Crystal Structure of Rat Heme Oxygenase-1 in Complex with Biliverdin-Iron Chelate

CONFORMATIONAL CHANGE OF THE DISTAL HELIX DURING THE HEME CLEAVAGE REACTION∗

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The crystal structure of rat heme oxygenase-1 in complex with biliverdin-iron chelate (biliverdin(Fe)-HO-1), the immediate precursor of the final product, biliverdin, has been determined at a 2.4-Å resolution. The electron density in the heme pocket clearly showed that the tetrapyrrole ring of heme is cleaved at the α-meso edge. The heme bound to HO-1, biliverdin-iron chelate, is located between the distal and proximal helices, but its accommodation state seems to be less stable in light of the disordering of the solvent-exposed propionate and vinyl groups. The middle of the distal helix is shifted away from the center of the active site in biliverdin(Fe)-HO-1, increasing the size of the heme pocket. The hydrogen-bonding interaction between Glu-29 and Gln-38, considered to restrain the orientation of the proximal helix in the heme-HO-1 complex, was lost in biliverdin(Fe)-HO-1, leading to relaxation of the helix. Biliverdin has a distorted helical conformation; the lactam oxygen atom of its pyrrole ring-A interacted with Asp-140 through a hydrogen-bonding solvent network. Because of the absence of a distal water ligand, the iron atom is five-coordinated with His-25 and four pyrrole nitrogen atoms. The coordination geometry deviates considerably from a square pyramid, suggesting that the iron may be readily dissociated. We speculate that the opened conformation of the heme pocket facilitates sequential product release, first iron then biliverdin, and that because of biliverdin’s increased flexibility, iron release triggers its slow dissociation.

Heme oxygenase (HO) catalyzes the oxygen-dependent degradation of heme to biliverdin IXα, iron, and carbon monoxide using reducing equivalents. In higher plants, algae, and cyanobacteria, HO functions in the biosynthesis of such open-chain tetrapyrroles as phytochrome chromophore and phycobilin (1), whereas in certain pathogenic bacteria, HO is used for iron uptake from heme obtained from the host (2). In contrast, in mammals, HO has a key function in physiological heme metabolism (3). Three isoforms of HO, HO-1, HO-2, and HO-3, are present in mammals. HO-1 (32 kDa) is highly expressed in spleen and liver and inducible by various substances including heme itself. This isoform is considered mainly to function in heme catabolism. In addition, HO-1 is involved in the defense against oxidative stress because it is induced by various oxidative stresses, removes the heme, pro-oxidant, and produces the antioxidants, biliverdin and bilirubin (4). In contrast, HO-2 (36 kDa) is expressed constitutively in the brain, testis, and the vascular systems. The principal role of HO-2 is speculated to be the production of CO as a neurotransmitter (5), but the role of CO as a signal agent is controversial (6). HO-3 also catalyzes heme degradation, but its function in vivo has yet to be determined (7, 8).

The reaction pathway of vertebrate HO consists of three sequential oxidation steps that utilize O2 and the reducing equivalents from NADPH-cytochrome P450 reductase (9, 10). In the first step, O2 bound to the heme iron is activated to produce oxygen as CO (11). Last, the oxygen bridge of verdoheme is cleaved to produce biliverdin-iron chelate (biliverdin(Fe)). Iron is released before the dissociation of biliverdin (12). In these reaction steps, heme acts as both substrate and cofactor. The crystal structures of the human, rat, and Neisseria meningitidis HO complexes with heme show that all HOs have similar overall structures, consisting mainly of α-helices (13–15). In the structure of rat HO-1 in complex with heme (heme-HO-1), the protein moiety consists of eight helices, A through H. The heme is sandwiched between the proximal helix, A (Leu-13–Glu-29), and the distal kinked helix, F (Leu-129–Met-155). The His-25 side-chain in the A helix contributes the proximal heme ligand, whereas H2O or OH− serves as the distal ligand. No dissociable residue is present on the distal side of the heme, but backbones of Gly-139 and Gly-143 in the F helix are close enough to form hydrogen-bonds with the distal ligand. We have reported that in the absence of heme, the proximal helix is disordered and the distal helix relaxed, and we proposed an induced-fitting model for the

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accommodation of heme into the heme pocket of HO (16). Recently, we determined the structure of the azide-bound form of rat heme-HO-1 (N_{3}^- -heme-HO-1) as an analog of the dioxygen-bound form and showed that the azide bound to the heme iron is nearly parallel to the heme plane and points toward the a-meso carbon of the heme (17). We also proposed that not only the amide group of Gly-143 but also the hydrogen-bonding network at the distal heme pocket has a crucial role in the formation of ferric hydroperoxide, the activated oxygen species for the a-meso hydroxylation step of the HO reaction (17).

