an electron transfer mechanism from CPR to HO that accompanies the dynamic conformational changes of CPR (Fig. 3). CPR is in a dynamic equilibrium between the open and closed conformations. When NADPH binds to CPR, FMN is readily reduced, and then CPR changes to the open conformation. The open CPR can bind to heme-HO-1, which transfers an electron from FMN to heme. FMN is subsequently re-oxidized and CPR changes to the closed conformation to release HO-1. The "closed-open transition" of CPR should be indispensable for smooth electron transfer from FAD to FMN and the dissociation of HO from CPR.

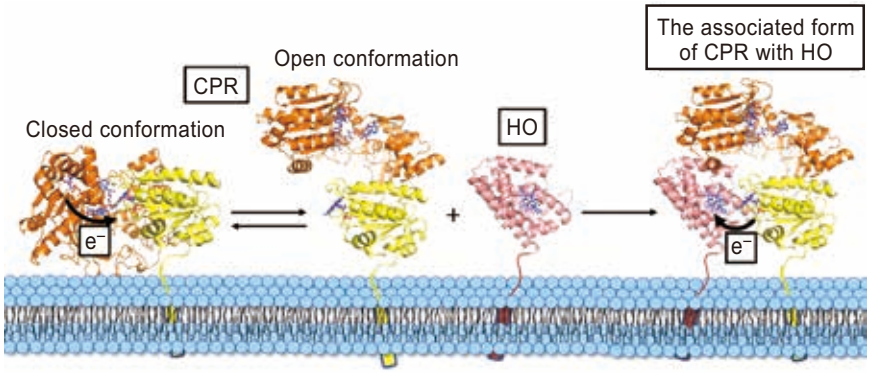

Fig. 3. Mechanism for electron transfer from CPR to heme-HO-1 that accompanies the dynamic conformation changes of CPR.

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