Assembly of Diethylenetriaminepentaacetatocobaltate(III) Complexes on the Surface Lysine Residues of Myoglobin

Keiichi Tsukahara,* Mari Nishimine, Kimiko Kiguchi, Yuka Shioyama, Miho Matsui, Naoko Kubota,† and Ryuichi Arakawa†
Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630-8506
†*Department of Applied Chemistry, Faculty of Engineering, Kansai University, Suita, Osaka 564-8680*

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Myoglobin, whose lysine residues are linked with diethylenetriaminepentaacetatocobaltate(III) complexes, was prepared and characterized.

Modification of metal complexes on the surface of metalloproteins, such as cytochrome *c* and myoglobin, is an important technique to study the intramolecular electron-transfer (ET) reaction of metalloproteins.[1] We have recently designed the modified myoglobin with a metal-chelating reagent, diethylenetriaminepentaacetic acid (DTPA).[2] To elucidate the effect of the number and site of metal complexes on the ET reactions of metalloproteins, we have inserted cobalt(III) ions into the DTPA moieties of metmyoglobin (metMb) and investigated the redox behavior in this communication.

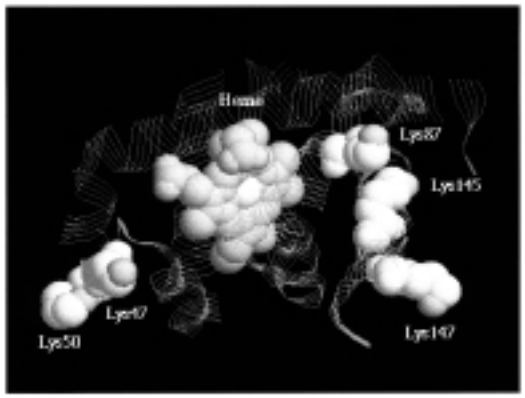

Figure 1. Display of heme with Lys residues 47, 50, 87, 145, and 147 of horse heart metMb from PDB data of Brookhaven National Laboratory by a RasMol v2.5 molecular visualization program.

The modified metMbs with DTPA (metMb(DTPA)$_n$, n = 1, 2, 4, and 5) were prepared by the previously described method.[2] The largest yield of each modified metMb(DTPA)$_n$ was collected and characterized. The modified Lys residues were determined by an amino-acid sequential analysis for lysyl endopeptidase digests.[2] The binding sites of Lys with DTPA are 87 for metMbDTPA, 87 and 145 for metMb(DTPA)$_2$, 47, 87, 145, and 147 for metMb(DTPA)$_4$ and 47, 50, 87, 145, and 147 for metMb(DTPA)$_5$, respectively (see Figure 1). Each modified myoglobin was treated with 1.5-fold excess of $CoCl_2 \cdot 6H_2O$ over the DTPA unit in a 10 mM Tris/HCl buffer at pH 8.0 and stood at room temperature for 24 h. The resulted solution was oxidized by 100-fold excess of potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) at room temperature for 2 h. The solution was passed through a Chelex 100 column at pH 8.0 to remove the excess Co(II) species and was dialyzed against a 10 mM phosphate buffer at pH 6.0. Then the metMb{Co^{III}(dtpa)}$_n$ species were purified with a DE-cellulose column chromatography at pH 6.0.[3] UV–vis and CD spectra were similar to those for native metMb, indicating that the heme environment and the conformation of metMb were not largely altered by modification.

The metMb{Co^{III}(dtpa)}$_n$ and metMb{Co^{II}(dtpa)}$_n$ species were distinguished by the following results; (1) the former was eluted faster than the latter on a DE-52 anion-exchange cellulose column with a Tris/HCl buffer at pH 8.0 and (2) the Co(II) ions were removed from metMb{Co^{II}(dtpa)}$_n$ with a Chelex 100 column at pH 8.0, although the Co(III) ions were not from metMb{Co^{III}(dtpa)}$_n$.

To confirm the presence of the [Co^{III}(dtpa)] moieties, a cyclic voltammetry was applied to metMb{Co^{III}(dtpa)}$_n$, where the modified gold electrode with bis(4-pyridyl) disulfide (BPD)[4] was used as a working electrode. Figure 2 shows cyclic

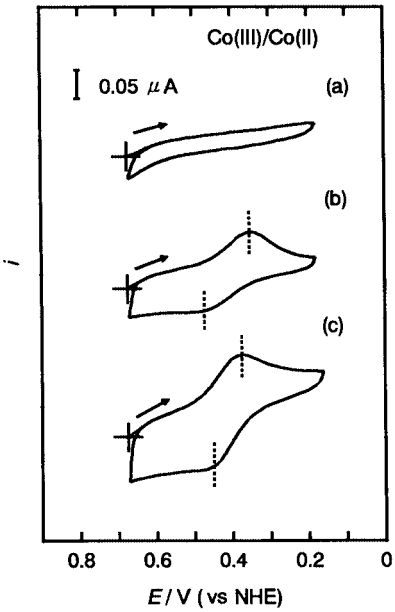

Figure 2. Cyclic voltammograms of metMb{Co^{III}(dtpa)}$_n$ (n = 1, 2, and 4) without background subtraction in a 10 mM phosphate buffer at 25 °C, pH 6.0, and the scan rate of 5 mV s^{-1} under nitrogen atmosphere. (a) metMb{Co^{III}(dtpa)}(Lys87) (1.0×10^{-4} M). (b) metMb{Co^{III}(dtpa)}$_2$ (1.0×10^{-4} M). (c) metMb{Co^{III}(dtpa)}$_4$ (6.6×10^{-5} M).

