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COMMUNICATIONS

Published on Web 07/18/2008

Control of the Coordination Structure of Organometallic Palladium Complexes in an apo-Ferritin Cage

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Metal clusters such as the Mn cluster of photosystem II, FeMoco of nitrogenase, and iron−sulfur clusters of ferredoxins play essential roles in many biological systems.[1−4] The coordination geometries and the functions of these clusters are influenced by amino acid residues in the active sites. Recently, preparation of non-native multinuclear metal clusters in the protein scaffolds have been reported.[5−8] Tri- or pentanuclear oxo-clusters of Zr(IV) and Hf(IV) have been identified within the dityrosyl cluster nucleation motif of ferric-ion-binding protein.[6,7] Several types of polynuclear tungsten oxide clusters are formed in the binding cavity of a Mo/W-storage protein.[8] We have also previously reported the utilization of the apo-ferritin (apo-Fr) cage for preparing a single Pd nanocluster as well as size selective olefin hydrogenation catalyzed by this composite.[9] These results suggest that multinuclear metal complexes with various coordination structures could be formed by designing amino acid residues on the protein surface. In this report, we describe the preparation of various organometallic multinuclear Pd complexes in the protein cages of apo-Fr and its mutants (Figure 1a) by their reactions with $[Pd^{II}(allyl)Cl]_2$ (allyl = η^3-C_3H_5), which catalyze organic reactions such as C−C bond formation reactions.[10,11] More importantly, we have successfully controlled the coordination structures of these multinuclear Pd^{II} complexes by replacing histidine residues at the binding sites.

Ferritin (Fr), an iron storage protein composed of 24 subunits, is a spherical protein assembly which has interior space with 8 nm in diameter (Figure 1b).[12,13] The space has been utilized for the deposition of metal particles and metal complexes such as FeS, CdS, CdSe, Pd, Ag, Gd-HPDO3A (gadolinium-[10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid]), and hexacyanoferrate(III).[9,14−19] These metal ions are incorporated into the Fr cage through the 3-fold channels located at the intersections of the three subunits (Figure 1c) and accumulated in the interior space to form metal nanoparticles by self-assembly or redox reactions.[9,14−16,19]

Recombinant L-chain apo-Fr from horse liver (10 μM, 5 mL in 50 mM Tris/HCl (pH8.0), 0.15 M NaCl) was treated with 100 equiv of $[Pd^{II}(allyl)Cl]_2$ (10 mM, 0.5 mL) in acetonitrile and the reaction mixture (9% acetonitrile aqueous solution) was stirred for 1 h at 25 °C. After dialysis against a 0.15 M NaCl aqueous solution, apo-Fr assembly containing Pd(allyl) complexes (**Pd(allyl)·apo-Fr**) was purified by a size exclusion column (ÄKTA design, Superdex G-200) to remove unbound Pd complexes. Inductively coupled plasma-optical emission spectrometry (ICP-OES) and bicinchoninate (BCA) analyses of the purified composite indicate that the number of Pd complexes incorporated in **Pd(allyl)·apo-Fr** is 103 ± 1.

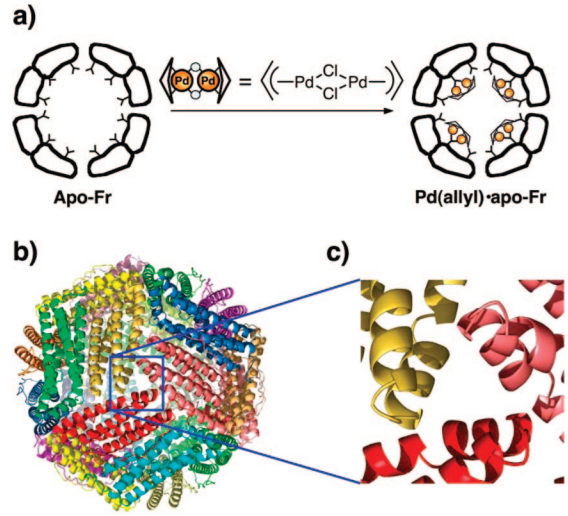

Figure 1. (a) Reaction scheme for the preparation of Pd(allyl)·apo-Fr; (b) entire structure of apo-Fr viewed down a 3-fold axis (PDB ID: 1DAT); (c) close up view of the 3-fold axis channel.

The composite was crystallized by a hanging drop vapor diffusion method in the presence of cadmium ions, which are essential for the crystallization of apo-Fr, as reported previously.[13,20] The crystal structure of **Pd(allyl)·apo-Fr** was refined to 1.7 Å resolution as shown in Figure 2a.[21] The root-mean-square deviation (rmsd) of the C^{α} atoms of **Pd(allyl)·apo-Fr** from that of apo-Fr is 0.47.[13] This indicates that the folding of apo-Fr is preserved even in the presence of the Pd complexes on the interior surface of apo-Fr.

