Crystal structure of rat haem oxygenase-1 in complex with ferrous verdohaem: presence of a hydrogen-bond network on the distal side

Hideaki SATO*†, Masakazu SUGISHIMA*†, Hiroshi SAKAMOTO‡, Yuichiro HIGASHIMOTO*, Chizu SHIMOKAWA*, Keiichi FUKUYAMA‡, Graham PALMER§ and Masato NOGUCHI*‡

*Department of Medical Biochemistry, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan; †Department of Bioscience and Bioinformatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology, 680-4 Kawazu, Iizuka 820-8502, Japan; ‡Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan, and §Department of Biochemistry and Cell Biology, Rice University, 6100 Main Street, Houston, TX 77005-1892, U.S.A.

INTRODUCTION

HO (haem oxygenase) (EC 1.14.99.3) is a microsomal enzyme that catalyses the O₂-dependent degradation of haem to biliverdin, CO and ferrous iron via three successive oxygenation reactions, i.e. haem to α-hydroxyhaem, α-hydroxyhaem to α-verdohaem and α-verdohaem to ferric biliverdin–iron chelate. In the present study, we determined the crystal structure of ferrous α-verdohaem–rat HO-1 complex at 2.2 Å (1 Å = 0.1 nm) resolution. The overall structure of the verdohaem complex was similar to that of the haem complex. Water or OH⁻ was coordinated to the verdohaem iron as a distal ligand. A hydrogen-bond network consisting of water molecules and several amino acid residues was observed at the distal side of verdohaem. Such a hydrogen-bond network was conserved in the structures of rat HO-1 complexes with haem and with the ferric biliverdin–iron chelate. This hydrogen-bond network may act as a proton donor to form an activated oxygen intermediate, probably a ferric hydroperoxide species, in the degradation of α-verdohaem to ferric biliverdin–iron chelate similar to that seen in the first oxygcnation step.

Key words: biliverdin–iron chelate, distal ligand, haem oxygenase, hydrogen-bond network, verdohaem.

Abbreviations used: CPR, NADPH–cytochrome P450 reductase; HO, haem oxygenase; rHO-1, rat HO-1.

1 These authors contributed equally to this study.

2 To whom correspondence should be addressed (email mnoguchi@med.kurume-u.ac.jp).

The co-ordinates and structure factors of the ferrous verdohaem–rat haem oxygenase-1 complex have been deposited in the Protein Data Bank with the accession code 2ZVU.
Figure 1  Degradation of haem catalysed by HO

(a) HO catalyses the degradation of haem to biliverdin IXα through three distinct intermediates, α-hydroxyhaem (α-hydroxylheme), α-verdohaem (α-verdoheme) and ferric biliverdin–iron chelate, at the expense of O₂ and electrons. (b) Resonance structures of ferrous α-verdohaem.

step of ring opening of verdohaem by HO [2,22,23]. Indeed, excess exogenous CO inhibits this oxygenation step [25], and the HO reaction substantially attenuates specifically at the verdohaem stage under a gaseous mixture of air and CO (e.g. air/CO = 5:1) [26]. This step is also inhibited by Ni²⁻ and CN⁻ [25], O₂⁻ and H₂O₂-bound forms on the verdohaem iron of ferrous verdohaem–rHO-1 complex have been reported [17,27]. All of these facts suggest that the degradation of verdohaem begins with O₂ binding to the verdohaem iron and then proceeds to the formation of ferric hydroperoxide, as seen in the first oxygenation step, haem to α-hydroxyhaem [17,27].

In the last decade, a variety of mammalian HO crystal structures have been described, including HO in complex with haem both with and without distal ligands [28–32], or in complex with haem-degradation intermediates [22,33]. For instance, rHO-1 in complex with ferric haem consists of eight α-helices, and the substrate haem is sandwiched between a proximal helix (Leu²⁻, Leu²⁵⁻, Glu²⁸⁻) and a distal helix (Leu²⁹⁻, Met³⁰⁻) [29]. The side chain of His³⁵⁻ in the proximal helix contributes the proximal haem ligand and water or OH⁻ serves as the distal ligand. No dissociable residue is present on the distal side of the haem, but the backbones of Gly¹⁹⁻ and Gly¹⁴³⁻ in the distal helix are close to the distal ligand.

