Synthesis and Characterization of a Peroxovanadium(V) Complex Containing *N*-Carboxymethylhistidine as a Model for the Vanadium Haloperoxidase Enzymes

Kan Kanamori,* Kazuya Nishida, Nanako Miyata, and Ken-ichi Okamoto†
Department of Chemistry, Faculty of Science, Toyama University, 3190 Gofuku, Toyama 930-8555
†*Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8571*

(Received September 17, 1998; CL-980720)

The peroxovanadium(V) complex containing *N*-carboxymethylhistidine (cmhist) was synthesized and structurally characterized by X-ray analysis as a model compound for the vanadium-dependent haloperoxidase enzymes. The complex takes a heptacoordinate structure consisting of a quadridentate cmhist ligand, a side-on bound peroxo group, and an oxo group. The coordination polyhedron is a distorted pentagonal bipyramid.

In 1983 Vilter isolated the haloperoxidase from the brown alga *Ascophyllum nodosum* and found that the enzyme is a non-heme vanadium-dependent one.[1] The vanadium-dependent haloperoxidase (VHPO) is now the subject attracting much attention of chemist, biologist, and physiologist. Several reviews have appeared focusing on VHPO.[2] Haloperoxidases are enzymes that catalyze the two-electron oxidation of a halide by hydrogen peroxide and the halogenation of organic substrates.

$$RH + HX + H_2O_2 \rightarrow RX + 2H_2O$$

In the absence of substrates the enzymes react with additional hydrogen peroxide to yield singlet oxygen. The catalytic cycle has been proposed.[3,4] The vanadium(V) ion in VHPO first reacts with peroxide to give the peroxovanadium(V) complex, which then functions halides to give oxidized halogen species such as OX^-.

Recently the crystal structures of the native and peroxide forms of vanadium chloroperoxidase from the fungus *Carvularia inaequalis* were revealed.[5,6] The vanadium atom in each form is coordinated by the imidazole nitrogen of a histidine residue. Although many peroxovanadium(V) complexes have been characterized by X-ray crystallography,[7] the structure of peroxovanadium(V) complex containing histidine or histidine derivatives have not been found. The sole relevant complex structurally characterized is a diperoxovanadium(V) complex with imidazole, $[VO(O_2)_2(imidazole)]^-$.[8] Here we report the X-ray crystal structure of K[VO(O$_2$)(DL-cmhist)]·H$_2$O (cmhist: *N*-carboxymethylhistidine). This complex was unstable in an acidic solution as described below. Therefore, we also prepared the protonated complex to obtain the information about the structure of the complex in an acidic solution.

DL-H_2cmhist was prepared according to an ordinary method using DL-histidine, monochloroacetic acid, and lithium hydroxide. Though more conveniently than the literature method,[9] the yield was lower. Vanadium(V) oxide (0.36 g; 2 mmol) was dissolved in 30 cm^3 of water by adding 4 cm^3 of 1 mol/dm^3 KOH (4 mmol) with heating and stirring. The resulting clear yellow solution was stirred at 0 °C. The quadridentate ligand, DL-H_2cmhist (0.89 g; 4 mmol) was added followed by an addition of 2.8 cm^3 of 10% H_2O_2 (8 mmol) to give a red solution. The solution was evaporated until ca. 70% of the initial volume remained and then kept in a refrigerator overnight. The orange powder precipitated was filtered out and ethanol was added to the filtrate. The solution was allowed to stand at room temperature for several days resulting the separation of red crystals, which were collected and air-dried to give 0.46 g of product in a 31% yield. Anal. Found: C, 25.71; H, 3.25; N, 11.11%. Calcd for K[VO(O$_2$)(cmhist)]·H$_2$O = $C_8H_{11}N_3KO_8V$: C, 26.16; H, 3.03; N, 11.45%. The synthesis using optically active ligand did not give crystals with a good quality enough for X-ray analysis. The protonated complex, [VO(O$_2$)(Hcmhist)(H$_2$O)] was obtained as fine orange crystals from the reaction mixture acidified (pH 3.5) with 60% $HClO_4$ in a 53% yield. Anal. Found: C, 28.92; H, 3.69; N, 12.39%. Calcd for [VO(O$_2$)(Hcmhist)(H$_2$O)] = $C_8H_{12}N_3O_8V$: C, 29.19; H, 3.68; N, 12.77%.