Although major progress has been made in understanding the nature of the first hydroxylation of heme, the mechanisms for the subsequent steps are still not clear. In the second step, whether the ferric α-hydroxyheme requires an electron before reacting with O_2 to yield verdoeheme remains unsettled (18–22). The final step is known to require O_2 and reducing equivalents (22–24), but little is known about the mechanism whereby biliverdin is produced from verdoeheme and finally released from the enzyme. For further investigation of the HO reaction mechanism, enzyme structures with heme degradation intermediates have been awaited with much interest. Herein we report the crystal structure of rat HO-1 in complex with biliverdin-iron chelate (biliverdin(Fe)-HO-1) at 2.4 Å resolution. This is the first report of the structure of biliverdin(Fe) in complex with protein.

**EXPERIMENTAL PROCEDURES**

Preparation of the Biliverdin(Fe)-HO-1 Crystal—Truncated rat HO-1 (Met-1–Pro-267) was expressed and purified as described elsewhere (17), and CN–heme-HO-1 crystals were prepared (45). Biliverdin(Fe)-HO-1 crystals were prepared by soaking CN–heme-HO-1 crystals in a solution of 4 mM sodium formate and 50 mM potassium phosphate, pH 7.4, with 100 mM sodium ascorbate as the reducing agent, until the crystal color changed to green (about 3 h). A small crystal (50 μm wide) was used for the data collection because large crystals (more than 100 μm wide) were apt to crack or dissolve during preparation.

**Data Collection and Processing**—The biliverdin(Fe)-HO-1 crystal was held in a cryo-loop and flash-cooled in a nitrogen gas stream at 100 K. Diffraction data were collected at 100 K using synchrotron radiation system. The crystal-to-CCD distance was 130 mm. The crystal was oscillated 1.5° with an exposure time of 40 s per frame; the total measurement angle was 120°. Diffraction data were processed, merged, and scaled with MOSFLM (25) and SCALA (26, 27). Crystallographic data and diffraction statistics are given in Table I.

**Model Building and Refinement**—Because the biliverdin(Fe)-HO-1 crystal was isomorphous with the CN–heme-HO-1 crystal, the structure of biliverdin(Fe)-HO-1 was determined using the structure of the protein moiety of CN–heme-HO-1 (45). Rigid-body, simulated annealing, and temperature-factor refinements were applied to the model using the 50.0–2.4-Å resolution data. XtalView (28) was used to refine the structure. In this step, the cleaved tetrapyrole ring was clearly visible in the electron density map. After one cycle of water picking and conjugate gradient energy minimization refinement, an additional electron density appeared around the N-terminal residue (Gln-11), to which the model from Ser-7 to Ser-10 was fitted. After one cycle of water picking, conjugate gradient energy minimization, and temperature-factor refinements, the model of biliverdin(Fe) moiety was fitted to the σ_A-weighted F_{o} – F_{c} map in the heme pocket. Last, conjugate gradient energy minimization and temperature-factor refinements were applied to the model, including biliverdin(Fe). Neither the torsion angles of the tetrapyrole rings in the biliverdin(Fe) moiety nor the coordination bonds to the heme iron were restrained in the refinements. All refinements were done by CNS (29). The stereochemical check of the model was carried out with PROCHECK (26, 30). Refinement statistics are given in Table I.

