Supplementary information

Ligand design for the improvement of the stability of metal complex•protein hybrids

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Experimental Section

Physical measurement. UV-vis spectra were recorded on a UV-2400PC UV-vis spectrometer (SHIMADZU). ^{1}H NMR spectra were recorded on a JNM-ECP500 and a JNM-GSX270 (JEOL). Concentration of iron and chromium ion was determined by a inductively coupled plasma spectrometer VISTA-PRO (varian). ESI-TOF mass spectra were measured on a LCT (micromass). Circular dichroism spectra were recorded on a JASCO model J-720 spectropolarimeter that was equipped with a JASCO model PTC-348WI Peltiert cooling temperature controller.

General Procedure and Materials. Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. 4-amino-3-hydroxyhydrocinnamic acid was synthesized followed by a reported method.[1] The expression and purification of heme oxygenase from *Corynebacterium diotheriae* were performed as described previously.[2]

Synthesis of Cr•2•HO To an ethanol solution of 4-amino-3-hydroxyhydrocinnamic acid (50 mg, 0.27 mmol, 2 ml) was added salicylaldehyde (34 mg, 0.33 mmol). This reaction mixture was refluxed for 2 hr. After cooling down to room temperature, **2** was

obtained as orange powder. : Yield: 86 %, δ_H(400 MHz; DMSO-d_6; Me_4Si) 13.9 (s, 1H, COOH), 9.70 (s, 1H, N=CH), 8.62 (s, 1H, Phe) 7.58 (d, J = 8.0 Hz, 1H, Phe), 7.37 (t, J = 6.8 Hz, 1H, Phe), 7.29 (d, J = 8.0 Hz, 1H, Phe), 6.93 (m, 2H, Phe), 6.81 (s, 1H, Phe), 2.77 (t, J = 7.6 Hz, 2H, Phe-CH_2CH$_2$), 2.53 (t, J = 8.0 Hz, 2H, CH$_2$CH_2COOH).

To a DMF solution of **2** (18 mg 0.0063 mmol, 1.0 ml) was added $CrCl_2$ (7.75 mg, 0.0063 mmol) under argon atmosphere and the mixture was refluxed for 2 hr. Then, the solution was stirred vigorously under air for 6h. Precipitate was removed by celite filteration. The diluted solution of **Cr•2•Cl** (18 mM, 80 μl) was slowly added to an aqueous solution of HO (0.72 μmol, 10 ml of 10 mM Tris/HCl buffer, pH 7.4) and gently stirred at 4°C for 30 minute. The mixture was dialyzed against 10 mM Tris/HCl buffer (pH 7.4) overnight at 4°C. After dialysis, the mixture was passed through Sephadex G25 (10 mM Tris/HCl buffer, pH 7.4). The composite was purified by DE52 (whatman) and HiTrapQ (GE healthcare) columns with liner gradients of Potassium Chloride (0-0.4 M) in 10 mM Tris/HCl buffer (pH 7.4). λ_{max}(10 mM Tris/HCl pH 7.4)/nm 474 (ε/dm^{3}mol^{-1}cm^{-1} 11100), 450 (12800), 279 (51400). *m/z* (ESI): 24341.9 (**Cr•2•HO** + H_2O requires 24338.6).

Fe•2•HO was also prepared by an almost same procedure. λ_{max}(10 mM Tris/HCl pH 7.4)/nm 393 (ε/dm^{3}mol^{-1}cm^{-1} 15200), 279 (40000).

Crystallization, X-ray Data Collection, and Crystallographic Refinement. **Cr•2•HO** was dialyzed against 20 mM MES buffer (pH 7.0), and concentrated to 19 mg/ml. Crystals were obtained by a hanging drop vapor diffusion method from a drop of the solution of the composite (1 μl) and a reservoir solution (1 μl) at 20°C. Reservoir solutions (500 μl) for **Cr•2•HO** contain polyethylene glycol 2000 monomethyl ester (30 % w/v) and ammonium sulfate (0.2 mM) in 100 mM MES buffer (pH 6.5). For cryogenic date collection, crystals were soaked into each reservoir solution containing 10 % v/v glycerol and flash-frozen by liquid nitrogen.

High resolution diffraction data of **Cr•2•HO** was collected with ADSC Quantum 4R using 1Å synchrotron radiation at BL6A of Photon Factory. The temperature around the crystals was maintained at 180K throughout the data collection. The oscillation angle, camera range and exposure time were 1°, 104.9 mm and 10 second, respectively. Data sets consisted of 180 frames. Data were integrated, merged and processed with HKL-2000.[3] Diffraction statistics are summarized in Table 2.

The structures were solved via molecular replacement with the structure of **Fe•1•HO** as an initial model for the refinements.[4] The refinement was carried out as described previously.[5] Complete statistics are summarized in Table 2. Several residues located in N- and C-terminal regions were not invisible due to their disorder. Figures were written by PayMOL.

Coordinates and structural factors of **Cr•2•HO** have been deposited in the Protein Data Bank under accession numbers of 2Z68.

Thermal stability. Melting Points (T_m) of the composites were determined according to a previously reported procedure.[6] The conditions are described below; sample concentration: 2.5 μM except for **Fe•2•HO** and **Cr•2•HO** (2 μM) in 10mM Tris/HCl buffer at pH 7.4 and 4°C and temperature range: 20°C–60°C (heating rate: 50°C/h). CD spectral changes against thermal increase were shown in Fig. 2.

References

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