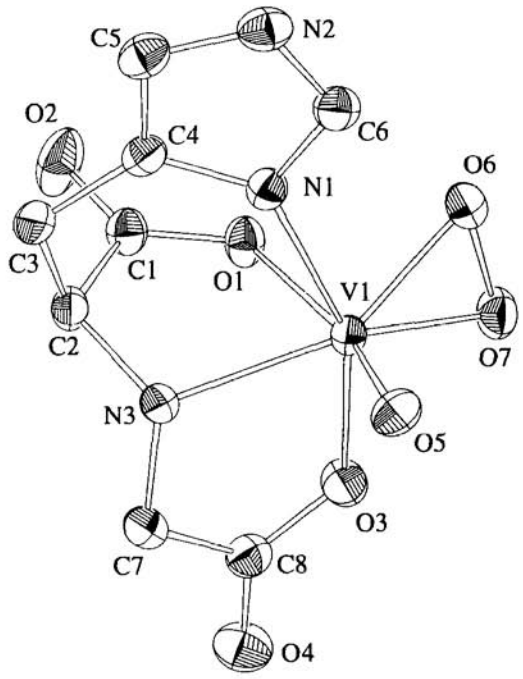

Figure 1. Structure and numbering scheme for the complex anion. Selected interatomic distances (Å) and angles (degree) are V1-O1 2.144(1), V1-O3 2.045(1), V1-O5 1.598(1), V1-O6 1.874(1), V1-O7 1.882(1), V1-N1 2.156(2), V1-N3 2.169(2), O6-O7 1.429(2), O1-V1-O3 84.01(5), O1-V1-N3 75.68(6), O1-V1-N1 82.29(5), O1-V1-O6 86.43(6), O1-V1-O7 90.09(5), O3-V1-O7 77.77(6), O3-V1-N3 77.60(6), N1-V1-N3 78.50(6), O6-V1-N1 78.62(6), O6-V1-O7 44.72(6), O3-V1-O5 95.73(6), O5-V1-N3 92.00(7), O5-V1-N1 93.14(6), O5-V1-O6 104.17(7), O5-V1-O7 102.12(7).

The structure of the complex was determined using X-ray crystallography.[10] The complex crystallizes in the orthorhombic space group *P*bca with Z=8. The eight units are interrelated by the crystallographic inversion centers and glide planes, indicating that the crystal is racemic. The coordination geometry around the vanadium atom approximates a pentagonal bipyramid. The peroxide binds in a side-on fashion to the vanadium center. The oxo group O3 and the carboxylate oxygen atom O1 of the histidine moiety occupy the axial positions. The two oxygen atoms of the peroxo group (O6 and O7), the imidazole nitrogen atom (N1), the amino nitrogen atom (N3), and the carboxylate oxygen atom (O3) of the incorporated acetate group complete the pentagonal plane. This heptacoordinate structure is commonly found in most peroxovanadium(V) complexes,[7] but is different from the coordination geometry of the peroxide form of the chloroperoxidase.[6] The vanadium atom in the enzyme adopts a pentacoordinate structure described as a distorted tetragonal pyramid. The oxo group occupies the apical position and the peroxide ion binds side-on in the equatorial plane to the vanadium atom. It is interesting to note that the arrangement of the oxo, the peroxo, and the imidazole groups in the present complex is similar to that found in the peroxide form of the enzyme. The coordination structure in the peroxide form of the enzyme can, therefore, be regarded as a structure in which the O1 and O3 sites in the present complex are empty.

The two bond lengths between the vanadium atom and the peroxo oxygen atoms (V1-O6 and V1-O7) are practically the same, indicating a symmetric coordination of the peroxide ion. These V-O(peroxo) lengths as well as the peroxo O-O bond length are similar to those found in the peroxide form of the chloroperoxidase[6] and lie within the range obtained for the previously characterized monoperoxovanadium(V) complexes.[3,7] The bond length between the vanadium atom and the imidazole nitrogen atom (V1-N1 2.156(1) Å) is slightly longer than that found in $[VO(O_2)_2(imidazole)]^-$ (2.092(2) Å).[8] The distance from the vanadium atom to the carboxylate O atom trans to the oxo group (V1-O1 2.144(1) Å) is longer than that to the O atom in the cis position (V1-O3 2.045(1) Å), reflecting the trans influence of the oxo group.