To investigate the chemistry of the verdohaem degradation, information on the three-dimensional structure of verdohaem–HO complex is indispensable. Recently Lad et al. [22], for the first time, solved the structure of human HO-1 in a complex with ferrous verdohaem. The distal ligand of the verdohaem iron was absent from this crystal structure. In addition, a hydrogen-bond network of water molecules, which is conserved in the structures of ferric haem [29], ferrous haem [31] and ferric biliverdin–iron chelate [34] complexes of rHO-1, was not observed in its distal side. Thus Lad et al. [22] concluded that the network of water molecules, which provides the required protons to activate the O₂-bound haem–HO-1 complex, is absent from the structure of the ferrous verdohaem–human HO-1 complex. However, according to resonance Raman spectra of a ferrous verdohaem–rHO-1 complex, the verdohaem iron seems to be six-co-ordinate and the distal ligand has been tentatively assigned as OH⁻ [35]. A ¹³C-NMR study of ferrous verdohaem in complex with HO from Neisseria meningitidis arrived at the same conclusion [36].
understand the detailed mechanism of verdohaem oxygenation by HO, it is important to clarify whether the hydrogen-bond network and the distal ligand are present or not.

In the present paper, we report the crystal structure of verdohaem–rHO-1 complex. Our results show the presence of a distal ligand to the verdohaem iron and a hydrogen-bond network consisting of water molecules and amino acid residues in the distal side of verdohaem. The role of the hydrogen-bond network in the degradation of verdohaem by HO is discussed.

**EXPERIMENTAL**

**Materials**

Haemin was purchased from Sigma. A solution of 5.0 M sodium formate (pH 7.0) was purchased from Hampton Research. Ferrous iron 5-oxa-protoporphyrin IX (verdohaem) was synthesized and purified as reported previously [14]. Formation of ferrous verdohaem was confirmed by its optical absorption spectrum (λmax in aqueous pyridine solution: 397, 505, 534 and 680 nm). Concentration of the bispyridine complex of verdohaem was determined spectrophotometrically using ε534 = 53.3 M−1 cm−1 [14]. All spectrophotometric analyses were conducted on a Varian Cary 50 Bio UV–visible spectrophotometer at 25 °C.

**Preparation of ferrous verdohaem–rHO-1 complex**

A soluble form of rHO-1 lacking the 22-amino-acid C-terminal hydrophobic segment was expressed in *Escherichia coli* and purified as described previously [37]. The reconstitution and purification of verdohaem–rHO-1 complex were performed as reported previously [14,38]. Unless otherwise stated, the following manipulations were carried out anaerobically in a Unilab glove box system (M. Braun Inertgas-Systeme GmbH) filled with nitrogen gas. Briefly, to 30 μM rHO-1 solution in 10 mM potassium phosphate buffer (pH 7.0) was added a slight excess of ferrous verdohaem dissolved in pyridine. The mixture was incubated at 2°C for 1 h, and then applied to a 1 ml hydroxyapatite (Bio-Rad Laboratories) column equilibrated with 10 mM potassium phosphate buffer (pH 7.0) [37]. To remove pyridine and unbound verdohaem, the column was washed with the same buffer, and eluted with 0.1 M potassium phosphate buffer (pH 7.0). The fractions containing the ferrous verdohaem–rHO-1 complex were combined and concentrated by ultrafiltration with Microcon YM-10 membrane (Millipore). The ferrous verdohaem–rHO-1 complex in 0.1 M potassium phosphate buffer (pH 7.0) was added a 5 mM sodium azide under anaerobic conditions. These crystal manipulations were carried out under anaerobic conditions in a custom-designed glove box to prevent the oxidative decomposition of verdohaem. After synchrotron irradiation, the absorption spectrum of the verdohaem–rHO-1 complex in crystals was monitored with a focused light beam designed to measure only the area of the enzyme crystal [39].

