VIII-D Modification of Myoglobin by Replacing the Native Heme with Metalloporphyrinoids

Functionalization of hemoproteins is one of the attractive subjects for creating a new biomaterial. Recently, we have prepared various artificial prosthetic groups and inserted them into apomyoglobin to obtain reconstituted myoglobins. For example, iron porphycene, a structural isomer of iron porphyrin, is a unique prosthetic group to modulate the myoglobin function, since the Lewis acidity of iron atom in the porphycene framework could be strong, and the dz^2 orbital level of the iron atom is stabilized due to the decrease of the macrocycle symmetry compared to porphyrin framework. The physicochemical properties of iron porphycene suggest that the replacement of the native heme in myoglobin with metalloporphycene will improve or convert the myoglobin function. From this project, it is found that the reconstitution of myoglobin with an artificial prosthetic group serves as a new way to create a functionalized hemoprotein.

VIII-D-1 Ligand Binding Properties of Myoglobin Reconstituted with Iron Porphycene: Unusual O_2 Binding Selectivity against CO Binding

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Sperm whale myoglobin, an oxygen storage hemoprotein, was successfully reconstituted with the iron porphycene having two propionates, 2,7-diethyl-3,6,12, 17-tetramethyl-13,16-bis(carboxyethyl)porphycenatoiron. The physicochemical properties and ligand binding events of the reconstituted myoglobin were investigated. The ferric reconstituted myoglobin shows the remarkable stability against acid denaturation and only a low-spin characteristic in its EPR spectrum. The Fe(III)/Fe(II) redox potential (–190 mV *vs*. NHE) determined by the spectroelectrochemical measurements was much lower than that of the wild-type. These results can be attributed to the strong coordination of His93 to the porphycene iron, which is induced by the nature of the porphycene ring symmetry. The O_2 affinity of the ferrous reconstituted myoglobin is higher by 2,600-fold than that of the wild-type, mainly due to the decrease in the O_2 dissociation rate, whereas the CO affinity is not so significantly enhanced. As a result, the O_2 affinity of the reconstituted myoglobin exceeds its CO affinity ($M' = K_{CO}/K_{O_2} < 1$). The ligand binding studies on H64A mutant support the fact that the slow O_2 dissociation of the reconstituted myoglobin is primarily caused by the stabilization of the Fe–O_2 σ-bonding. The high O_2 affinity and the unique characteristics of the myoglobin with the iron porphycene indicate that the reconstitution with a synthesized heme is a useful method not only to understand the physiological function of myoglobin but also to create a tailor-made function on the protein.

VIII-D-2 Unusual Ligand Discrimination by a Myoglobin Reconstituted with a Hydrophobic Domain-Linked Heme

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New reconstituted horse heart myoglobins possessing a hydrophobic domain at the terminal of the two heme-propionate side chains were constructed. The O_2 and CO bindings for the reconstituted deoxymyoglobins were examined in detail by laser flash photolysis and stopped-flow rapid mixing techniques. The artificially created domain worked as a barrier against exogenous ligand penetration into the heme pocket, whereas the bound O_2 was stabilized in the reconstituted myoglobin as well as in the native one. In contrast, the CO dissociation rate constant for the reconstituted myoglobin increased by 20-fold compared to the native protein, suggesting that the incorporation of the hydrophobic domain onto the heme pocket perturbs the distal site structure of the reconstituted myoglobin. As a result, the substantial ligand selectivity for the reconstituted myoglobin significantly increases in favor of O_2 over CO with the M' value ($= K_{CO}/K_{O_2}$) of 0.88. The present work concludes that the O_2 selectivity of myoglobin over CO is markedly improved by chemically modifying the heme-propionates without any mutation of the amino acid residues in the distal site.

VIII-D-3 Enhancement of Peroxidase Activity of Myoglobin Reconstituted with Iron Porphycene: Compound III Formation due to the Reaction of Ferric Myoglobin with Hydrogen Peroxide

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The replacement of native heme with an artificially created metal complex is one of the attractive studies in a series of hemoprotein modifications. However, the number of studies that demonstrate the conversion of myoglobin to peroxidase using the reconstitution by non-native prosthetic group have been quite limited. To enhance the peroxidase activity of myoglobin, we

focused on iron porphycene as a structural isomer of iron porphyrin. Reconstituted myoglobin with 2,7-diethyl-3,6,12,17-tetramethyl-13,16-dicarboxyethylporphycenatoiron(III) accelerated the H_2O_2-dependent oxidation of substrates such as guaiacol, thioanisole, and styrene. At pH 7.0, and 20 °C, the initial rate of the guaiacol oxidation is 10-fold faster than that observed for native myoglobin. This finding clearly suggests that the replacement of native heme with iron porphycene enhances the peroxidase activity. In addition, the guaiacol oxidation catalyzed by the reconstituted myoglobin was accelerated at the higher pH values. Moreover, the stopped-flow technique demonstrated that two reaction intermediates, the compound II-like species and compound III, formed from compound II with the excess amounts of H_2O_2, were detected in the absence of a substrate. It is the first example that compound III is formed *via* compound II in myoglobin chemistry. The enhancement of peroxidase activity and the formation of the stable compound III in myoglobin with iron porphycene could be due to the strong coordination of the Fe–His93 bond.

VIII-D-4 Preparation and O_2 Binding Study of Myoglobin Having a Cobalt Porphycene

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Sperm whale myoglobin, an oxygen-storage hemoprotein, was reconstituted with 2,7-diethyl-3,6,12,17-tetramethyl-13,16-bis(carboxyethyl)porphycenatocobalt(II) in order to investigate the reactivity of a cobalt porphycene in a protein matrix. Similar to the previously reported finding for the myoglobin with the iron porphycene, the reconstituted myoglobin with the cobalt porphycene was also found to have a higher O_2 affinity by two orders of magnitude when compared to the myoglobin possessing cobalt protoporphyrin IX. The EPR spectra of the deoxy and oxy myoglobins having the cobalt porphycene at 77 K also have similar features to the myoglobin with cobalt protoporphyrin IX. These spectra suggest that the porphycene cobalt in the deoxy form is coordinated by one nitrogenous ligand postulated to be the imidazole ring of His93, and that the bond configuration of Co^{II}–O_2 is regarded as the Co^{III}–$O_2^{\bullet -}$ species.