A Substrate-Specific α-Hydoxylation of Dipeptides Mediated upon a Co(III)-terpyridine Complex: A Functional Model for Peptidylglycine α-Hydroxylating Monooxygenase

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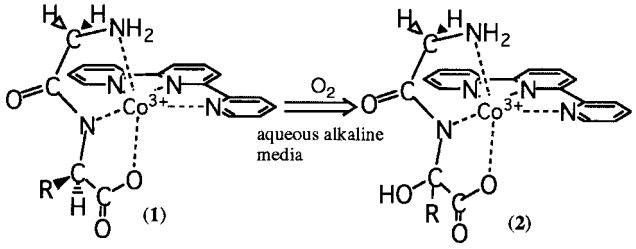
(Received October 19, 2000; CL-000952)

A substrate-specific α-hydroxylation of dipeptides has been found out as a functional model for peptidylglycine α-hydroxylating monooxygenase (PHM), in the reaction of the Co(III) ternary complexes containing terpyridine and dipeptide ligands under aerobic and slightly alkaline conditions.

The biologically active peptides, such as hormones, neurotransmitters and growth factors, carry an amide group at their carbonyl termini, of which nitrogen atom is derived from the terminal glycine residue of the precursor.[1–3] Peptidylglycine α-amidating monooxygenase (PAM) catalyzes oxidative removal of the two carbon atoms from a glycine-extended precursor through a two-step process.[4] At the first step, peptidylglycine α-hydroxylating monooxygenase (PHM) catalyzes an α-hydroxylation of C-terminal glycine and the second enzyme, peptidylamido-glycolate lyase (PAL), promotes decomposition of the hydroxylated amimo acid residue to a peptide amide. Recently, an X-ray analysis of the active site of PHM with a substrate revealed an intriguing picture of its catalytic machinery.[5] There have been several reports on the catalytic activities of PAM in the transformation of the biological compounds.[6–8] However, the PHM model reaction for oxidative hydroxylation of C-terminal peptide by a metal complex has scarcely been reported on account of difficulty of control of molecular oxygen.[9] In the course of study on the transformation of amino acid, we previously discovered some interesting hydroxylation mediated on the Co(III) complex under aerobic condition.[10–12] Moreover, the novel type transamination from salicylidene–alanine to α-hydroxy salicylidene-*o*-hydroxybenzylamine was promoted by the ternary complexes with 2,2':6',2"-terpyridine (terpy) ligand.[13] On the basis of these findings, we have carried out an α-hydroxylation of dipeptide (dp), which is expected to be accelerated by the π-orbital character of terpy on the ternary cobalt complex.[14] Here, we describe the substrate-specific α-hydoxylation of dipeptide mediated upon the Co(III)-terpy complex as a functional model for PHM.

The starting Co(III) ternary complexes, $[Co(dp)(terpy)]^+$ (**1**), were prepared by the reaction of $[Co(CO_3)(OH)(terpy)]$[15] (2 mmol) with an equimolar amount of dp (dp = gly–ala (**a**), gly–val (**b**), gly–leu (**c**), gly–phe (**d**), gly–trp (**e**), and gly–phg (**f**)) at pH 8.5 and 40°C for 6 h in an aqueous solution (50 mL). After treating with SP Sephadex C-25 column (H^+ form, 3.6 cmϕ × 28 cm) with 0.1 M NaCl solution and desalting with methanol, red colored complexes, $[Co(dp)(terpy)]^+$ (**1a**–**1f**), were obtained.[16] Their structures were confirmed by the ^1H NMR and electronic absorption spectra.

The treatment of **1a** at pH 10.5 and 40 °C under aerobic condition for 12 h gave a ternary Co(III) complex (**2a**) containing glycyl-α-hydroxy-alanine (gly-Oala) ligand, whose structure was determined by ^1H NMR and FAB-mass spectra as follows: In the ^1H NMR spectra, a quartet peak of alanine α-proton for **1a** observed at 4.94 ppm disappeared completely in **2a** and a doublet peak of the alanine methyl protons for **1a** at 1.95 ppm was replaced to a singlet one at 2.24 ppm, although the other proton peaks did not show any significant difference between them. Clearly, the α-position of C-terminal amino acid residue was substituted by the other functional group after the reaction. In order to elucidate its functional group, FAB-mass spectrum of **2a** was measured and compared with that of **1a**. Although a parent peak of complex **1a** was observed at *m/z* = 436, that in **2a** was detected at 452. The increase of 16 mass numbers suggests that an oxygen atom was incorporated in the product complex. Interestingly, this hydroxylation has exhibited a substrate-specificity for dipeptides employed, as shown in Figure 1. The reactions of **1a**, **1d**, **1e**, and **1f** gave the Co(III) complexes containing glycyl-α-hydroxy amino acid (**2a**, **2d**, **2e**, and **2f**), while those of **1b** and **1c** with a bulky aliphatic side chain scarcely afforded such a hydroxylated complex. Also, the complexes with aromatic side chain (**1d** and **1e**) were quickly oxidized, and the formation rate of **2f** was the fastest of all.