**Crystallographic data**

Diffraction data were collected at 100 K using synchrotron radiation [λ = 1.0000 Å (1 Å = 0.1 nm)] at the BL38B1 beamline of SPring-8 and the RIKEN/MSC Jupiter 210 detector. Diffraction data were processed and scaled with HKL2000 [40]. The crystal belonged to the space group *P*321 with the unit-cell dimensions *a* = *b* = 65.4 Å and *c* = 120.2 Å. The structure of the ferrous verdohaem–rHO-1 complex was determined using the molecular replacement method with MolRep [41,42] and the protein moiety of the ferrous haem–rHO-1 structure (PDB accession code 1UBB) [31] as a search model. The structure of the protein moiety was refined with Refmac5 [42,43]. The resultant *Fo* − *Fc* electron-density map showed significant electron density for the verdohaem and the sixth ligand of verdohaem iron. Then, verdohaem was added to the model and refined. Finally, water molecules and a formate ion were added to the model and refined.

**RESULTS**

**Characterization of the crystals obtained in the co-crystallization of rHO-1, ferrous verdohaem and azide**

The verdohaem–rHO-1 complex in solution showed a Soret absorption peak at 400 nm and visible peaks at 690 and 534 nm.

**Table 1** Diffraction and refinement statistics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>50–2.20</td>
</tr>
<tr>
<td>Number of observations</td>
<td>76000</td>
</tr>
<tr>
<td>Number of unique reflections</td>
<td>15371</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>98.9 (90.7)</td>
</tr>
<tr>
<td>Mean λ/σ (I)</td>
<td>12.5 (2.9)</td>
</tr>
<tr>
<td>Rfree (%)</td>
<td>6.8 (34.2)</td>
</tr>
<tr>
<td>R-factor/Rfree (%)</td>
<td>18.9/24.0</td>
</tr>
<tr>
<td>Number of protein atoms</td>
<td>1727</td>
</tr>
<tr>
<td>Number of ligand atoms (verdohaem)</td>
<td>43</td>
</tr>
<tr>
<td>Number of solvent molecules (formate/water)</td>
<td>1/109</td>
</tr>
<tr>
<td>Average atomic B-factors (Å²) (protein/ligand/solvent molecules)</td>
<td>35.5/36.2/41.6</td>
</tr>
<tr>
<td>Root mean square deviations from ideal values</td>
<td></td>
</tr>
<tr>
<td>Bond length (Å)</td>
<td>0.011</td>
</tr>
<tr>
<td>Bond angle (°)</td>
<td>1.79</td>
</tr>
<tr>
<td>Ramachandran plot</td>
<td>94.2</td>
</tr>
<tr>
<td>Most favoured (%)</td>
<td>5.8</td>
</tr>
<tr>
<td>Additionally allowed (%)</td>
<td></td>
</tr>
</tbody>
</table>

© The Authors Journal compilation © 2009 Biochemical Society
in the absence of sodium azide, and the peaks shifted to 404 and 707 nm respectively in the presence of 10 mM sodium azide (Figure 2a). This spectral shift is similar to that of N$_3^-$-bound ferrous verdohaem–rHO-1 complex as reported previously [20]. To protect verdohaem from non-enzymatic decomposition, 10 mM sodium azide was included in the verdohaem–rHO-1 complex solution during crystallization. The ligation of N$_3^-$ to verdohaem complexed with rHO-1 was confirmed by the colour change from green to brown. Rod-shaped crystals appeared after a few weeks and reached a maximum size (approx. 0.5 mm × 0.05 mm × 0.05 mm) in 2 months. Unexpectedly, the crystal colour was not brown, but green, indicating that N$_3^-$ had dissociated from verdohaem iron during crystallization. The possibility that verdohaem bound to rHO-1 was degraded to biliverdin–iron chelate by the residual dioxygen was eliminated by the analysis of X-ray diffraction and spectroscopic measurements of the crystal.

