Electron Spin−Echo Envelope Modulation Study of Imidazole-Coordinated Oxovanadium(IV) Complexes Relevant to the Active Site Structure of Reduced Vanadium Haloperoxidases

Kouichi Fukui,* Hiroaki Ohya-Nishiguchi, and Hitoshi Kamada

Institute for Life Support Technology, Yamagata Technopolis Foundation, Matsuei 2-2-1, Yamagata 990, Japan

Received November 18, 1997

Some haloperoxidases contain vanadium ion essential for their enzymatic acitivity.[1] The vanadium ion is in the +5 oxidation state in the native form, and the reduction of the vanadium to the +4 oxidation state results in an inactivation of the enzyme. Although the X-ray structure of the V^V form of V−CPO[2] was recently solved,[3] the vanadium site structure of the reduced enzyme is still an open question. X-ray absorption results revealed a substantial structural change of the vanadium site upon reduction.[4] Very recently, Hamstra et al.[5] proposed that two imidazoles are ligated in the reduced form whereas one imidazole is ligated in the native form.[3] Such a structural change is supposed to explain the inactivation of the enzyme.[5] ESEEM spectroscopy is a powerful tool to investigate metal site structures, and ESEEM data of V^{IV}−BrPO indicate imidazole ligation to the VO^{2+} ion.[6] However, ESEEM spectral properties of imidazole-coordinated VO^{2+} complexes are still poorly understood. To the best of our knowledge, the imidazole-coordinated VO^{2+} complexes so far studied by ESEEM or ENDOR spectroscopy are only $VO(L)_4^{2+}$,[7−11] $VO(acac)_2(L)$[10−12] (L = imidazoles), and $VO(His)_2$.[7,9] Of these, only $VO(mim)_4Cl^+$ was studied in detail and the ^{14}N HFC and NQC tensors were determined by computer simulation.[11] Here we report ESEEM results of another type of imidazole-coordinated VO^{2+} complexes, $VO(Himac)_2$ (**1**) and VO(salimH)(acac) (**2**).[13] The ^{14}N HFC and NQC tensors of **1** have been determined from orientation selection ESEEM results. Also interesting is the structural change of **2** induced by an addition of acid. It was reported that a new species of larger $A_{||}(^{51}V)$ forms when acid is added to **2**,[14] and V^{IV}−BrPO exhibits a similar CW EPR spectral change when pH is lowered.[15] Although it is not clear from the CW EPR results which ligand atom is protonated and/or detached from the VO^{2+} ion, the present ESEEM results enable us to identify the detached nitrogen. In addition to the relevance to vanadium haloperoxidases, this study will provide a basis for interpretation of ESEEM data of VO^{2+}-substituted proteins where VO^{2+} ion is used as a spectroscopic probe.[16]

Figure 1 shows three-pulse (stimulated echo) ESEEM spectra of **1** measured at the $m_I(^{51}V) = -^7/_{2||}$ (a), $-^3/_{2\perp}$ (b), and $^1/_{2\perp}$ (c) lines. Peaks due to the coordinating imidazole ^{14}N nucleus are observed in the region of 0−10 MHz. From the simulations[17] of the spectra, the ^{14}N HFC **A** and NQC **Q** tensors have been determined as $|A_X|$ ($\approx|A_{zz}|$) = 7.05, $|A_Y|$ = 6.55, and $|A_Z|$ = 5.8 MHz ($|A_{iso}|$ = 6.5 MHz), and Q_X ($\approx Q_{zz}$) = −0.2, Q_Y = −0.9 and Q_Z = 1.1 MHz (e^2qQ/h = 2.2 MHz and η = 0.64), where the **A** and **Q** tensors are assumed as coaxial and their principal axes are labeled as *XYZ*. In the best fits, the two-axis systems, **g**;*xyz* and **A** (**Q**);*XYZ*, are not coaxial, forming Euler angles of β = 80° and γ = 20°. The simulations confirm the assignments of the 5.7 and 8.8 MHz peaks in Figure 1a, 5.0 and 8.6 MHz peaks in Figure 1b, and 4.8 and 8.6 MHz peaks in Figure 1c to the double-quantum (DQ) lines. Figure 1c additionally exhibits single-quantum (SQ) lines at 3.4 and 5.3 MHz. (The higher frequency SQ line almost overlaps with the 5.0 MHz DQ line in Figure 1b.) Quite interestingly, the peak frequencies in Figure 1c are very similar to the ESEEM frequencies of V^{IV}−BrPO, 3.0, 4.7, 5.3, and 8.6 MHz,[6] obtained at almost the same field of B = 317 mT. Therefore the 4.7 and 8.6 MHz peaks and the 3.0 and 5.3 MHz peaks of V^{IV}−BrPO are most likely the DQ and SQ lines, respectively, on the basis of the assignments of **1**. The fact that the lower-frequency SQ peak is more prominent for V^{IV}−