The anomalous difference Fourier maps show that dinuclear Pd(allyl) complexes are formed at two Pd binding sites per single Fr subunit. In other words, there are 48 Pd binding areas on the interior surface of **Pd(allyl)·apo-Fr** that contribute to the capture of 96 Pd atoms (Figure 2a−c). The total number of Pd atoms observed in the crystal structure is almost identical to that of Pd atoms determined by quantitative analyses by ICP and BCA methods.

The two binding areas are located at the 3-fold axis channel and at a metal binding domain called as the "accumulation center" (Figure 2a).[13] Pd1 of wild type apo-Fr (PdW1) and PdW2 located at the 3-fold axis channel have a thiol-bridged dinuclear structure stabilized by the coordination of N^{ϵ} of His114 and the O atom of

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10.1021/ja802463a CCC: $40.75

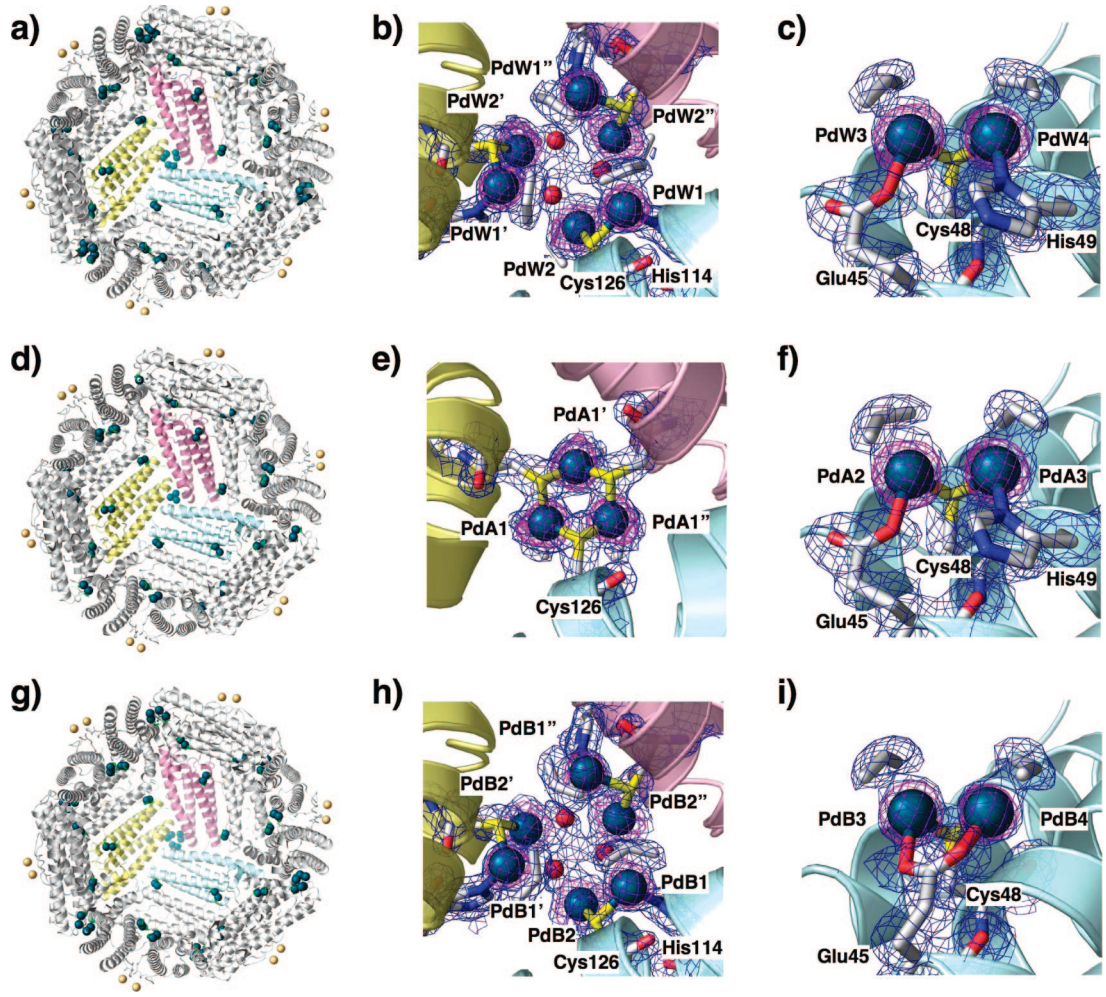

Figure 2. Crystal structures of **Pd(ally)·apo-Frs**. The interior structures of (a) **Pd(allyl)·apo-Fr**, (d) **Pd(allyl)·apo-H114AFr**, and (g) **Pd(allyl)·apo-H49AFr**. Close views of 3-fold channels and accumulation centers of **Pd(allyl)·apo-Fr** (b and c), **Pd(allyl)·apo-H114AFr** (e and f), **Pd(allyl)·apo-H49AFr** (h and i), respectively. The Pd and Cd atoms are indicated as greenish blue and beige spheres, respectively. The anomalous difference Fourier maps at 4.0 σ indicate the positions of palladium atoms which are shown in magenta. The selected $2|F_o| - |F_c|$ electron density maps at 1.0 σ are shown in blue.

a water molecule. PdW1-S^{γ}_{Cys126}, PdW2-S^{γ}_{Cys126}, PdW1-N^{ϵ}_{His114}, and PdW2-O_{H2O} distances are 2.47, 2.38, 2.14, and 2.34 Å, respectively (Figure 2b). PdW3 and PdW4 at the accumulation center are bound to O^{ϵ} of Glu45 and N^{δ} of His49, respectively, while the thiol-group of Cys48 serves as a bridging ligand between PdW3 and PdW4 with PdW3-S^{γ}_{Cys48}, PdW4-S^{γ}_{Cys48}, PdW3-O^{ϵ}_{Glu45}, and PdW4-N^{δ}_{His49} distances of 2.31, 2.57, 2.30, and 2.17 Å, respectively (Figure 2c). The distances of PdW1−PdW2 (3.68 Å) and PdW3−PdW4 (3.12 Å) are too long to directly interact each other.[22,23] Moreover, the η^3-allyl ligands of PdW1, PdW3, and PdW4 give clear electron density maps near each Pd atom, although the electron density map of the allyl ligand of PdW2 is slightly weak (Figure 2b and 2c). In addition, the *B*-factor of the PdW2 atom is larger (42.19) than the *B*-factor of the PdW1, PdW3, and PdW4 atoms (24.80, 19.86, 22.54, respectively).

