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Coordination Structure of Active Site in Synthetic Hemoprotein (Albumin-Heme) with Dioxygen and Carbon Monoxide

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Summary: Recombinant human serum albumin (rHSA) incorporating the tetraphenylporphinatoiron(II) derivative with a covalently linked proximal base (FeP) [albumin-heme (rHSA-FeP)] is a synthetic hemoprotein, which can bind and release dioxygen (O_2) reversibly under physiological conditions. The coordination structure and spin-state of the active site in rHSA-FeP with O_2 and carbon monoxide (CO) were revealed by magnetic circular dichroism (MCD), resonance Raman (RR), and infrared (IR) spectroscopy. Under an N_2 atmosphere, the MCD spectrum of rHSA-FeP showed the formation of the five-coordinate ferrous high-spin complex of FeP. Upon exposure of this solution to O_2 or CO, the spectral pattern immediately changed to that of a six-coordinate ferrous low-spin species. The vibration stretching frequencies of the coordinated O_2 (ν_{O2}) and CO (ν_{CO}) were observed at 1158 cm^{-1} and 1964 cm^{-1}, respectively. The electronic structures of the O_2- and CO-adduct complexes of FeP in the hydrophobic pocket of albumin are both identical to those for FeP itself in toluene solution.

Keywords: albumin-heme; biomimetic; infrared spectroscopy; magnetic circular dishroism spectroscopy; O_2-adduct complex

Introduction

Human serum albumin (HSA) is the major plasma protein in our blood circulatory system, and it binds a great variety of metabolites and drugs.[1] The most well-known ligands for HSA are fatty acids, billilubin, and hemin. Hemin dissociated from the methemoglobin is immediately scavenged by HSA and safely transported to the liver for metabolism. Recently, Carter et al. first succeeded in revealing the single crystal structure of HSA complexed with a hemin in its subdomain IB with a 1.9 Å resolution.[2] From the viewpoint of clinical application, dioxygen

(O_2)-carrying albumin could be of extreme medical importance, however, it has also been known that the HSA-hemin hybrid is not able to reversibly coordinate O_2.

We have found that eight molecules of 2-[8-(2-methyl-1-imidazolyl)octanoyloxymethyl]-5,10,15,20-tetrakis[(α,α,α,α-*o*-pivaloylamino)phenyl]porphinatoiron(II) (FeP) are efficiently incorporated into recombinant HSA (rHSA),[3] which is now manufactured on a large scale by expression in *Pichia pastoris* as a host cell (Fig. 1).[4] The obtained synthetic hemoprotein (albumin-heme: rHSA-FeP) can bind and release O_2 under physiological conditions similar to hemoglobin and myoglobin, and showed a good compatibility with human whole blood. Hence, rHSA-FeP is recognized to be a synthetic O_2-carrying hemoprotein and one of the most promising materials not only as a blood replacement composition, but also as an O_2-delivering medicine.[5] In order to characterize the coordination structure of the active site in rHSA-FeP with O_2 and carbon monoxide (CO), we employed magnetic circular dichroism (MCD), resonance Raman (RR), and infrared (IR) spectroscopies. The electronic property and spin-state of the dioxygenated or carbonyl FeP in the rHSA host are described herein.

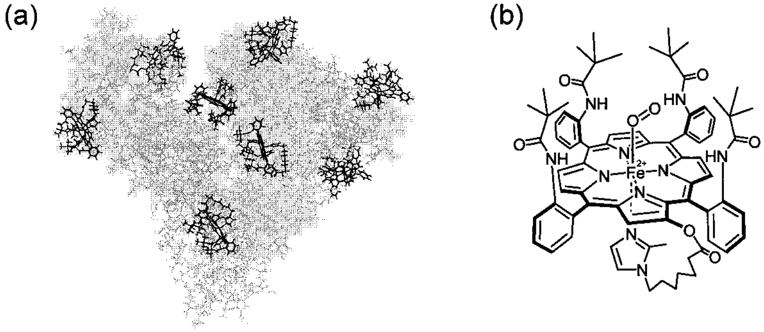

Figure 1. (a) The predicted three dimensional structure of rHSA included eight FeP molecules, (b) molecular structure of dioxygenated FeP.