**RESULTS**

**Protein Structure**—The structure of biliverdin(Fe)-HO-1 was refined using 2.4 Å resolution data to the R factor of 0.194 and the free R factor of 0.239. Six residues at the N terminus and 46 residues at the C terminus are disordered in the biliverdin(Fe)-HO-1 crystal. Main-chain atoms from Ser-7 to Met-9 were ordered compared with heme-HO-1, but the side-chain of Met-9 was disordered. The structure of biliverdin(Fe)-HO-1 consists of eight helices, A through H (Fig. 1a), and is similar to that of heme-HO-1 (13–15) (Fig. 1b), and the root-mean-square deviation for Ca atoms was 0.26 Å. The biliverdin(Fe) is wedged between A and F helices at a position corresponding to the heme in heme-HO-1 (14). The plot of the deviations of Ca atoms between biliverdin(Fe)-HO-1 and heme-HO-1 (Fig. 1c, solid line) shows that residues with a large deviation (>0.8 Å) are localized in the middle of the F-helix and in the FG and GH loops. These two loops are located on the molecular surface and can easily adopt different conformations depending on the crystal packing force. Indeed, the structures of heme-HO-1 and N₃⁻-heme-HO-1 (17), although determined from different space groups, differ in the conformation of these two loops, but the rest of their structures are similar. In particular, the heme pocket structure and positions of the water molecules involved in the distal hydrogen-bonding network, considered essential for catalysis (31, 32), are well conserved (14, 16). The plot of the deviations for the Ca atoms between biliverdin(Fe)-HO-1 and N₃⁻-heme-HO-1 (Fig. 1c, dashed line) (both isomorphous crystals) shows a large deviation only in the middle of the F-helix. Therefore, essential structural differences between the substrate (heme-) and product (biliverdin(Fe))-bound forms are chiefly restricted to the middle of the F-helix.

**Distal Helix**—The F-helix of biliverdin(Fe)-HO-1 was bent (~50°) at Leu-141 and Ser-142, as in heme-HO-1 and N₃⁻-heme-HO-1, but the middle of the helix, Gly-143 to Gln-145, was shifted away from the heme pocket compared with that in heme-HO-1 and N₃⁻-heme-HO-1. The distance between Gly-143 N and the iron atom was elongated by 0.9 Å in biliverdin(Fe)-HO-1 compared with that in heme-HO-1 and N₃⁻-heme-HO-1, whereas the distance between Gly-139 O and the iron atom showed no change in the three structures (Table II). Notably, in biliverdin(Fe)-HO-1, the amide group of Gly-143 was directed toward the carbonyl group of Gly-139, forming an α-helical hydrogen-bond (2.9 Å). The backbone of Gly-144 and Asp-140 were also hydrogen-bonded (2.8 Å) (Fig. 2, left). In N₃⁻-heme-HO-1, however, the amide group of Gly-143 is pointed
FIG. 1. Structural comparison of heme-HO-1, N$_5$-heme-HO-1, and biliverdin(Fe)-HO-1. a, ribbon diagram of biliverdin(Fe)-HO-1. Alphabets show labels of helices in heme-HO-1 (14). b, superimposition of C$_\alpha$ traces of heme-HO-1 (black) and biliverdin(Fe)-HO-1 (green) in stereo diagram. The view is identical to that in a. c, plots of the deviations of C$_\alpha$ atoms between biliverdin(Fe)-HO-1 and heme-HO-1 (Protein Data Bank code 1DVE) (solid line) and between biliverdin(Fe)-HO-1 and N$_5$-heme-HO-1 (Protein Data Bank code 1IVJ) (dashed line). Solid horizontal bars represent the helices in heme-HO-1 (14). All figures were prepared with MOLSCRIPT (42), RASTER3D (43), and CONSCRIPT (44).
Fig. 2. Comparison of the heme pocket structures of biliverdin(Fe)-HO-1 and N₃-heme-HO-1. Ball and stick models of the heme pockets of biliverdin(Fe)-HO-1 (left) and N₃-heme-HO-1 (right; Protein Data Bank code 1IVJ). For clarity, in the F helix only main-chain atoms are shown. Dashed lines indicate hydrogen-bonds that stabilize the two conformers. Numerals show distances between atoms in Angstroms.

Fig. 3. Ramachandran plot for residues around the kinked point of the distal helix. Plots of Leu-138 through Val-146 residues are joined within the corresponding residues of heme-HO-1 (●, Protein Data Bank code 1DVE), N₃-heme-HO-1 (●, Protein Data Bank code 1IVJ), and biliverdin(Fe)-HO-1 (▲). Leu-141, Ser-142, and Gly-143 are shown in black, the other residues in gray.