(1) (a) De Boer, E.; van Kooyk, Y.; Tromp, M. G. M.; Plat, H.; Wever, R. *Biochim. Biophys. Acta* **1986**, *869*, 48−53. (b) DeBoer, E.; Tromp, M. G. M.; Plat, H.; Krenn, G. E.; Wever, R. *Biochim. Biophys. Acta* **1986**, *872*, 104−115. (c) van Schijndel, J. W. P. M.; Vollenbroek, E. G. M.; Wever, R. *Biochim. Biophys. Acta* **1993**, *1161*, 249−256.

(2) Abbreviations: BrPO, bromoperoxidase; CPO, chloroperoxidase; ENDOR, electron−nuclear double resonance; ESEEM, electron spin−echo envelope modulation; HFC, hyperfine coupling; NQC, nuclear quadrupole coupling; Hacac, acetylacetone; HimacH, 4-imidazoleacetic acid; mim, 1-methylimidazole; H_2salen, *N*,*N*′-bis(salicylidene)ethylenediamine; HsalimH, 4-(2-(salicylideneamino)ethyl)imidazole.

(3) Messerschmidt, A.; Wever, R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 392−396.

(4) Arber, J. M.; de Boer, E.; Garner, C. D.; Hasnain, S. S.; Wever, R. *Biochemistry* **1989**, *28*, 7968−7973.

(5) Hamstra, B. J.; Houseman, A. L. P.; Colpas, G. J.; Kampf, J. W.; LoBrutto, R.; Frasch, W. D.; Pecoraro, V. L. *Inorg. Chem.* **1997**, *36*, 4866−4874.

(6) de Boer, E.; Keijzers, C. P.; Klaassen, A. A. K.; Reijerse, E. J.; Collison, D.; Garner, C. D.; Wever, R. *FEBS Lett.* **1988**, *235*, 93−97.

(7) Mulks, C. F.; Kirste, B.; van Willigen, H. *J. Am. Chem. Soc.* **1982**, *104*, 5906−5911.

(8) Dikanov, S. A.; Burgard, C.; Hüttermann, J. *Chem. Phys. Lett*. **1993**, *212*, 493−498.

(9) Dikanov, S. A.; Samoilova, R. I.; Smieja, J. A.; Bowman, M. K. *J. Am. Chem. Soc*. **1995**, *117*, 10579−10580.

(10) Reijerse, E. J.; Shane, J.; de Boer, E.; Collison, D. In *Electron Magnetic Resonance of Disordered Systems*; Yordanov, N. D., Ed.; World Scientific: Singapore, 1989; pp 189−204.

(11) Reijerse, E. J.; Shane, J.; de Boer, E.; Höffer, P.; Collison, D. In *Electron Magnetic Resonance of Disordered Systems*; Yordanov, N. D., Ed.; World Scientific: Singapore, 1991; pp 253−271.

(12) Eaton S. S.; Dubach, J.; More, K. M.; Eaton, G. R.; Thurman, G.; Ambruso, D. R. *J. Biol. Chem.* **1989**, *264*, 4776−4781.

(13) Faint purple powder of **1** was prepared by mixing aqueous solutions of $VOSO_4 \cdot xH_2O$ (x = 3 assumed), 2.5 equiv of HimacH, and 2 equiv of NaOH. This complex is readily oxidized when dissolved in H_2O under atmospheric conditions, so that the samples for spectroscopic measurements were prepared with N_2 gas bubbling. Anal. Calcd (found) for $C_{10}H_{10}N_4O_5V$: C, 37.87 (37.52); H, 3.18 (3.28); N, 17.67 (17.51). Vis IR (in 2:1 v/v H_2O/ethylene glycol solution); λ_{max}/nm (ϵ/M^{-1} cm^{-1}), 760 (53), 565 (36). CW EPR (in H_2O/ethylene glycol 2: 1 v/v glass); $g_\perp$ = 1.979, $g_{||}$ = 1.951, $|A_\perp(^{51}V)| = 60.5 \times 10^{-4}$, $|A_{||}(^{51}V)| = 164.0 \times 10^{-4}$ cm^{-1}. The complex **2**·MeOH was prepared according to the method of ref 14.

