# Novel Hydroperoxidation of the α-Position in Pyridoxylidene-Amino Acid Schiff Base–Cobalt Complex by Hydrogen Peroxide

**Koichiro Jitsukawa,* Tetsuya Yamamoto, Tomokazu Atsumi, Hideki Masuda* and Hisahiko Einaga**

*Department of Applied Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466, Japan*

The reaction of pyridoxylidene-L-phenylalanine with $Co^{II}$ ion in the presence of $H_2O_2$ generates a novel $Co^{III}$ ternary complex, [Co(pyridoxylidene-α-hydroxyphenylalaninato)(pyridoxylidene-α-hydroperoxyphenylalaninato)]$^-$, which has been characterized on the basis of the analysis of its $^1H$ NMR, positive-ion FAB MS and X-ray structure.

Amino acid transformations catalysed by pyridoxal coenzyme have been non-enzymatically mimicked by various systems of metal ions and pyridoxylidene-amino acid Schiff bases.[1–5] The metal ions act as the centre for the fixation of Schiff base intermediate and the electronic activation of the α-position of the amino acid moiety through the aldimine–ketimine equilibrium.[1] Recently we reported a novel transformation of an amino acid mediated by $Co^{III}$ ion under aerobic conditions, in which the α-position of pyridoxylidene-amino acid was hydroxylated by oxygen, species which originated from molecular oxygen to generate the [Co(pyridoxylidene-α-hydroxyamino acidato)$_2$].[6] In further studies of such an amino acid transformation, an attractive $Co^{III}$ ternary complex with pyridoxylidene-α-hydroperoxyamino and pyridoxylidene-α-hydroxyamino acids has been synthesized by the reaction of pyridoxylidene-L-amino acid Schiff base and $Co^{II}$ ion in the presence of $H_2O_2$. The resulting complex has been characterized on the basis of the analysis of its $^1H$ NMR, positive-ion FAB mass spectra and X-ray structure. This is a novel type of hydroperoxidation of pyridoxylidene-amino acid.

To 150 ml of a yellowish aqueous solution of pyridoxylidene-L-phenylalanine Schiff base (PFS) [prepared from L-phenylalanine (1.65 g, 10 mmol), pyridoxal hydrochloride (2.04 g, 10 mmol) and NaOH (0.40 g, 10 mmol)] was added $Co(NO_3)_2 \cdot 6H_2O$ (1.46 g, 5 mmol), and then 10 ml of 30% $H_2O_2$ solution was added dropwise at room temperature under nitrogen atmosphere. The resulting mixture was stirred overnight at pH 4 to give a dark-brown solution, and the solution was passed through a cation exchange column with SP-Sephadex C-25 resin ($H^+$ form, 3.6 cm ϕ × 25 cm) pretreated to pH 3–4 with acetic acid. After the elution of neutral and anionic compounds with water, a single adsorbed band was fractionated with a 1 mol dm$^{-3}$ NaCl solution, and the fraction was allowed to stand for several weeks to give single crystals of **1** suitable for X-ray structure analysis. The electronic absorption spectrum of **1**, showing $\lambda_{max}$ at 515 nm with a shoulder at 655 nm, reveals that the $Co^{II}$ ion has been oxidized to $Co^{III}$ species.

The $^1H$ NMR spectrum of **1** showed the absence of any peak assignable to the α-proton of phenylalanine of the PFS ligand in the spectral range usually expected for the Schiff base complex (δ *ca.* 5),[6] although all other proton peaks in **1** were assignable to the Schiff base complex. In order to elucidate the structural details of **1**, we tried to analyse its X-ray structure. The crystal structure established (Fig. 1) demonstrated that the two Schiff base ligands coordinated to the $Co^{III}$ ion in *mer-trans*(*N*) configuration.† Judging from the double bond character of the azomethine bond [C(4M)=N(7) 1.33(1), 1.27(1) Å], the coordination structures of these two Schiff base ligands are both in aldimine form, this is also supported by the electronic absorption spectrum of **1**, being similar to that of the binary $Co^{III}$ complex of PFS with a typical aldimine form.[6]

The most striking structural feature of **1** is found at the two α-positions of the phenylalanine moiety. The positions, as suggested from the $^1H$ NMR spectrum, have already been substituted by some other groups, whose difference Fourier map showed one and two larger peaks at the respective positions. These substituents were assigned to hydroxy and hydroperoxy groups from the magnitude of electron density of the located peaks and their bond lengths; the C–O bond in the former was 1.41(1) Å and the C–O and O–O bonds in the latter were 1.40(1) and 1.44(1) Å, respectively. The length of the diatomic bond characterized as O–O is consistent with that reported for alkylhydroperoxide [1.477(3) Å].[7] The structure of **1** was also confirmed by its positive-ion FAB mass spectrum; the parent ion peak found at $m/z$ = 733 was assignable to [Co(pyridoxylidene-α-hydroxyphenylalaninato)(pyridoxylidene-α-hydroperoxyphenylalaninato)]$^-$ ion ({H[Co(phfs)(pohfs)] + H}$^+$).