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voltammograms of metMb{Co^{III}(dtpa)}$_n$ (n = 1, 2 and 4) in a 10 mM phosphate buffer at pH 6.0.[5] The redox wave observed at 0.42 V vs NHE for n = 2 and 4 corresponds to the Co(III)/Co(II) couple[6] and that for the Fe(III)/Fe(II) couple of the heme prosthetic group was not found on the BPD-modified Au electrode.[7] Moreover, neither the Lys87-modified metMb-{Co^{III}(dtpa)} (n = 1, see Figure 2(a)) nor ethylenediaminetetraacetatocobaltate(III) ([Co^{III}(edta)]$^-$) which is a model compound for the Co(III) moiety showed any redox wave for the Co(III)/Co(II) couple. The redox wave for metMb{Co^{III}-(dtpa)}$_4$ as well as that for metMb{Co^{III}(dtpa)}$_5$ showed a reversible nature; the peak separation after background subtraction was independent of the scan rate (v) and the peak current (i_p) was linearly dependent on the square root of the scan rate defined by eq 1:[8]

$$i_p = 2.69\times10^5 n^{3/2} A D^{1/2} v^{1/2} c \qquad (1)$$

where n, A, D, and c are the number of electron, the surface area of the electrode, the diffusion coefficient, and the concentration of the substrate, respectively. The diffusion coefficients of metMb{Co^{III}(dtpa)}$_n$ (n = 4 and 5) can be therefore evaluated to be 4.7×10^{-7} cm^2 s^{-1} (for n = 4) and 4.0×10^{-7} cm^2 s^{-1} (for n = 5) at 25 °C, respectively. These values are smaller than those for native metMb (1.1×10^{-6} cm^2 s^{-1})[7,9] and metal complexes (($5.0—8.0) \times 10^{-6}$ cm^2 s^{-1}).[10]

On the other hand, the peak separation (ΔE_p) for metMb{Co^{III}(dtpa)}$_2$ was dependent on the scan rate, indicating a quasi-reversible nature. The standard heterogeneous rate constant (k^0) was evaluated to be (2.2 ± 0.5) $\times 10^{-4}$ cm s^{-1} (for n = 2) at 25 °C by the Nicholson's equation:[11]

$$k^0 = 6.24\psi(D\pi v n)^{1/2} \qquad (2)$$

where ψ is the dimensionless rate parameter at the transfer coefficient of 0.5.

The different reversibility of the Co(III)/Co(II) couple for metMb{Co^{III}(dtpa)}$_n$ (n = 1, 2, 4, and 5) and the model compound ([Co^{III}(edta)]$^-$) on the BPD-modified Au electrode may be explained by the following reasons. No response for [Co^{III}(edta)]$^-$ indicates no appreciable interaction between the Co(III) complex and the thiolpyridine group on the Au electrode. Singly Lys87-modified metMb{Co^{III}(dtpa)} did not show any redox waves, suggesting that the region far from Lys87 of metMb{Co^{III}(dtpa)} approaches to the surface of the Au electrode. The reversible nature for metMb{Co^{III}(dtpa)}$_4$ (Lys47, 87, 145, and 147) and metMb{Co^{III}(dtpa)}$_5$ (Lys47, 50, 87, 145, and 147), and the quasi-reversible response of metMb{Co^{III}(dtpa)}$_2$ (Lys87 and 145) suggests that the interaction area contains at least Lys47 and Lys147 and that Lys145 is oriented to the surface of Au electrode more closely than Lys87. From the above consideration the interacting area is suggested to be the bottom region of myoglobin shown in Figure 1, where the heme iron is located 19 Å from the surface of myoglobin and both cationic residues of Lys42, Lys98, Lys102, and His36 and aromatic Tyr103 and Phe106 may contact with the pyridine groups of the modified Au electrode. In this model the Lys87 residue is located at 17.8 Å from Lys147, while Lys145 is ca. 5 Å closer.

We designed metMb{Co^{III}(dtpa)}$_n$ whose Lys residues are modified with Co(III)DTPA complexes and found the different electrochemical reversibility for the Co(III)/Co(II) couple, depending on the binding site of the Co(III) moiety. Further studies are currently in progress on the interaction between metMb{Co^{III}(dtpa)}$_n$ and the surface of the electrodes.

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