(14) Cornman, C. R.; Kampf, J.; Lah, M. S.; Pecoraro, V. L. *Inorg. Chem*. **1992**, *31*, 2035−2043.

(15) de Boer, E.; Boon, K.; Wever, R. *Biochemistry* **1988**, *27*, 1629−1635.

(16) ESEEM studies of VO^{2+}-substituted proteins where imidazole ligation is expected or proposed are found in ref 12 and in the following: (a) Gerfen, G. J.; Hanna, P. M.; Chasteen, N. D.; Singel, D. J. *J. Am. Chem. Soc*. **1991**, *113*, 9513−9519. (b) Dikanov, S. A.; Tyryshkin, A. M.; Hüttermann, J.; Bogumil, R.; Witzel, H. *J. Am. Chem. Soc.* **1995**, *117*, 4976−4986. (c) Buy, C.; Matsui, T.; Andrianambinintsoa, S.; Sigalat, C.; Girault, G.; Zimmermann, J.-L. *Biochemistry* **1996**, *35*, 14281−14293.

(17) For the procedure of the computer simulation, see ref 18 and references therein.

S0020-1669(97)01453-5 CCC: $15.00
Published on Web 04/21/1998

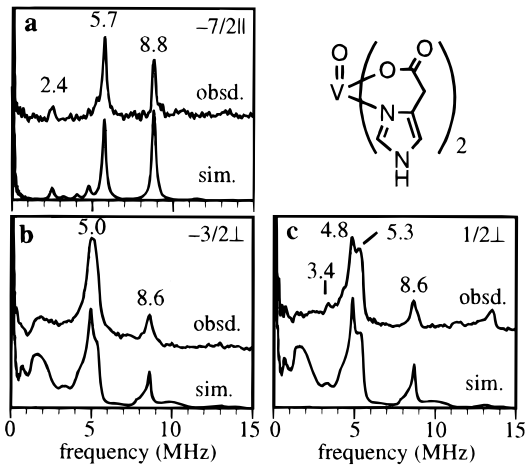


Figure 1. Three-pulse ESEEM spectra and their simulations of 2 mM VO(Himac)$_2$ in 2:1 v/v H_2O/ethylene glycol glass. Conditions: $\nu = 8.84$ GHz, $T = 77$ K; (a) $\tau = 270$ ns, $B = 260.5$ mT; (b) $\tau = 300$ ns, $B = 305.5$ mT; (c) $\tau = 300$ ns, $B = 318.5$ mT.

BrPO may indicate more severe structural distortion in the enzyme. de Boer et al.[6] reported that VO(2-methyl-8-quinolinolate)$_2$ exhibits ESEEM spectra similar to those of V^{IV}−BrPO. However, this complex does not contain imidazole ligation. Thus **1** is the first imidazole-coordinated VO^{2+} complex which models ESEEM spectral properties of V^{IV}−BrPO.

It is believed that the directions of the NQC axes are mainly connected with the imidazole moiety itself;[16b,19] i.e., the maximum amplitude axis is always along the lone-pair orbital (visually along the V−N bond) and the minimum amplitude axis is always normal to the ring plane. According to this criterion, the solution-state structure of **1** is such that the imidazole rings fairly lie in the equatorial plane with the dihedral angle between the imidazole ring and equatorial plane being ∼20°. Comparison between the HFC tensors of **1** and VO(mim)$_4$Cl$^+$ suggests that the directions of the HFC axes are also mainly connected with the imidazole moiety with the maximum amplitude axis normal to the ring plane (and the minimum amplitude axis along the V−N bond as pointed out previously).[20] For VO(mim)$_4$Cl$^+$, where the imidazole rings are expected to be normal to the equatorial plane,[21] the maximum amplitude HFC axis is normal to the g_z axis. On the other hand, the corresponding axis is almost parallel to the g_z axis for **1**, where the imidazole rings are supposed to lie fairly in the equatorial plane.