The structure of the protonated form of the peroxovanadium(V)-cmhist complex was estimated based on the infrared spectra. The protonated complex exhibits a new band at 1668 cm^{-1} in addition to the original band at 1629 cm^{-1} in the antisymmetric COO stretching vibration region. This new band can be assigned to the unionized uncoordinated COO stretching band.[11] It can, therefore, be considered that one of the carboxylate groups in the cmhist ligand is protonated and free from the coordination in the protonated complex. So we tentatively formulate the protonated complex as $[VO(O_2)(Hcmhist)(H_2O)]$. The O1 atom would be released from the coordination more easily than the O3 atom since the V1-O1 bond is weakened by the trans influence of the oxo group in $K[VO(O_2)(cmhist)]$.

The peroxovanadium(V) complex is fairly stable in a neutral aqueous solution but decomposes slowly in acidic solutions. For example, the peroxo-to-vanadium charge-transfer band observed at 420 nm disappeared in about 48 h when the complex was dissolved in a solution of pH 3, suggesting the cleavage of the vanadium-peroxo bond. Thus, with regard to the stability of the peroxo binding the present complex is distinct from $[VO(O_2)(nta)]^{2-}$ and related complexes where the vanadium-peroxo bond remains intact under a similar condition.[3] Study of detailed solution properties of the cmhist complex including reaction with halides is in progress.

References and Notes

1 H. Vilter, K. -W. Glombitza, and A Grave, *Bot. Mar.*, **26**, 331 (1983); H. Vilter and K. -W. Glombitza, *Bot. Mar.*, **26**, 341 (1983); H. Vilter, *Bot. Mar.*, **26**, 451 (1983); H. Vilter, *Phytochemistry*, **23**, 1387 (1984).

2 R. Wever and B. E. Krenn, in "Vanadium in Biological Systems," ed by N. D. Chasteen, Kluwer Academic Publisher, Dordrecht (1990), pp. 81-97; A. Butler and J. V. Walker, *Chem. Rev.*, **93**, 1937 (1993); H. Vilter, in "Metal Ions in Biological Systems. Vol. 31 Vanadium and Its Role in Life," ed by H. Sigel and A. Sigel, Marcel Dekker, New York (1995), pp. 325-362; R. Wever and W. Hemrika, in "Vanadium in the Environment," ed by J. V. Nriagu, John Wiley & Sons, New York (1998), pp. 285-305.

3 G. J. Colpas, B. J. Hamstra, J. W. Kampf, and V. L. Pecoraro, *J. Am. Chem. Soc.*, **118**, 3469 (1996).

4 B. J. Hamstra, G. J. Colpas, and V. L. Pecoraro, *Inorg. Chem.*, **37**, 949 (1998).

5 A. Messerschmidt and R. Wever, *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 392 (1996).

6 A. Messerschmidt, L. Prade, and R. Wever, *Biol. Chem.*, **378**, 309 (1997).

7 A. Butler, M. J. Clague, and G. E. Meister, *Chem. Rev.*, **94**, 625 (1994).

8 D. C. Crans, A. D. Keramidas, H. Hoover-Litty, O. P. Anderson, M. M. Miller, L. M. Lomoine, S. Pleasic-Williams, M. Vandenberg, A. J. Rossomando, and L. J. Sweet, *J. Am. Chem. Soc.*, **119**, 5447 (1997).

9 L. A. Meiske and R. J. Angelici, *Inorg. Chem.*, **21**, 738 (1982).

10 Crystal data: formula, $C_8H_{11}N_3O_8VK$; fw, 367.23; orthorhombic; space group, *P*bca (No. 61); a = 14.917(4) Å, b = 26.373(9) Å, c = 6.390(4) Å; Z = 8; Dcalcd = 1.94 g/cm^3; R(Rw) = 0.030(0.056). A total of 2547 independent reflections with $|Io|>2\sigma(Io)$ were used for the calculation.

11 K. Nakamoto, "Infrared and Raman Spectra of Inorganic and Coordination Compounds," 5th ed, John Wiley & Sons, New York (1997).