Supporting Information

Process of Accumulation of Metal Ions on the Interior Surface of apo-Ferritin: Crystal Structures of a Series of apo-Ferritins Containing Variable Quantities of Pd(II) Ions

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Experimental:

Materials: Reagents were purchased from TCI, Wako, Nacalai Tesque, and Sigma-Aldrich and used without further purification. Recombinant L-chain apo-Fr from horse liver (apo-rHLFr) was prepared in NovaBlue competent cells (Novagen) transformed with the expression vector pMK2 kindly supplied by Prof. Ichiro Yamashita. The culture and purification of apo-rHLFr and apo-H49A-rHLFr were performed according to previous reports[1,2].

Preparation of H-Pd^{II}•apo-rHLFr: An apo-rHLFr solution (1 mM, 30 ml) in 0.15 M NaCl aqueous solution was adjusted to pH 8.5 with 0.01 N NaOH, followed by the addition of aliquots of K_2PdCl_4 aqueous solution (40 mM, 150 ml). After stirring for 30 min, the solution was concentrated to approximately 10 ml and subsequently purified using a gel filtration column (Superdex G-200) equilibrated with 0.15 M NaCl aqueous solution**.** The metal ion content in apo-rHLFr was determined by ICP-OES and the BCA methods. **H-Pd^{II}•apo-H49A-rHLFr** was prepared with the same procedure with apo-H49ArHLFr. **L-, I-Pd^{II}•apo-rHLFrs** and apo-rHLFr reacted with 500 equiv. of Pd(II) ions were also prepared with the same procedure except for volume of the K_2PdCl_4 solution (37.5, 75, and 375 mL, respectively) reacted with apo-rHLFr.

Crystallization of L-, I-, and H-Pd^{II}•apo-rHLFrs, and data collection: Crystallization was performed using a hanging drop vapor diffusion method as described in a previous report[3]. Purified Pd^{II}•apo-rHLFr solution was concentrated to 10-20 mg ml^{-1} and drops were produced by mixing an equal volume (1 µl) of the protein solution (20 mM Tris/HCl, 0.15 M NaCl aq.) and the precipitant solution (0.5-1 M $(NH_4)_2SO_4$, 20-30 mM $CdSO_4$), and equilibrated against the precipitant solution (1 ml) at 20℃. The crystals were obtained within one day. Before data collection, single crystals were

immersed in a precipitant solution containing 30 % (w/w) glycerol and subsequently frozen in liquid nitrogen. X-ray diffraction data of each crystal were collected at 100K at beamline BL41XU at SPring-8 using X-ray wavelengths of 0.5086 Å, and 0.4639 Å which represent the peak wavelengths of Pd and Cd X-ray absorption, respectively, in order to distinguish Pd atoms from Cd atoms which are essential for crystallization. Apo-rHLFr reacted with 500 equiv. of Pd(II) ions could not be crystallized.

Structure analysis: Data were processed with the program *HKL2000* in the cubic *F*432 space group. The structures were solved by molecular replacement with *MOLREP* using apo-rHLFr structure (pdb code: 1DAT) as an initial model. Refinement of the protein structure was performed using the program *REFMAC5* with higher resolution data of each crystal (**L-Pd^{II}•apo-rHLFr**, **I-Pd^{II}•apo-rHLFr,** and **H-Pd^{II}•apo-rHLFr**) collected at 0.5086 Å (1.65 Å, 2.10 Å, and 2.50 Å, respectively). Model biases were reduced by composite omit maps calculated with *CNS*[4]. Rebuilding was carried with *COOT*. The solvent was identified to fit residual (F_o-F_c) density peaks with a lower cut-off of 3 σ. Residues Lys172, His173, and Asp174 of **L-Pd^{II}apo-rHLFr**, Ser1 of **I-Pd^{II}apo-rHLFr**, Ser1, and Asp174 of **H-Pd^{II}apo-rHLFr** were not decided because of their disordered electron densities. Residues His49, Asp127, Glu130, Ser131, Glu136, Gln158, and Asp174 of **I-Pd^{II}apo-rHLFr**, His49, Arg52, Glu56, Cys126, Asp127, Glu130, Ser131, Lys143, Lys172, and His173 of **H-Pd^{II}apo-rHLFr** were replaced to Ala because electron density of these residues are missing.

Anomalous analysis: Metal ions in crystals were identified using the differences in anomalous scattering effect of two wavelengths as reported[5]. The data collected at 0.5086 Å, for the Pd peak wavelength, gives anomalous data with Pd f'' = 3.6 e and Cd f'' = 0.65 e. The other data set collected at 0.4639 Å, for the Cd peak wavelength, gives anomalous data with Pd f'' = 3.1 e and Cd f'' = 3.7 e. Anomalous difference Fourier maps were calculated for all data sets at lower resolution (**L-Pd^{II}•apo-rHLFr**: 1.70 Å, **I-Pd^{II}•apo-rHLFr**: 2.15 Å**,** and **H-Pd^{II}•apo-rHLFr**: 2.60 Å), and the coordination structures of the Pd and Cd binding sites were picked up at 4 σ. Anomalous peak heights at the Pd binding sites are almost the same but were quite different at Cd binding sites. Pd binding sites were identified by this difference between the two wavelengths. The occupancy values of all palladium atoms were refined to make the B-factors of the Pd atoms below 60 Å^2.