Results and Discussion

a. MCD Spectroscopy

The application of MCD to natural hemoproteins and model hemes has been extensively studied as a fingerprint method for determining the oxidation state, spin state, and coordination structure of the heme.[6] Under an N_2 atmosphere, the MCD spectrum of rHSA-FeP showed the formation of the five-coordinate ferrous high-spin complex of FeP with intramolecular coordinated proximal imidazole (Fig. 2). This result showed no ligation of the amino-acid residues of the protein, *ex.* histidine, tyrosine, cysteine, to the six-coordination site of the heme.[6a),c)] The X-ray analytical data of ferric hemin incorporated into the subdomain IB of HSA showed that the central Fe(III) ion is tightly bound to Tyr-161 to form a five-coordinate complex.[2] The bulky pivalamido-fences on the porphyrin plane could prevent access of the neighboring peptide residues to the six-coordination site of FeP. Upon exposure of this solution to O_2, the spectrum immediately changed and showed an S-shaped A-term MCD in the Soret region, which indicates a transformation to the ferrous low-spin complex.[6b)] The carbonyl rHSA-FeP also exhibited a similar A-term MCD band in the same region with a much stronger intensity. The wavelength where the value of $[\theta]_M$ is zero for the O_2- and CO-adduct complex coincided well with the absorption maxima in their corresponding UV-vis. spectra (Table 1). In all cases, the complicated shapes in the Q-band regions are similar to those of the previously reported studies (not shown).[6b)]

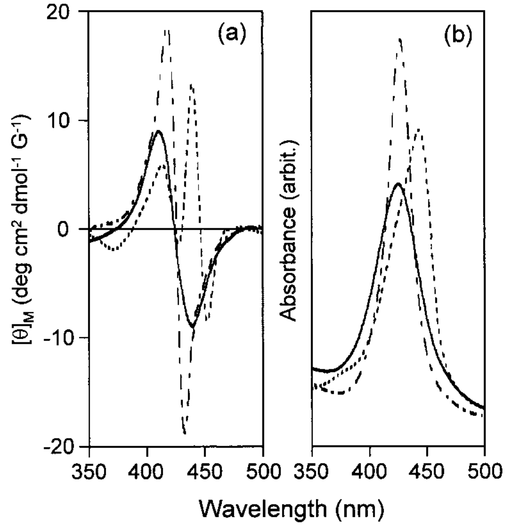

Figure 2. (a) MCD and (b) UV-vis. spectral changes of rHSA-FeP in phosphate buffer solution (pH 7.3) at 25°C: under N_2:
- - - - , under O_2: —— , under CO: - · - · -.

Table 1. Absorption maximam wavelength (nm) of MCD ($[\theta]_M$) and UV-vis. spectra of rHSA-FeP in phosphate buffered solution (pH 7.3) at 25 °C.

	MCD	UV-vis.
N_2	371 (–4.6), 414 (+9.4), 428 (–1.1), 440 (+22), 452 (–13)	440
O_2	411 (+15), 435 (–8.6)	426
CO	418 (29), 433 (–7.1)	426

(+) positive, (–) negative.

b. RR and IR Spectroscopy

The RR spectrum of the rHSA-FeP solution under an N_2 atmosphere showed a medium but distinct band at 201 cm^{-1}, which corresponded to the characteristic Fe(II)-N(imidazole) stretching mode ($\nu_{Fe\text{-}N\varepsilon}$) of the five-coordinate ferrous high-spin porphyrin, and was quite identical to that of FeP in CH_2Cl_2 (Table 2).[3a),b),7,8] This means that unusual hindrance of the axial imidazole coordination of FeP is never accomplished in albumin. After exposure to O_2, the $\nu_{Fe\text{-}O2}$ vibration of dioxygenated FeP appeared at 564 cm^{-1}, suggesting an end-on type O_2-coordination.[7,8] The deformation modes of the porphyrin ring (ν_8 and ν_4) were also upshifted from 376 and 1356 cm^{-1} to 393 and 1371 cm^{-1}, respectively. These changes further supported the conversion from the five-coordinate high-spin to the six-coordinate low-spin spcies.