TABLE II
Selected distances of atoms at the distal heme pocket

|                  | Gly-139 O-Fe | Gly-143 N-Fe | Gly-139 O–Gly-143 N | Gly-139 O–Gly-144 N | Asp-140 O–Gly-144 N |
|------------------|--------------|--------------|---------------------|---------------------|---------------------|
| Rat              | 4.9          | 5.2          | 2.9                 | 3.8                 | 2.8                 |
| Biliverdin(Fe)-HO-1 | 5.1          | 4.3          | 3.1                 | 2.9                 | 3.8                 |
| N₃-heme-HO-1     | 5.0          | 4.3          | 3.2                 | 3.0                 | 3.8                 |
| Human            | 4.8          | 5.3          | 3.2                 | 4.3                 | 3.1                 |
| Heme-HO-1 (closed form) | 4.9          | 6.0          | 3.1                 | 4.8                 | 2.9                 |

Val-146 may clarify the difference in the F-helix conformations between heme-HO-1 and biliverdin(Fe)-HO-1 (Fig. 3). In heme-HO-1 and N₃-heme-HO-1, all the residues examined fell within the region for a right-handed α-helical conformation except Leu-141 ((φ, ψ) = (−89°, 6°) in heme-HO-1, (φ, ψ) = (−87°, 4°) in N₃-heme-HO-1) and Ser-142 ((φ, ψ) = (−120°, −90°) in heme-HO-1, (φ, ψ) = (−123°, −98°) in N₃-heme-HO-1); the former was on the periphery of the α-helical region and the latter was outside of the region. Leu-141 ((φ, ψ) = (−74°, −12°)) in biliverdin(Fe)-HO-1 was also on the periphery of the region, but the dihedral angles of Ser-142 ((φ, ψ) = (−98°, −58°)) deviated less from the α-helical region compared with those in heme-HO-1 and N₃-heme-HO-1. Furthermore, the dihedral angles of the following Gly-143 ((φ, ψ) = (−101°, 14°)) differed distinctly from those of heme-HO-1 and N₃-heme-HO-1 ((φ, ψ) = (−71°, −25°) in heme-HO-1, (φ, ψ) = (−60°, −28°) in N₃-heme-HO-1). The dihedral angles of the other biliverdin(Fe)-HO-1 residues were similar to those in heme-HO-1 and N₃-heme-HO-1. That is, the deviations of the dihedral angles between the heme and biliverdin(Fe) complexes with HO are limited to two consecutive amino acid residues, Ser-142 and Gly-143. Thus, the conformational change in the backbone at these residues in biliverdin(Fe)-HO-1 caused the outward shift of F-helix from the heme pocket after this kink and also brings about rearrangement of the hydrogen-bonds in the kinked region.

Proximal Helix—The proximal helix of biliverdin(Fe)-HO-1 is shifted slightly away from the heme pocket compared with the helices in heme-HO-1 and N₃-heme-HO-1. The His-25 ligand of biliverdin(Fe)-HO-1 maintains a conformation similar to those of heme-HO-1 and N₃-heme-HO-1, and the distance between the iron chelated by biliverdin and Ne of His-25 (2.4 Å) is roughly comparable with the corresponding distances in heme-HO-1 (2.3 Å) and N₃-heme-HO-1 (2.1 Å) (Fig. 2). In heme-HO-1 and N₃-heme-HO-1, the amide side chain of Gln-38 of the B-helix is close enough to form a hydrogen-bond...
with the carbonyl group of the backbone of Glu-29 in the A-helix (2.9 Å in heme-HO-1, 2.7 Å in N₃-heme-HO-1), which indicates that it contributes to fixing the two helices on the proximal side of the heme. In biliverdin(Fe)-HO-1, however, the side-chain of Gln-38 is no longer hydrogen-bonded to the main-chain of Glu-29 (distance between Gln-38 N/H₉₂₈₀ and Glu-29 O is 4.4 Å) and has moved closer to the pyrrole ring-A of biliverdin(Fe). A similar conformational change in the side chain of Gln-38 occurs in apo-HO (16).