Crystallization, data collection, structure analysis of H-Pd^{II}•apo-H49A-rHLFr: Crystallization was performed using the hanging drop vapor diffusion method as described above. Purified Pd^{II}•apo-H49ArHLFr solution was concentrated to 10-20 mg ml^{-1} and drops were produced by mixing an

equal volume (1 μl) of the protein solution (20 mM Tris/HCl, 0.15 M NaCl aq.) and the precipitant solution (1 M $(NH_4)_2SO_4$, 10 mM $CdSO_4$), and equilibrated against the precipitant solution (1 ml) at 20℃. The crystals were obtained within one day. Before data collection, single crystal was immersed in a precipitant solution containing 30 % (w/w) glycerol and subsequently frozen in liquid nitrogen. X-ray diffraction data was collected at 100K at beamline BL38B1 at SPring-8 using X-ray wavelengths of 1.0 Å. The data was processed with the program *HKL2000* in the cubic *F*432 space group. The structures were solved by molecular replacement with *MOLREP* using apo-rHLFr structure (pdb code: 1DAT) as an initial model. Refinement of the protein structure was performed using the program *REFMAC5*. Model biases were reduced by composite omit maps calculated with *CNS*[4]. Rebuilding was carried with *COOT*. The solvent was identified to fit residual (F_o-F_c) density peaks with a lower cut-off of 3 σ. The coordination structures of Pd binding sites were picked up at 4 σ according to anomalous Fourier difference maps and geometric parameters. Although Cd atoms are observed in the anomalous Fourier difference maps, the Pd binding sites were determined in comparison with **H-Pd^{II}•apo-rHLFr** structure. The occupancy values of all palladium atoms were refined to make the B-factors of the Pd atoms below 60 Å^2. Residues Ser1, and Asp174 of **H-Pd^{II}apo-H49A-rHLFr** were not decided because of their disordered electron densities. Residues Ser2, Gln3, Cys126, Asp127, Glu130, and Glu136 of **H-Pd^{II}apo-H49A-rHLFr** were replaced to Ala because electron densities of these residues are missing.

The procedures were done twice using two crystals for each composite to confirm the reproducibility of the X-ray structural analyses.

Physical Measurements. Absorption spectra were recorded on a Shimazu UV-2400PC UV-vis spectrometer. Metal concentrations of **Pd^{II}•apo-rHLFr**s and **Pd^{II}•apo-H49A-rHLFr** were determined by using an inductively coupled plasma optical emission spectrometers (Varian ICP-OES Vista-PRO). $PdCl_2$ in 0.1 M HCl (1.01 g/L) was used as calibration standard and $Y(NO_3)_3$ in 0.1 M HNO_3 (1.01 g/L) was applied for an internal standard.

Determination of Protein Concentrations. Protein concentrations were determined with bicinchoninic acid (BCA) protein assay. It was done three times for each loading condition and mutant. To calibrate the effect of Pd(II) ions, the standard curves were obtained with apo-rHLFr in the presence of $KPdCl_4$.

References:

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Table S1. Crystallographic data

	L- Pd^{II}•apo-rHLFr		I- Pd^{II}•apo-rHLFr		H- Pd^{II}•apo-rHLFr		H- Pd^{II}•apo-H49ArHLFr
	Pd Peak	Cd Peak	Pd Peak	Cd Peak	Pd Peak	Cd Peak	
Wavelength (Å)	0.5086	0.4639	0.5086	0.4639	0.5086	0.4639	1.0
Resolution range (Å)	50.0-1.65 (1.69-1.65)	50.0-1.70 (1.76-1.70)	50.0-2.10 (2.16-2.10)	50.0-2.15 (2.23-2.15)	50.0-2.50 (2.59-2.50)	50.0-2.60 (2.69-2.60)	30.0-1.80 (1.86-1.80)
Space group	*F*432	*F*432	*F*432	*F*432	*F*432	*F*432	*F*432
Crystal cell (Å)	180.790	180.890	180.570	180.630	181.593	181.655	180.701
Observations	664854	607039	650546	603344	195568	175020	512012
Unique reflections	57490 (5750)	52652 (5235)	27717 (2749)	25818 (2583)	16816 (1681)	15011 (1488)	44243 (4381)
Redundancy	11.6 (11.6)	11.5 (11.6)	23.5 (23.5)	23.4 (23.5)	11.6 (11.8)	11.7 (11.7)	11.6 (11.4)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	99.9 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
I/s	30.3 (10.8)	30.5 (9.36)	54.3 (12.7)	52.5 (11.5)	33.9 (7.2)	29.1 (7.7)	57.4 (8.0)
Rmerge	0.084 (0.292)	0.084 (0.325)	0.084 (0.255)	0.082 (0.298)	0.092 (0.302)	0.098 (0.316)	0.089 (0.300)

Values in parentheses are for the highest-resolution shell.

Table S2. Refinement statistics.

	L-Pd^{II}•apo-rHLFr	I-Pd^{II}•apo-rHLFr	H-Pd^{II}•apo-rHLFr	H- Pd^{II}•apo-H49ArHLFr
Resolution range (Å)	34.8-1.65	40.4-2.10	45.4-2.50	21.3-1.80
Reflection used	29369	14423	8894	22765
R-factor (%)	17.0	19.9	22.3	19.7
Free R-factor (%)	18.8	25.4	28.7	22.5
R.m.s. deviations from ideal				
Bond length (A)	0.009	0.018	0.026	0.012
Bond angles (º)	1.144	1.489	2.262	1.252
Chiral-center restraints ($Å^3$)	0.090	0.126	0.202	0.094