Notably, this hydroxylation proceeds neither under argon atmosphere in slightly alkaline media nor under an aerobic condition in slightly acidic or neutral media. Under higher pressure of oxygen, the yield of the hydroxylated complexes increased; those of **2a** after 12 h under oxygen pressures of 1 and 2 atms at pH 10.5 were 53 and 73%, respectively, although it was only 20 % under atmospheric condition. The reaction was also dependent on the concentration of hydroxide ion; the yield of **2a** under atmospheric condition was 8% at pH 10.0, whereas those were raised up to 20 and 29 % at pH 10.5 and 11.0, respectively. At pH 9.5, no oxidized product was obtained, whereas at higher pH under aerobic condition, the proportion of $[Co(terpy)_2]^{3+}$ complex in the products increased. It is sure that this reaction requires both molecular oxygen and hydroxide ion, although the detailed mechanism is not clear yet. In addition, the characteristic CD spectrum ($\Delta\varepsilon$ = –0.9 at 19kcm^{-1}, $\Delta\varepsilon$ = –0.3 at 27kcm^{-1}, and $\Delta\varepsilon$ = +1.7 at 33kcm^{-1}) observed in **1a** disappeared in the course of hydroxylation to **2a**, indicating that the C-terminal α-position of **1a**, an asymmetric center, was attacked by some species during the reaction. Such a reaction did not proceed in the Co(III) ternary complexes with *N*-methyldipicolylamine

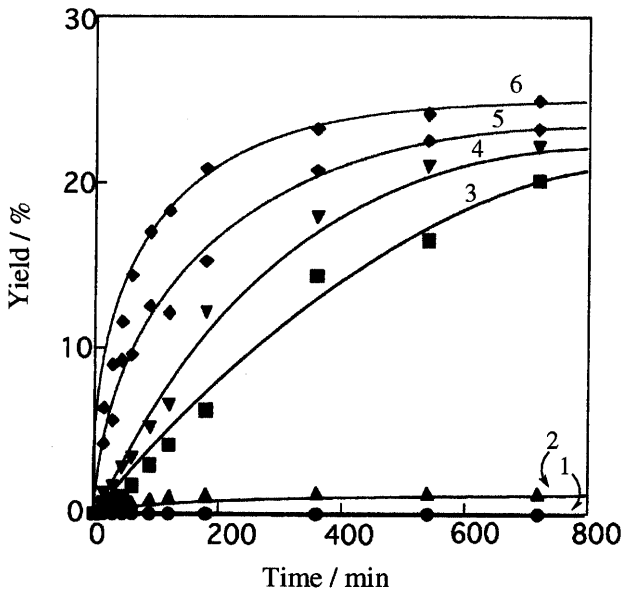


Fig. 1. Formation rates for the Co(III) ternary complexes with terpy and glycyl-α-hydroxy amino acid as followed by HPLC using ODS-C18 reverse-phase column; line 1, **2b** (gly-Oval); line 2, **2c** (gly-Oleu); line 3, **2a** (gly-Oala); line 4, **2e** (gly-Otrp); line 5, **2d** (gly-Ophe); and line 6, **2f** (gly-Ophg).

instead of terpy, which may be attributable to the terpy ligand with the vacant low-lying π* orbitals attracting electron density from the metal.[17–20]

The most interesting finding in this reaction is a substrate-specific α-hydroxylation of dipeptides as mentioned above. We can consider that the interligand interaction between the C-terminal amino acid sidechain and terpy ring is one of the possible control factors. Because the solvent effect of the NMR chemical shift has been observed for the starting complexes of **1b** and **1c**. That is, the α-H peak of leucine residue of **1c** observed at 4.88 ppm in D_2O exhibited a higher-field shift to 4.74 ppm in less polar solvent (D_2O:dioxane-d_8 = 1:3) although α-H at 2.19 pm and δ-Hs at 1.08 and 1.16 ppm shifted to lower-field region (2.29 ppm and 1.08, 1.19 ppm), respectively. This finding indicates that the α- and β-Hs in **1c** are located at the just side of terpy ring and the γ- and δ-Hs of leucine residue are situated over the ring. Such a characteristic interaction was not detected in the complexes **1a**, **1d**, **1e**, and **1f**. The hydrophobic interaction in an aqueous solution will be much stronger and the shielding effect will be enhanced.[21] This substrate-specific hydroxylation will be explainable that an intramolecular interaction between the alkyl sidechain of C-terminal amino acid and terpy ligand prevents the approach of nucleophilic reagent such as OH^- that can abstract the α-H of dp.

In conclusion, the PHM model reaction, the oxidative transformation of C-terminal amino acid residue, has been modeled on the ternary Co(III) complexes with terpy and dipeptide under the presence of molecular oxygen in slightly alkali media, which furthermore demonstrates the substrate specificity.

We thank Chemical Material Centre, the Institute for Molecular Science, for measurement of mass spectra. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture.

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16 The yields of the resulting ternary complexes were 79% for **1a**, 81% for **1b**, 70% for **1c**, 83% for **1d**, 70% for **1e**, and 66% for **1f**, respectively.
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