A larger *B*-factor value of PdW2 indicates that the PdW2(allyl) complex could be fluctuating or changing locations because the PdW2 atom is coordinated only by a single Cys residue at position 126. Thus, the PdW2(allyl) complex is not strongly fixed relative to the other three Pd complexes. Each Pd atom (PdW1−PdW4) in apo-Fr has square-planar geometry with an allyl ligand and amino acid residues or a water molecule (Figure 2b and 2c). These structures are similar to those reported for dinuclear Pd allyl complexes.[4,24]

Two mutants, apo-C126AFr and apo-C48A/H49AFr, were prepared to elucidate the effect of the cysteine residues for the ligation of the Pd(allyl) complex in apo-Fr. When apo-C126AFr mutant was treated with 100 equiv of $[Pd^{II}(allyl)Cl]_2$ under the same conditions as apo-Fr, the quantitative analyses show that the number of Pd atoms in apo-C126AFr (37 ± 4) was smaller than the number of Pd atoms (103 ± 1) in apo-Fr. This indicates that Pd(allyl) complexes can be bound to the accumulation center in spite of the deletion of Cys126. In the case of apo-C48A/H49AFr, the number of Pd atoms in apo-C48A/H49AFr (54 ± 5) was about half of Pd atoms in apo-Fr, suggesting that Pd(allyl) complexes are bound to the binding site at the 3-fold channel. These results indicate that the cysteine residues are crucial to stabilize the coordination of Pd(allyl) complexes at these sites.

To alter the coordination structures of Pd(allyl) complexes of **Pd(allyl)·apo-Fr**, two mutants, in which His114 or His49 was replaced with Ala, were prepared. The mutants, apo-H114AFr and apo-H49AFr, were treated with 100 equiv of $[Pd^{II}(allyl)Cl]_2$ under the same conditions used for analyses of apo-Fr. The quantitative analyses of the composites show that the numbers of Pd atoms retained in **Pd(allyl)·apo-H114AFr** and **Pd(allyl)·apo-H49AFr** are 78 ± 6 and 99 ± 4, respectively.