Figure 2a shows the three-pulse ESEEM spectrum of **2** recorded at the $-^7/_{2||}$ line. In Figure 2a, two pairs of DQ lines are resolved in accordance with the presence of two types of coordinating nitrogens, Schiff base nitrogen and imidazole nitrogen. One pair comprises 5.0 and 8.1 MHz peaks, and these frequencies coincide well with the DQ frequencies of VO(salen), 5.1 and 8.1 MHz,[18] obtained under practically the same conditions. Thus this pair is attributed to the Schiff-base nitrogen. The frequencies of the other pair, 5.7 and 8.7 MHz, agree with the DQ frequencies of **1** in Figure 1a, so that this pair is attributed to the imidazole nitrogen. Furthermore, the spectrum recorded at the $-^3/_{2\perp}$ line was roughly the sum of the corresponding spectra of **1** and VO(salen) (see Supporting Information). The finding that the imidazole nitrogens exhibit similar ESEEM signals in **1** and **2** is consistent with the

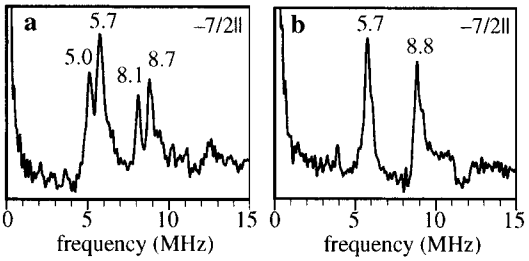
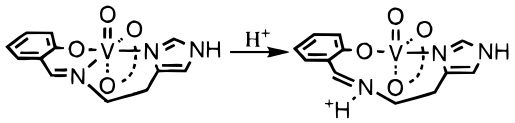


Figure 2. (a) Three-pulse ESEEM spectrum of 5 mM VO(salhisH)(acac) in 1:1 v/v DMF/toluene glass. Conditions: $\nu = 8.83$ GHz, $T = 77$ K, $\tau = 280$ ns, $B = 261.6$ mT. (b) Spectrum after addition of 1 equiv of aqueous HCl. Conditions: $\nu = 8.84$ GHz, $T = 77$ K, $\tau = 280$ ns, $B = 260.2$ mT.

Scheme 1



above-noted relation between the nitrogen HFC axes and imidazole orientation. The X-ray data of **2**[14] show that the imidazole ring is fairly parallel to the equatorial plane as is expected for **1** (the dihedral angle between the mean imidazole ring and equatorial plane is 10.7° in **2**).

Addition of 1 equiv of acid to **2** caused a change of CW EPR spectrum as reported previously.[14] The three-pulse ESEEM spectrum recorded at the new $-^7/_{2||}$ line is shown in Figure 2b, where one pair of DQ lines disappears completely. This shows unequivocally that one nitrogen is detached from vanadium. This nitrogen is identified as the Schiff base nitrogen on the basis of the above assignment. On the contrary, the peaks due to the imidazole nitrogen remain unchanged in Figure 2b. Thus, the imidazole nitrogen must stay attached to vanadium with the ring plane still fairly in the equatorial plane (Scheme 1). The present results therefore support the previous conclusion that one nitrogen of **2** is protonated and detached from vanadium upon addition of 1 equiv of acid (though the detached nitrogen is the Schiff base nitrogen as opposed to the previous scheme).[14]

V^{IV}−BrPO undergoes a similar CW EPR spectral change when pH is lowered to 4.2,[15] which may be also due to the detachment of one nitrogen ligand. Then at least two protein-derived ligands should be ligated in the reduced enzyme around neutral pH because the VO^{2+} ion is still protein-bound at low pH.[15] In contrast, as shown by the X-ray study,[3] one imidazole is the only protein-derived ligand in V^V−CPO. The two enzymes, V−BrPO and V−CPO, have high similarities in amino acid sequence, and the vanadium site structures of these enzymes will be very similar.[3] This situation favors the previous proposition that another imidazole becomes ligated upon reduction.[5] Hamstra et al.[5] further suggested that one imidazole is equatorially ligated and the other is axially ligated. In this regard, however, the present ESEEM results, showing a good correspondence between **1** and V^{IV}−BrPO rather, imply that both imidazoles are equatorially ligated in the reduced enzyme as in **1**.

Supporting Information Available: Figures of two- and three-pulse ESEEM spectra and their simulations of **1** and the three-pulse spectra of **2** recorded at the $-^3/_{2\perp}$ line (3 pages). Ordering information is given on any current masthead page.

IC971453G

(18) Fukui, K.; Ohya-Nishiguchi, H.; Kamada, H. *Inorg. Chem.* **1997**, 36, 5518−5529.
(19) Ashby, C. I. H.; Paton, W. F.; Brown, T. L. *J. Am. Chem. Soc.* **1980**, *102*, 2990−2998.
(20) Scholes, C. P.; Falkowski, K. M.; Chen, S.; Bank, J. *J. Am. Chem. Soc.* **1986**, *108*, 1660−1671.
(21) Calviou, L. J.; Arber, J. M.; Collison, D.; Garner, C. D.; Clegg, W. *J. Chem. Soc., Chem. Commun.* **1992**, 654−656.