Complex **1** was synthesized by the reaction of PFS with $Co^{II}$ ion in the presence of $H_2O_2$ under a nitrogen atmosphere. In the absence of $H_2O_2$, however, a $Co^{II}$ binary complex with pyridoxylidenephenylalanine was produced (positive-ion FAB mass spectrum $m/z$ = 683). The reaction of the binary $Co^{III}$ complex, [Co(pfs)$_2$]$^-$, with $H_2O_2$ did not give such a peroxidation product. Therefore, the formation mechanism of complex **1** is clearly different from that of the [Co(phfs)$_2$]$^-$ complex previously reported.[6] The present complex **1** might have been formed by the attack of OH and/or OOH radical species on the α-position of PFS, because the reaction of $H_2O_2$ with $Co^{II}$ ion generates the OH and OOH radicals with oxidation to $Co^{III}$ ion.[8] Interestingly, in the positive-ion FAB mass spectrum of the reaction product, the peak assignable to the bis(hydroperoxidized ligand) complex, [Co(pohfs)$_2$]$^-$, was not detected, which may suggest that such a complex is unstable for isolation or that one of the two hydroperoxy

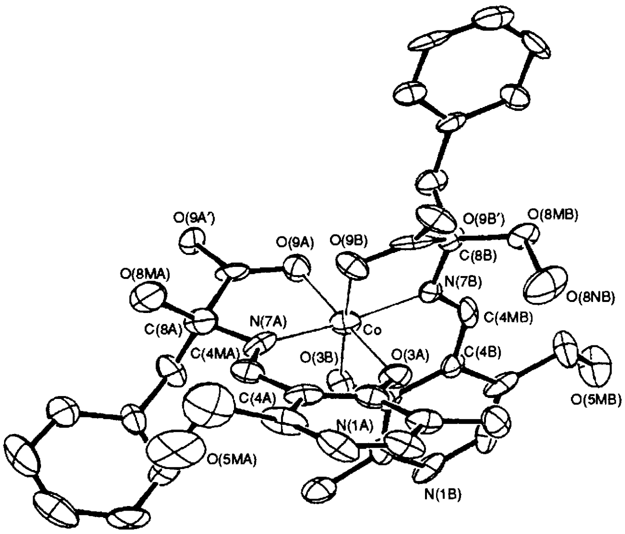
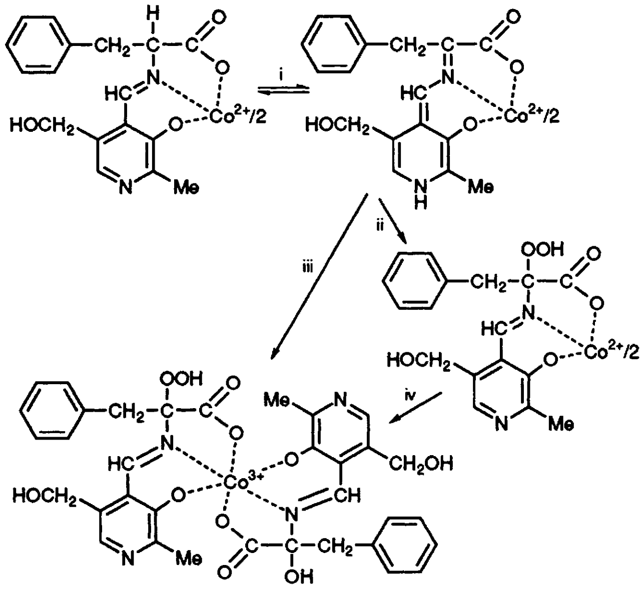