The crystal structures of **Pd(allyl)·apo-H114AFr** and **Pd(allyl)·apo-H49AFr** show that the numbers of Pd atoms in the cages are 72

and 96 (Figure 2d and 2g), respectively,[21] which are almost identical to the values obtained by the quantitative analyses performed using ICP and BCA methods. **Pd(allyl)·apo-H114AFr** has thiolato-bridged trinuclear Pd complexes at the 3-fold channels because the deletion of His114 causes a 2.4 Å shift of the position of the C^{α} atom of Cys126 toward the center of the 3-fold channel (Figure 2e). The PdA1−PdA1′ distance (3.24 Å) indicates that the complexes have no direct bonding interaction among the Pd atoms.[25,26] The coordination geometry of each Pd atom is a typical square-planar structure with an allyl ligand and two S^{γ} atoms at the 3-fold axis channel with a six-membered-ring structure as a reported Pd trinuclear complex (Figure 2e).[27] The coordination structures of PdA2 and PdA3 are identical to those of **Pd(allyl)·apo-Fr** (Figure 2f).

The crystal structure of **Pd(allyl)·apo-H49AFr** shows that the coordination structure of the dinuclear Pd complex at the accumulation center is altered from that of **Pd(allyl)·apo-Fr**, although the structures of PdB1 and PdB2 are the same as that of **Pd(allyl)·apo-Fr** (Figure 2h and 2i). PdB3 and PdB4 form a dinuclear structure even after the deletion of His49 (Figure 2i). However, the distance between PdB3 and PdB4 is 2.81 Å, which is shorter than that of both **Pd(allyl)·apo-Fr** (3.12 Å) and a reported thiolato-carboxylato Pd dinuclear complex (3.30 Å)[28] because each $O^{\epsilon}{}_1$ and $O^{\epsilon}{}_2$ atom of the carboxylate moiety of Glu45 is bound to PdB3 and PdB4.

A Suzuki coupling reaction of 4-iodoaniline and phenylboronic acid to afford 4-phenylaniline was examined to evaluate the catalytic activities of these composites,[29] since Pd(allyl) complexes are known to catalyze the Suzuki coupling reaction.[30] The turnover frequencies (TOF = [product (mol)] per **Pd(allyl)·apo-Frs** per hour) of the coupling reactions were determined by ^{1}H NMR based on the consumption of 4-iodoaniline and the formation of the product. The size exclusion column chromatography of the reaction solution showed that the spherical 24-mer assembly of the composite is maintained during the reaction. The activity of **Pd(allyl)·apo-Fr** (3500 ± 400) is almost identical to that of **Pd(allyl)·apo-H49AFr** (3400 ± 300). On the other hand, the activity of **Pd(allyl)·apo-C48A/H49AFr** (1900 ± 100) is 1.8-fold lower than that of **Pd(allyl)·apo-Fr** due to deletion of binding sites at the accumulation center. These results show that each Pd dinuclear complex at both binding areas has similar activity of Suzuki coupling. **Pd(allyl)·apo-H114AFr** (900 ± 50) shows about 4-fold lower activity than that of **Pd(allyl)·apo-Fr**. The lower reactivity of **Pd(allyl)·apo-H114AFr** suggested that the trinuclear Pd cluster at the 3-fold channel interferes with the penetration of substrates or that the geometry of Pd complexes at the 3-fold channel is different from **Pd(allyl)·apo-Fr**. **Pd(allyl)·apo-C126AFr** (830 ± 70) shows lower activity than the expected value (1750), which is half the TOF of **Pd(allyl)·apo-Fr**; the reason is not clear at this moment. Although the catalytic activities per Pd atoms of **Pd(allyl)·apo-Fr** are about twice lower than that of $[Pd^{II}(allyl)Cl]_2$ under the same conditions,[31] the catalytic activities of the **Pd(allyl)·apo-Frs** could be improved by increasing the number of Pd(allyl) complexes in apo-Fr by the introduction of Cys residues on the interior surface of apo-Fr.

In summary, we have demonstrated that a variety of coordination structures of multinuclear Pd complexes such as dinuclear and trinuclear complexes are constructed by protein engineering of the interior surface of apo-Fr. These results suggest that multinuclear metal complexes with various coordination structures could be prepared by the deletion or introduction of key residues such as His, Glu, and Cys residues at appropriate positions on protein surfaces. Further work on the design of polynuclear metal complexes and catalytic reactions is currently in progress.

Acknowledgment. This work was supported by the Global COE Program in Chemistry, Nagoya University for S.A., International Research Training Group (DFG, JSPS) "Complex Functional Systems" (Münster and Nagoya), Grant-in-Aid for Scientific Research (Grant No. 18685019 for T.U.) and on Priority Areas (Grant No. 16033226, Chemistry of Coordination Space for Y.W.) from Ministry of Education, Culture, Sports, Science and Technology, Japan, and PRESTO, Japan Science and Technology Agency (JST), and Amano Enzyme Inc.

Supporting Information Available: Experimental details, X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA802463A