We collected X-ray diffraction data of this green crystal and determined its structure at a resolution of 2.2 Å. The structure of the protein moiety consisting of eight α-helices was similar to that of rHO-1 in complex with haem reported previously [29]. The electron-density map clearly showed that a planar compound similar to the haem is bound in the haem pocket of rHO-1, which was easily assignable to verdohaem as shown in Figure 3(a). Thus it appears that this green crystal is that of RHO-1 in complex with ferrous verdohaem. To confirm this conclusion and evaluate the effect of X-ray irradiation, we measured the absorption spectrum of another crystal that was obtained under crystallization conditions similar to those used for the X-ray data collection and irradiated with a similar X-ray dose (Figure 2b). An absorption peak at 685 nm indicated that ferrous verdohaem in complex with rHO-1 had not ring-opened and that N$_3^-$ had dissociated from verdohaem iron. The spectrum also exhibited an absorption peak at 470 nm (Figure 2b) that was similar to that of ferrous verdohaem π-neutral radical species in complex with rHO-1 observed in electrochemical titration experiments (Figure 2c) [24]. We suggest that a part of the oxaporphyrin ring of ferrous verdohaem in complex with rHO-1 has been photoreduced to form the π-neutral radical species as a consequence of the synchrotron irradiation.

**Structure of the verdohaem-binding site**

The imidazole N$_\varepsilon$ of His$^{25}$ was co-ordinated to the verdohaem iron and five water molecules, W1–W5 (Figure 3b), formed the distal hydrogen-bond network (Figure 3) observed previously in the structure of haem–rHO-1 complex [29], as well as in its distal-ligand bound forms [30,31]. This hydrogen-bond network was also observed in the structure of RHO-1 complexed with the biliverdin–iron chelate [33]. These facts suggest that the distal hydrogen-bond network is conserved throughout the HO reaction. Although N$_3^-$ was not co-ordinated to the verdohaem iron, some electron density was located on the distal side of verdohaem iron (Figure 3a). The shape of this density was fitted well to a monoatomic molecule, but not to a linear triatomic molecule, such as N$_3^-$H$_2$. It is most likely that a water molecule or a OH$^-$ ion (W0, Figure 3b) is directly bound to the verdohaem iron, which is consistent with the absorption spectrum of the crystal and previous spectroscopic studies [35,36]. The fact that the refined temperature factor of W0 (31.2 Å$^2$) is similar to that of verdohaem iron (34.5 Å$^2$) is also consistent with this conclusion. The refined structure shows that the distance between the verdohaem iron and W0 is 2.7 Å (Figure 3b), which is longer than the bond between haem iron and bound OH$^-$, 2.2 Å, in the ferric haem–rHO-1 complex [29]. The angle of the N$_\varepsilon$(His$^{25}$)–Fe–W0 linkage is 161$^{\circ}$ (Figure 3b), indicating that this linkage is highly bent compared with that of the ferric haem–rHO-1 complex. The side chains of Arg$^{103}$ and Tyr$^{158}$ displayed electrostatic interaction and hydrogen-bonding with a propionate group of verdohaem (Figure 3b), as observed in ferric haem complexed with rHO-1 [29]. Residual electron density around the methyl groups of the model of verdohaem (Figure 3a) may indicate that a second binding mode of verdohaem that is rotated 180$^{\circ}$ about the α–γ axis of the verdohaem exists in this crystal, as seen in the haem–HO-1 complex [30,34].

**Structural comparison of the ferrous verdohaem–rHO-1 complex with the ferric, ferrous and CO-bound haem–rHO-1 complexes**

Structural comparison of the ferrous haem–rHO-1 complex and the ferrous verdohaem–rHO-1 complex shows that verdohaem...
slides slightly toward the $\alpha$-meso direction of the porphyrin ring (Figure 4a). Upon binding of CO to the ferrous haem–rHO-1 complex, a similar structural change occurs, and the distal helix is also shifted in the $\gamma$-meso direction to avoid the steric collision between the distal ligand and the distal helix [31]. However, such a massive structural shift of the distal helix does not occur upon verdohaem binding. In the ferric haem–rHO-1 complex, the distal OH$^-\,ion bound to the haem iron can form hydrogen bonds to the carbonyl oxygen of Gly$^{139}$ and the amide nitrogen of Gly$^{143}$ [29]. But the hydrogen bond between W0 and the amide nitrogen of Gly$^{143}$ is lost in the verdohaem–rHO-1 complex because of the slight shift of verdohaem and the tilt of the Fe–W0 bond with respect to the verdohaem plane (Figure 3b). Instead, W0 forms a hydrogen bond to W1.