Table 2. The stretching frequencies (cm^{-1}) of coordinated O_2, CO and Fe(II)-N_ε, and Fe(II)-O_2 of rHSA-FeP at 25°C.

	solvent	$\nu^{16}{}_{O2}$	$\nu^{18}{}_{O2}$	ν_{CO}	$\nu_{Fe\text{-}N\varepsilon}$	$\nu_{Fe\text{-}O2}$
rHSA-FeP	p.b.[a)] (pH 7.3)	1158	1079	1964	201	564
FeP[b)]	CH_2Cl_2	1160	1079	1964	201	565
FepivP(DIm)[c)]	Nujol/CH_2Cl_2	1159	1093	1969	200	564
Myoglobin[d)]	p.b.[1)] (pH 7.2)	1103	1065	1944	221	572
O_2 or CO (gas)[e)]	–	1556	–	2143	–	–
O_2^- (metal-O_2^-)[e)]	–	1100–1150	–	–	–	–

[a)] p.b.: Phosphate buffer. b) Ref. [8]. c) FepivP: 5,10,15,20-Tetrakis[(α,α,α,*o*-pivaloylamino) phenyl]porphinatoiron(II), DIm: 1,2-Dimethylimidazole, Ref. [9]. d) Ref. [10], [11]. e) Ref. [12].

From the difference IR spectrum of the rHSA-FeP solution under $^{16}O_2$ and CO, we assigned the vibration stretching modes of the coordinated O_2 ($\nu^{16}{}_{O2}$) and CO (ν_{CO}) to 1158 cm^{-1} and 1964 cm^{-1}, respectively (Table 2). Under $^{18}O_2$, the $\nu^{18}{}_{O2}$ appeared at 1079 cm^{-1}; the frequency shifting between the coordinated $^{16}O_2$ and $^{18}O_2$ is in good agreement with the calculated value from the harmonic oscilator prediction of the O-O stretching vibration. The ν_{O2} value was close to those of ionic superoxides and the end-on type O_2 bonding to the similar tetraphenylporphinatoiron(II) derivatives.[9] It is remarkable that the ν_{O2} and ν_{CO} values of rHSA-FeP are almost the same as those of FeP itself in CH_2Cl_2 solution. If the coordinated O_2 and CO have some weak interaction with the surrounding amino-acid residue, they should be siginificantly shifted. Nevertheless, it has not been seen. Based on these findings, we can conclude that (i) the coordination geometry of O_2 to FeP in albumin is a bent/end-on configuration in the same manner as hemoglobin and myoglobin, and (ii) the micro-protein environment around FeP does not affect the coordinated O_2 and CO ligands.

Conclusion

The coordination structure and spin-state of the active site in the synthetic O_2-carrying hemoprotein, *albumin-heme*, has been elucidated by MCD, RR, and IR spectroscopy. Under an N_2 atmosphere, all the FePs produce a five-coordinate high-spin complex with an intramolecularly coordinated axial imidazole similar to FeP itself in a non-ligated solvent, such as toluene and CH_2Cl_2. In the hydrophobic cavity of rHSA, the amino-acid residues may bind to the six-coordination site of the intercalated FeP, however, the four rigid pivaloyl-fences on the porphyrin ring protect the access of the peptide residue as a base. This is in contrast to the fact that planar protoporphirinatoiron(III) tightly coordinates to the Tyl-161 in the subdomain IB of HSA.[2] The coordination structure and electronic state of the dioxygenated and carbonyl complex of FeP in rHSA also showed a similarity to those in the organic solvents. Although the association kinetics of these gaseous ligands to rHSA-FeP are largely affected by the protein environment around the indivisual FeP,[3c)] the coordinated O_2 and CO are efficiently shielded by the four pivalamido-substituents which are presented as the nearest neighbor, and not influenced by the surrounding peptide residues.

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