Supramolecular Porphyrin Arrays Mediated by Hemoprotein Matrix

Takashi Hayashi, Koji Oohora, and Akira Onoda
Department of Applied Chemistry, Graduate School of Engineering, Osaka University
2-1 Yamadaoka, Suita 565-0871, Japan

Protoporphyrin IX, heme *b*, is one of the most well-known iron porphyrin cofactors in nature. Famous hemoproteins such as myoglobin, hemoglobin, horseradish peroxidase, cytochrome P450cam possess heme *b* as a cofactor. In addition, the cofactor is bound in the protein matrix via non-covalent interaction and Fe–axial ligand coordination. Therefore, the heme cofactor is able to be removed from the heme pocket under the acidic conditions, and then it is possible to reconstitute with an artificial or modified heme analogue. To functionalize a hemoprotein, our group has recently focused on the heme-substitution with several porphyrinoid metal complexes.[1)] For example, it has been found that myoglobin reconstituted with metal porphycene shows not only extremely high O_2 affinity but also high peroxidase and/or peroxygenase activities rather than that observed by native myoglobin. Furthermore, we have also introduced a heme moiety onto the myoglobin surface via covalent linkage to obtain a supramolecular protein polymer via interprotein heme–heme pocket interaction. In this presentation, we report the construction of supramolecular porphyrin arrays mediated by the heme–heme pocket interaction.[1,2)]

We first used cytochrome b_{562} mutant, H63C cyt b_{562}, where His63 was replaced with cysteine, because wild type cyt b_{562} does not have any cysteine residues in the protein matrix. Modified heme containing a maleimide group at the terminal of the heme-propionate via a flexible linker was reacted with H63C cyt b_{562} to afford the covalently heme-attached protein. After the removal of the native heme *b* cofactor from the heme pocket, an external heme-linked apohemoprotein was yield. Upon the neutralization, the supramolecular hemoprotein polymer was obtained as shown in Figure 1. The result obtained by size exclusion chromatography suggests the formation of the cyt b_{562} array, and a series of AFM (atomic force microscopy) images indicate the formation of hemoprotein fibers with the length of 300–1000 nm on a HOPG substrate.[3)] Since myoglobin also has no cysteine, an A125C mutant was expressed from *E.coli* and then external heme-attached myoglobin was prepared by the same method. The modified myoglobin is also found to provide the protein self-assembly structure. Furthermore, we prepared not only one-dimensional hemoprotein fiber but also two-dimensional hemoprotein network and three-dimensional hemoprotein cluster.[4,5)]

Next, the oxygen binding parameters for supramolecular myoglobin polymer were measured to determine the inherenet myoglobin function of binding affinity for dioxygen. From the kinetic parameters, on-rate and off-rate, the dioxygen binding constants for native and polymeric deoxymyoglobins were determined to be 8.6 x 10^5 and 5.0 x 10^5 M^{-1} at 25 °C, pH 7.0, respectively, indicating that the polymerization has no serious influence on the physiological property.[5)]

We further tried to immobilize the hemoprotein polymer on a gold surface. Particularly, the heme-immoblized gold surface gave the hemoprotein assemblies with the number of 6–8 protein layers on the electrode upon the addition of the heme-linked apohemorpoteins as shown in Figure 2. The modified gold electrode with zinc cyt b_{562} reconstituted with zinc protoporphyrin IX showed efficient photocurrent generation in the presence of methyl viologen as an electron mediator.[6)] Moreover, it is found that heme-immobilized gold nanoparticles give a unique self-assembly of the gold nanoparticle upon the addition of apohemoprotein dimer.[7)] The supramolecular porphyrin array mediated by hemoprotein matrix serves as a new way to create bionanomaterials.

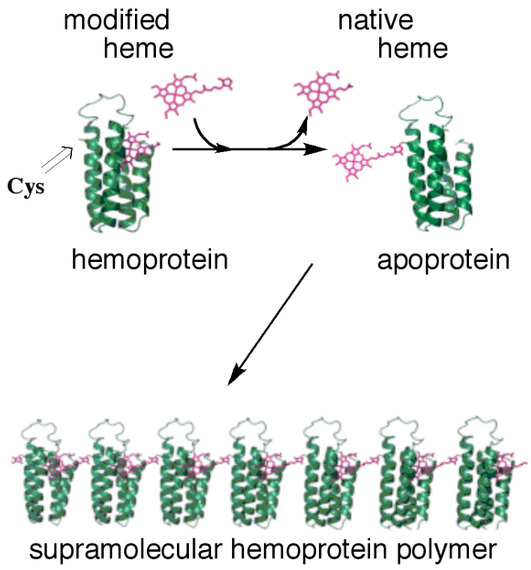

Fig. 1. Scheme of supramolecular hemoprotein polymer.

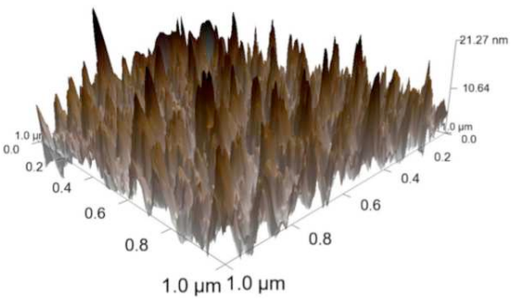

Fig. 2. 3D image obtained by AFM measurement of immobilized cyt b_{562} assembly on a gold surface.

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