![Figure 3](image3.png) **Figure 3** Structure of the ferrous verdohaem–rHO-1 complex

Verdohaem (green), His$^{25}$ and Thr$^{135}$–Gly$^{144}$ (yellow) are shown as stick models. Water molecules of distal hydrogen-bond network are shown as spheres (red). (a) Omit map of the verdohaem, its distal ligand and water molecules of the distal hydrogen-bond network. Residual electron-density map (contoured at 3.5 $\sigma$, blue), in which verdohaem and its distal ligand are omitted from the model is superimposed on the model. Residual electron density map (contoured at 3.0 $\sigma$, brown) in which water molecules of the distal hydrogen-bond network are omitted is also superimposed on the model. (b) Close-up view of the verdohaem-binding site of rHO-1. Co-ordination bonds of the verdohaem iron and distal hydrogen-bond network are shown as continuous and broken lines respectively. Figure prepared using PyMOL (DeLano Scientific; http://pymol.sourceforge.net/).

![Figure 4](image4.png) **Figure 4** Structural comparison of ferrous verdohaem–, ferric haem–, ferrous haem–, CO-bound haem– and ferric biliverdin–iron chelate–rHO-1 complexes

Haem, verdohaem, their distal ligands and biliverdin–iron chelate are shown as stick models. C$\alpha$ traces are shown for the other residues. (a) The structures of ferric haem–rHO-1 (yellow, PDB accession code 1DVE), ferrous haem–rHO-1 (orange, PDB accession code 1UBB) and CO–haem–rHO-1 (black, PDB accession code 1IX4) are superimposed on the structure of ferrous verdohaem–rHO-1 (green) to minimize the RMSD (root mean square deviations) of the positions of the C$\alpha$ atoms (RMSDs = 0.53 Å for ferric haem–rHO-1, 0.22 Å for ferrous haem–rHO-1 and 0.36 Å for CO–haem–rHO-1). (b) The structure of ferric biliverdin–iron chelate–rHO-1 (magenta, PDB accession code 1J2C) is superimposed on the structure of ferrous verdohaem–rHO-1 (green) to minimize the RMSDs of the positions of the C$\alpha$ atoms (RMSD = 0.42 Å). Several residues (His$^{25}$ and Leu$^{135}$–Gly$^{144}$) are shown as stick models. Water molecules of the distal hydrogen-bond network are shown as spheres (orange, ferrous verdohaem–rHO-1; magenta, ferric biliverdin–iron chelate–rHO-1). Figure prepared using PyMOL (DeLano Scientific; http://pymol.sourceforge.net/).

**DISCUSSION**

It has been suggested that the hydrogen-bond network on the distal side plays important roles in the first oxygenation step of the HO reaction, where ferric hydroperoxide (Fe$^{3+}$-OOH) is proposed as an activated oxygen species. Although the ferric hydroperoxide species has a short lifetime and high reactivity, it has been detected in the haem–rHO-1 complex by EPR study at 77 K [44]. Protons required for the formation of the active species could be provided by the hydrogen-bond network. For the conversion of ferrous verdohaem into the biliverdin–iron chelate, the ferric hydroperoxide species has also been proposed as a possible reactive intermediate in the HO catalytic cycle. In the crystal structure of the ferrous verdohaem–rHO-1 complex, a hydrogen-bond network consisting of five water molecules (W1–W5) and surrounding amino acid residues such as Thr$^{135}$, Arg$^{136}$, Asp$^{140}$ and Tyr$^{58}$ is observed in the distal pocket (Figure 3b).
therefore propose that the hydrogen-bond network is involved in the catalytic conversion of verdohaem into biliverdin–iron chelate, as seen in the oxygenation of haem to α-hydroxyhaem. The distance between the distal ligand W0 and the water molecule W1 is only 2.9 Å, and W2 makes hydrogen bonds to W1 and the carboxyl side chain of Asp319, the replacement of which by alanine or phenylalanine significantly decreases the catalytic activity from verdohaem to biliverdin [17]. Thus this hydrogen-bond network appears to play a key role in the proton relay.