**Biliverdin-Iron Chelate Structure**—The wire-frame model around the heme pocket of biliverdin(Fe)-HO-1 is shown in Fig. 4. The Fₘหนาว=F map was clearly assignable to the electron density of biliverdin(Fe), consistent with the fact that heme cleavage occurs at the α-meso carbon. One propionate group of the biliverdin(Fe) adopted the same conformation as that of the heme in heme-HO-1 and was buried in the heme pocket, whereas the other group, which is exposed to the solvent in heme-HO-1, was disordered. The vinyl group of pyrrole ring-A, which is also exposed to solvent in heme-HO-1, was not visible. In addition, the temperature factors for the atoms of ring A are higher than those for the atoms of the other three rings. Taking into account that all four pyrrole rings of the heme in heme-HO-1 have similar temperature factors, the biliverdin(Fe) in biliverdin(Fe)-HO-1 seems to be accommodated less tightly in the heme pocket than the heme in heme-HO-1. Biliverdin was in helical conformation with the lactam oxygen atoms of ring A and ring B that point upward and downward, respectively. By nature, biliverdin assumes a helical structure to avoid collision with the lactam oxygen atoms. The helical pitch of the biliverdin in biliverdin(Fe)-HO-1, defined as the distance between the two oxygen atoms, was 3.4 Å, comparable with that (3.34 Å) in the crystal structure of biliverdin dimethyl ester (34). A crystallographic study of the iron(III) complex of octaethylbilindione (OEB) (35), a chemical model of biliverdin(Fe), showed that it forms a dimeric structure, [Fe³⁺(OEB)]₄, in which each iron is five-coordinate with bonds to four nitrogen atoms in one tetrapyrrole unit and with a bond to a lactam oxygen atom in the partner. Each OEB molecule in [Fe³⁺(OEB)]₄ has the analogous helical pitch of 3.22 Å, but relatively shorter helical pitches have been reported in biliverdin crystallized with proteins (e.g. for apomyoglobin (36), 2.9–3.0 Å; for biliverdin IXβ reductase (37), 2.8 Å). The iron atom in biliverdin(Fe) has no distal ligand, unlike the heme iron of heme-HO-1, the distal ligand of which is a water molecule or hydroxide. The coordination geometry to the iron atom is shown in Fig. 5. The distances between the iron atom and the nitrogen atoms of the four pyrrole rings vary considerably, from 2.0 to 2.3 Å, and the coordination angles of the four nitrogen atoms about the Fe-N axis (N₃,Fe-N₄; 91.4°, N₅,Fe-N₆ angle; 130.9°, N₇,Fe-N₈; 84.5°). This asymmetry of the iron coordination reflects the distorted helical structure of the biliverdin in biliverdin(Fe)-HO-1; the exposed ring A deviates markedly from the mean plane through the biliverdin(Fe) molecule to the distal side, whereas the buried ring B is shifted slightly from the plane to the proximal side.
that the conformation of the F helix changes to open the heme facilitates both substrate binding and product release. The heme in the holo state (14, 17) but partially fluctuates in HO reaction (13–15, 17). This helix is in close contact with the network with Asp-140, Arg-136, and Thr-135. This network is formed by Met-34, Phe-37, and Phe-214. These residues remain unchanged in biliverdin(Fe)-HO-1. On the opposite side of the heme in heme-HO-1, Lys-179, Arg-183, and Lys-18 interact electrostatically with the propionate groups of the heme. In biliverdin(Fe)-HO-1, despite disordering of one of the two propionate groups, these basic residues retain conformations similar to those in heme-HO-1. In the distal heme pocket of heme-HO-1, five water molecules (W1–W5) form a hydrogen-bonding network with Asp-140, Arg-136, and Thr-135. This network is completely conserved in N₃⁻–heme-HO-1, and W1 is hydrogen-bonded with the azide ligand (Fig. 6). In biliverdin(Fe)-HO-1, three of the five water molecules (W1–W3) form a hydrogen-bonding network with Asp-140 and Arg-136 (Fig. 6). It should be noted that W1 in biliverdin(Fe)-HO-1 is close enough to form a hydrogen-bond (2.6 Å) with the ring-A oxygen of biliverdin. On the proximal side of biliverdin, there are no water molecules, and the ring-B oxygen does not interact with the protein moieties.