**Fig. 1** Molecular structure of the complex anion, [Co(phfs)(pohfs)]$^-$. Only the important interatomic distances (Å) and angles (°) are given: Co–O(3A) 1.880(5), Co–O(3B) 1.882(5), Co–N(7A) 1.858(7), Co–N(7B) 1.886(6), Co–O(9A) 1.886(6), Co–O(9B) 1.890(6), C(4MA)–N(7A) 1.33(1), C(4MB)–N(7B) 1.27(1), C(8A)–N(7A) 1.50(1), C(8B)–N(7B) 1.47(1), C(8A)–O(8MA) 1.41(1), C(8B)–O(8BM) 1.40(1), O(8BM)–O(8BN) 1.44(1); O(3A)–Co–N(7A) 94.9(3), O(3B)–Co–N(7B) 94.7(2), O(3A)–Co–O(9A) 178.7(2), O(3B)–Co–O(9B) 176.2(2), N(7A)–Co–O(9A) 86.2(3), N(7B)–Co–O(9B) 85.8(3), C(4A)–C(4MA)–N(7A) 120.5(8), C(4B)–C(4MB)–N(7B) 124.7(8), C(4MA)–N(7A)–C(8A) 119.4(7), C(4MB)–N(7B)–C(8B) 121.2(7).



**Scheme 1** i, aldimine–ketimine equilibrium; ii, $H_2O_2$ attack at α-position; iii, OH, OOH radical attack at α-position; iv, intramolecular oxidation

groups initially substituted has been supplied by the oxidation of the central metal ion.

The present α-hydroperoxidation of pyridoxylidene-amino acid, to our knowledge, is a novel type of fixation of active oxygen species onto the Schiff base ligand. Such a fixation does not occur in the reaction of $Co^{II}$ ion with the analogous Schiff bases containing salicylaldehyde in place of pyridoxal. Pyridoxal may play an important role for the hydroxylation and hydroperoxidation through activation of the C–H bond at the α-position of the amino acid, which is of interest in connection with a model for the enzymatic transformation of amino acid.[9,10] From the above results, we presume that, at the initial stage of this reaction, peroxide species generated from hydrogen peroxide would attack at the α-position of the Schiff base, which is in equilibrium between aldimine and ketimine forms (Scheme 1). Further details of the mechanism will be reported elsewhere.

Thanks are due to the Chemical Material Centre, the Institute for Molecular Science, for assistance in obtaining the positive-ion FAB mass spectra. The present study was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. 05209212) from the Ministry of Education, Science and Culture of Japan.

*Received, 5th July 1994; Com. 4/04101A*

---

## Footnote

† *Crystal data* for **1**: $C_{34}H_{32}CoN_4NaO_{11}\cdot5H_2O$, $M = 844.65$, monoclinic, $P2_1/c$, $a = 11.505(3)$, $b = 22.266(4)$, $c = 14.732(3)$ Å, $\beta = 94.27(2)°$, $Z = 4$, $V = 3763.4$ Å$^3$, $D_c = 1.49$ g cm$^{-3}$; Mo-K$\alpha$ ($\lambda = 0.71073$ Å); $\mu = 5.95$ cm$^{-1}$, $3 < 2\theta < 55°$. A total of 6352 unique reflections were collected on an Enraf-Nonius CAD4-EXPRESS four-circle diffractometer. 2466 reflections having $I > 3\sigma(I_o)$ were used in the structure solution and refinement using the SDP-MolEN program system. Absorption correction was applied by DIFABS. The structure was solved by the heavy-atom method and refined anisotropically. The hydrogen atoms, except those of hydroxy, hydroperoxy, and a part of water molecules, were located on Fourier difference maps, which were not refined, but included in the calculation. Final $R$ and $R_w$ factors were 0.063 and 0.069, respectively. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

## References

1 A. E. Martell, *Acc. Chem. Res.*, 1989, **22**, 115, and references cited therein.

2 D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, 1954, **76**, 648.

3 H. Kuno, K. Okamoto, J. Hidaka and H. Einaga, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 2824.

4 V. M. Shanbhag and A. E. Martell, *Inorg. Chem.*, 1990, **29**, 1023.

5 I. I. Mathews, P. A. Joy, S. Vasudevan and H. Manohar, *Inorg. Chem.*, 1991, **30**, 2181.

6 K. Jitsukawa, Y. Yamamoto, H. Masuda and H. Einaga, *Chem. Lett.*, 1993, 693.

7 F. S. El-Feraly, D. A. Benigni and A. T. MsPhail, *J. Chem. Soc., Perkin Trans. 1*, 1983, 355.

8 R. A. Sheldon and J. K. Kochi, *Metal-Catalyzed Oxidation of Organic Compounds*, Academic, New York, 1981.

9 *Vitamin B$_6$*, ed. K. Dakshinamurti, Annals of the New York Academy of Science, 1990.

10 D. M. Smith, N. R. Thomas and D. Gani, *Experientia*, 1991, **47**, 1104.