A comparison of the crystal structures of the ferrous verdohaem–rHO-1 and ferric biliverdin–iron chelate–rHO-1 complexes [33] reveals that the tetrapyroles are accommodated in the haem pocket with the same orientation in both crystals, probably due to an ionic interaction of Arg319 and a hydrogen bond of Tyr334 to a propionate group of these intermediates (Figure 3b). Verdohaem in complex with rHO-1 is nearly planar, and W0 contacts the hydrogen-bond network. On the other hand, the ferric biliverdin–iron chelate in complex with rHO-1 is ruffled, and some water molecules (W1, W2 and W3) are conserved in the distal side of the ferric biliverdin–iron chelate–rHO-1 complex (Figure 4b). Pyrrole A of the ferric biliverdin–iron chelate is shifted to the distal side, and its lactam oxygen makes a hydrogen bond with W1. Thus it would be reasonable to conclude that the hydrogen-bond network is essentially conserved during the ring opening of ferrous verdohaem to the ferric biliverdin–iron chelate.

The previous resonance Raman and 13C-NMR studies of ferrous verdohaem complexed with the HO from rat and Neisseria meningitidis respectively had suggested that the verdohaem iron was six-co-ordinate and the distal ligand was tentatively assigned as OH− [35,36]. In contrast, the distal water ligand was not observed in the crystal structure of the ferrous verdohaem–human HO-1 complex reported earlier [22] (see Supplementary Figure S1 at http://www.BiochemJ.org/bj/419/bj4190339add.htm). In the present structure of the ferrous verdohaem–rHO-1 complex, coordination of W0 to the verdohaem iron was observed. The long bond between the iron and W0 and the skewed co-ordination geometry (Figure 3b) suggest that the co-ordination of W0 to the verdohaem iron is weak. Because ferrous verdohaem has resonance structures as shown in Figure 1(b), the oxidation state of the central iron lies between +2 and +3. In fact, CN−, N3− and CO can bind to iron of the verdohaem–HO-1 complex as axial ligands [20]. Thus the bond between the verdohaem iron and W0 might be weakened in the ferrous verdohaem–rHO-1 complex relative to that in the ferric haem–rHO-1 complex. Differences in crystallization conditions may affect the distribution of charge between the central iron and the porphyrin macrocycle and change the co-ordination of the verdohaem iron. In addition, the distal hydrogen-bond network found in the structure of the ferrous verdohaem–rHO-1 complex (Figure 3b) was absent from the structure of the ferrous verdohaem–human HO-1 complex reported previously [22] (Supplementary Figure S1 at http://www.BiochemJ.org/bj/419/bj4190339add.htm). It is unclear why such differences in the distal ligand and hydrogen-bond network are observed between the complexes of ferrous verdohaem with rHO-1 and human HO-1. As reported previously, however, using ascorbate as a reductant, the haem-degradation reaction could proceed to the biliverdin–iron chelate stage in ferric haem–rHO-1 crystals [33]. Because the HO activity is not severely inhibited under these crystallization conditions and by the crystal packing, it is likely that the crystal structure is essentially identical with the solution structure. The ferrous verdohaem–rHO-1 crystals in the present study were obtained under similar crystallization conditions as the haem–rHO-1 crystals. The obtained ferrous verdohaem–rHO-1 crystal was isomorphous with that of the haem–rHO-1 complex. Thus we presume that our structure of the ferrous verdohaem–rHO-1 complex properly reflects the intermediate state of the HO reaction.

During co-crystallization of ferrous verdohaem–rHO-1 complex with N3−, the ligand dissociated from the sixth co-ordination site. The crystal structure and the absorption spectrum of the crystal showed that W0 had replaced the azide. Compared with the position of haem in the structure of haem–rHO-1 complex, ferrous verdohaem in rHO-1 shifts slightly toward the α-meso direction of the porphyrin ring (Figure 4a). This structural difference suggests that the distal side of the ferrous verdohaem–rHO-1 complex is too narrow to accommodate N3− in the crystal. In the crystal structure of N3−-bound haem–rHO-1 complex, N3− is oriented nearly parallel to the haem plane by the steric constraint of the distal helix [30]. Thus the small change of the position of iron in the haem pocket may affect the affinity of N3− to the verdohaem iron in the verdohaem–rHO-1 crystal.