**Protein-Biliverdin Interactions**—In biliverdin(Fe)-HO-1, the carbonyl group of Gly-139 and the amide group of Gly-143 are no longer in contact with the iron atom because of the outward shift of the F helix and absence of the distal ligand. The α-meso edge of the heme in heme-HO-1 faces a hydrophobic wall formed by Met-34, Phe-37, and Phe-214. These residues remain unchanged in biliverdin(Fe)-HO-1. On the opposite side of the heme in heme-HO-1, Lys-179, Arg-183, and Lys-18 interact electrostatically with the propionate groups of the heme. In biliverdin(Fe)-HO-1, despite disordering of one of the two propionate groups, these basic residues retain conformations similar to those in heme-HO-1. In the distal heme pocket of heme-HO-1, five water molecules (W1–W5) form a hydrogen-bonding network with Asp-140, Arg-136, and Thr-135. This network is completely conserved in N₃⁻–heme-HO-1, and W1 is hydrogen-bonded with the azide ligand (Fig. 6). In biliverdin(Fe)-HO-1, three of the five water molecules (W1–W3) form a hydrogen-bonding network with Asp-140 and Arg-136 (Fig. 6). It should be noted that W1 in biliverdin(Fe)-HO-1 is close enough to form a hydrogen-bond (2.6 Å) with the ring-A oxygen of biliverdin. On the proximal side of biliverdin, there are no water molecules, and the ring-B oxygen does not interact with the protein moieties.

**DISCUSSION**

**Conformational Change in the Distal Helix**—One of the intriguing features of the HO-1 structure is the kinked conformation of the distal F-helix, which furnishes α-selectivity to the HO reaction (13–15, 17). This helix is in close contact with the heme in the holo state (14, 17) but partially fluctuates in the apo state (16), indicating that the flexibility of this helix facilitates both substrate binding and product release. The biliverdin(Fe)-HO-1 structure presented here clearly shows that the conformation of the F helix changes to open the heme pocket when the α-meso edge of the heme is cleaved. This is the first evidence that conformational change in the F helix actually occurs during the HO reaction. The amide group of Gly-143 is in close contact with the heme distal ligand in heme-HO-1, whereas in biliverdin(Fe)-HO-1, its direction is altered to form a hydrogen-bond with the carbonyl group of Gly-139, resulting in the outward shift of Gly-143 and the next several residues from the heme pocket. This movement is attributable to steric hindrance between F helix and pyrrole ring-A; Gly-143 will collide with ring A unless the backbone conformation of the F helix changes. Another possible factor for re-direction of the amide bond of Gly-143 in biliverdin(Fe)-HO-1 is loss of the distal water ligand in biliverdin(Fe). Such ligand-dependent conformational changes are consistent with the presence of two conformers, opened and closed forms, in an asymmetric unit of human heme-HO-1 crystals (13) grown at pH 7.5, which is near the pK₅ of the water ligand bound to the heme iron. Previous resonance Raman studies of heme-HO-1 complexes suggested that the distal ligand exchange from water to hydroxide occurs at pK₅, accompanied by high-to-low spin transition of the heme iron (38, 39).

**Comparison with Human HO-1 Structures**—Structural comparison of biliverdin(Fe)-HO-1 with human heme-HO-1 molecules (Fig. 7) shows that the distal helix conformation of biliverdin(Fe)-HO-1 is similar to that of the opened form (molecule B) of human heme-HO-1, consistent with the fact that this human isoform has an open heme pocket with loose interaction between the distal helix and heme (13). In this isoform, as in biliverdin(Fe)-HO-1, hydrogen-bonds are formed between Gly-139 oxygen and Gly-143 nitrogen and between Asp-140 oxygen and Gly-144 nitrogen (Fig. 2, Table II). Interestingly, the distal helix of the other human isoform, the closed form (molecule A), has a conformation similar to that in biliverdin(Fe)-HO-1 rather than to the conformations in heme-HO-1 and N₃⁻–heme-HO-1. No hydrogen-bond exists between Gly-139 oxygen and Gly-144 nitrogen in either human HO-1 isoform. This agrees with the fact that contacts between the distal helix and heme are less tight in the human HO-1 closed form compared with rat HO-1 complexes with heme. The human closed form therefore is considered to be a conformation intermediate between that of the rat heme-HO-1 and human opened form (13, 14), and the biliverdin(Fe)-HO-1 structure should be in the conformational group with an open heme pocket.

**Dissociations of Iron and Biliverdin**—Iron release is believed to occur before biliverdin dissociation (12). The coordination geometry of the iron in biliverdin(Fe)-HO-1 showed substantial distortion from either of the two ideal forms, square-pyramidal, and trigonal-bipyramidal, for five-coordinate metal clusters, indicative of the susceptibility to lose iron. The iron coordination in (Fe⁵⁺(OEB))₂ (35) approximates a trigonal bipyramid, but that compound is readily demetalated; the iron site has a partial occupancy of 0.56; therefore, the crystal is apparently composed of a mixture of (Fe⁴⁺(OEB))₂ and (Fe⁵⁺(OEB)) (H₂OEB) in which one of the iron ions is lost. Although the iron site was fully occupied in the biliverdin(Fe)-HO-1 crystal we studied, iron was partially lost when the crystal was overincubated with sodium ascorbate (data not shown). Conceivably, the distorted geometry of iron coordination in biliverdin(Fe)-HO-1 could facilitate the release of the iron from biliverdin(Fe).
Based on early work on the degradation of the heme bound to HO in a reaction system with ascorbate (12), the iron in the biliverdin(Fe)-HO-1 crystal is probably in the ferric state. A kinetic study of the HO reaction (40) showed that product release from HO first requires the reduction of Fe$^{3+}$-biliverdin to Fe$^{2+}$-biliverdin; on reduction of iron, rapid release of iron occurs and the slower dissociation of biliverdin follows. Reduction of the iron in biliverdin(Fe)-HO-1 may not affect its distorted geometry because the iron(II) complex of a methoxy derivative of OEB, [Fe$^{2+}$(OEOB)$_2$]$_2$ (41), has a structure very similar to that of [Fe$^{3+}$OEB]$_2$. The superior solubility of ferrous to ferric iron may be favorable for iron release (10, 40).

The nature of the binding of biliverdin(Fe) in the heme pocket of HO, together with the structure of the heme pocket in the apo state (16), gives an insight into a possible mechanism for the final product release from the enzyme. Upward movement of the distal helix and relaxation of the proximal helix allow the active site to be opened, but several residues (Met-34, Phe-37, Phe-214, Lys-179, and Arg-183) that surround the biliverdin(Fe) in biliverdin(Fe)-HO-1 have conformations similar to those in heme-HO-1 and N$_2$-heme-HO-1. Conserved interactions between the biliverdin(Fe) and protein moieties of HO are consistent with the finding that even in the apo state, these residues roughly retain their conformations. The position of biliverdin(Fe) also remains unchanged compared with the heme positions in heme-HO-1 and N$_2$-heme-HO-1. The binding of biliverdin(Fe) to the apo heme pocket, however, is less tight than that of the heme; the propionate and vinyl groups that are exposed to the solvent are invisible in biliverdin(Fe)-HO-1. The loose accommodation of biliverdin is accounted for by the opened conformation of the distal helix and the decreased rigidity of the tetrapyrrole caused by $\beta$-meso cleavage. As stated earlier in this article, the helical pitch of the biliverdin(Fe) in biliverdin(Fe)-HO-1 is longer than that of the biliverdin bound to apomyoglobin (36). In contrast to the partial exposure of the biliverdin in biliverdin(Fe)-HO-1, the biliverdin in the biliverdin-apomyoglobin complex is buried in the myoglobin heme pocket. Comparison of both biliverdin structures bound to proteins shows the marked deviation of ring A from the mean plane of the tetrapyrrole in biliverdin(Fe)-HO-1, indicating that interaction between the ring-A oxygen atom and distal hydrogen-bonding network produces the distorted helical structure of biliverdin(Fe). Upon release of the iron, the mechanism of which is unknown, the proximal helix should be more relaxed because of loss of the coordination bond between the iron and His-25. Iron release would therefore render biliverdin more flexible, compelling it to a less stable accommodation state in the heme pocket. Therefore, the final product release would be mainly caused by the increased flexibility of biliverdin assisted by the widening of the active site. This scheme suggests slow dissociation of biliverdin from the HO active site, which is consistent with the finding that biliverdin release is the rate-limiting step of single turnover in the HO reaction (10, 40).

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Crystal Structure of Rat Heme Oxygenase-1 in Complex with Biliverdin-Iron Chelate: CONFORMATIONAL CHANGE OF THE DISTAL HELIX DURING THE HEME CLEAVAGE REACTION
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