In the present study, we have clearly shown that a water molecule or an OH− ion is co-ordinated to the verdohaem iron as a distal ligand, and is involved in a hydrogen-bond network in the distal side of verdohaem–rHO-1 complex. This distal hydrogen-bond network is conserved in haem–, verdohaem– and biliverdin–iron chelate–rHO-1 complexes, and could provide protons that are required for active species formation in verdohaem degradation. We have also shown that the orientation of haem and its reaction intermediates, verdohaem and biliverdin–iron chelate, in HO are all the same. This is controlled by the interaction of a propionate group of the substrate and intermediates with the side chains of Arg319 and Tyr334 via ionic and hydrogen bonds respectively. It is known that, except for α-verdohaem, the other verdohaem isomers, β-, γ- and δ-isomers, cannot serve as substrates for HO [25]. This can be explained by the fact that only the α-isomer can be accommodated in the haem pocket in the proper orientation for α-regiospecific cleavage.

ACKNOWLEDGEMENTS

We thank Dr Kazumasa Muramoto (University of Hyogo, Hyogo, Japan) and Dr Eiki Yamashita (Osaka University, Osaka, Japan) for their help with the collection of absorption spectra of the verdohaem–rHO-1 crystal using a microspectroscopic technique. We also thank Dr Seiki Baba of JASRI (Japan Synchrotron Radiation Research Institute) for his aid with data collection using synchrotron radiation at SPring-8 (Proposal No. 2008A1096).

FUNDING

This work was supported in part by Grants-in-Aid for Young Scientists [grant numbers 19750150 (to H. Sato), 20770092 (to M. S.) and 18770121 (to Y. H.)] from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, by Grants-in-Aid for Scientific Research [grant numbers 18590278 (to M. N.), 20370037 (to K. F.) and 18550153 (to H. Sakamoto)] from the Japan Society for the Promotion of Science, by National Institute of Health [grant number GM080575 (to G. P.)], and by a grant from the Ishibashi Foundation for the Promotion of Science (to H. Sato).

REFERENCES

Hydrogen-bond network in ferrous verdohaem–rat HO-1 complex

SUPPLEMENTARY ONLINE DATA
Crystal structure of rat haem oxygenase-1 in complex with ferrous verdohaem: presence of a hydrogen-bond network on the distal side

Hideaki SATO*, Masakazu SUGISHIMA*, Hiroshi SAKAMOTO†, Yuichiro HIGASHIMOTO*, Chizu SHIMOKAWA*, Keiichi FUKUYAMA‡, Graham PALMER§ and Masato NOGUCHI*2
*Department of Medical Biochemistry, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan, †Department of Bioscience and Bioinformatics, Graduate School of Computer Science and Systems Engineering, Kyushu Institute of Technology, 680-4 Kawazu, Iizuka 820-8502, Japan, §Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan, and 2Department of Biochemistry and Cell Biology, Rice University, 6100 Main Street, Houston, TX 77005-1892, U.S.A.

Figure S1 Structural comparison of verdohaem–rHO-1 and verdohaem–human HO-1 complexes

One structure of verdohaem–human HO-1 (dark cyan, PDB accession code 1TWN, chain A) is superimposed on the structure of verdohaem–rHO-1 (green) to minimize the RMSDs (root mean square deviations) of the positions of the Cα atoms (RMSD = 1.19 Å). Verdohaem and several residues (His25 and Arg136–Gly144) are shown as stick models. Cα traces are shown for the other residues. Iron-co-ordinated distal water and water molecules of the distal hydrogen-bond network in the verdohaem–rHO-1 complex are shown as red spheres.

REFERENCE


Received 24 November 2008/14 January 2009; accepted 20 January 2009
Published as BJ Immediate Publication 20 January 2009, doi:10.1042/BJ20082279

1 These authors contributed equally to this study.
2 To whom correspondence should be addressed (email mnoguchi@med.kurume-u.ac.jp).

The co-ordinates and structure factors of the ferrous verdohaem–rat haem oxygenase-1 complex have been deposited in the Protein Data Bank with the accession code 2ZVU.

© The Authors Journal compilation © 2009 Biochemical Society