Two-Dimensional ESEEM Study of VO^{2+} Complexes with Imidazole and Histidine: Histidine Is a Polydentante Ligand

Sergei A. Dikanov,*,†,‡ Rimma I. Samoilova,†,‡
Joanne A. Smieja,§ and Michael K. Bowman†

Macromolecular Structure and Dynamics, Environmental Molecular Sciences Laboratory, Pacific Northwest Laboratory, Richland, Washington 99352
Institute of Chemical Kinetics and Combustion Russian Academy of Sciences, Novosibirsk 630090, Russia
Department of Chemistry, Gonzaga University Spokane, Washington 99258

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The oxovanadium cation, VO^{2+}, has been successfully employed as a spin probe[1] in electron−nuclear double resonance (ENDOR)[2,3] and electron spin echo envelope modulation (ESEEM)[3,4] studies of metalloprotein active sites. These techniques are often used to detect coordination by side chains of histidine residues.[5,6] Interpretation usually relies on the ENDOR study by Mulks et al.[7] of oxovanadium complexes of imidazole or histidine. Unfortunately, the nitrogen ENDOR spectra were not completely analyzed, and the hyperfine coupling to the remote imidazole nitrogen has been questioned.[8] Here we demonstrate that the complex of VO^{2+} with histidine has been misinterpreted and that the dominant ESEEM pattern is produced by the α-amino group. Nevertheless, we show that histidine side chain ligands can be identified from either nitrogen hyperfine or quadrupole couplings.

Samples were prepared as described by Mulks et al.[7] Imidazole and histidine with natural abundance (Aldrich) or ^{15}N-labeled in the ring (Cambridge Isotope Laboratory, isotopic purity >95% and >98%, respectively) were used. Experiments were performed on a Bruker ESP 380 spectrometer at 30 K.

Figure 1 shows a stack plot of three-pulse ESEEM spectra as a function of the time τ between the first pair of pulses for the perpendicular hyperfine component of VO^{2+}-(imidazole)$_4$ and VO^{2+}-(histidine)$_n$ complexes. Upon isotopic substitution of ^{15}N for ^{14}N, the spectrum of the VO^{2+}-(imidazole)$_4$ complex completely changes. Instead of two broad lines at 5 and 9 MHz due to the two double-quantum (dq) transitions of directly coordinated ^{14}N nitrogens,[8,9] only a single line at the ^{15}N Zeeman frequency (ν_I = 1.46 MHz) has appreciable intensity. This line has been attributed to the noncoordinated, remote ^{15}N of the imidazole, with a hyperfine coupling of ~0.5 MHz.[8] The coordinated ^{15}N nitrogen signals in three-pulse spectra are expected to be broad, weak lines between 2 and 5.5 MHz. The indicated features are enhanced in 2D, four-pulse HYSCORE

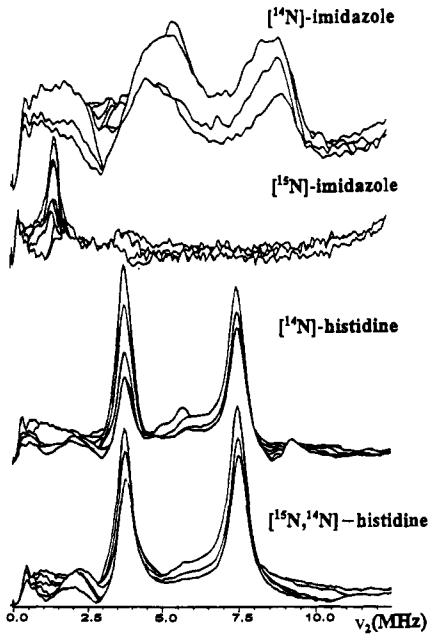

Figure 1. Superimposed plot of a set of three-pulse ESEEM spectra as the modulus of the Fourier transforms along time T axis recorded at the perpendicular component ($m_I^V = -3/2$) of the EPR spectrum: VO^{2+}-([^{14}N]imidazole)$_4$ (3373 G), VO^{2+}-([^{15}N]imidazole)$_4$ (3400 G), VO^{2+}-([^{14}N]histidine)$_2$ (3405 G), and VO^{2+}-([^{15}N,^{14}N]histidine)$_2$ (3397 G). The initial τ is 88 ns in the nearest trace, incremented by 16 ns in the successive traces. The microwave frequency was 9.68−9.73 GHz.

experiments.[10] A contour plot of the HYSCORE spectrum of VO^{2+}-([^{14}N]imidazole)$_4$ (Figure 2) shows two intense cross peaks in the (+,−) quadrant corresponding to the two main peaks in the three-pulse spectrum, confirming their assignment as dq transitions. The peaks along both diagonals result from pulse imperfections and spectrometer noise. The streaks flanking the diagonal in the (+,−) quadrant are single-quantum−single-quantum (sq) transitions, as are the blobs near (+2,+5) and (+5,+2) MHz.

The contour HYSCORE spectrum of VO^{2+}-([^{15}N]imidazole)$_4$ in Figure 2 demonstrates *both* nitrogens of the imidazole ligand. The coordinated nitrogens appear as two weak but clearly new arcs in the (+,−) quadrant centered at ~(+5.5,−2.5) and (+2.5,−5.5) MHz. The remote nitrogens produce the intense peak in the (+,+) quadrant on the diagonal at ν_I = 1.46 MHz. However, an arc perpendicular to the diagonal can be seen in the contour of this peak. The contour line form permits estimation of both the isotropic and anisotropic hyperfine couplings.[11] Analysis[11] gives the maximum component of the hyperfine tensor as 6.3 MHz for the coordinated nitrogen, in good agreement with the coupling of 6.0−6.5 MHz from the ^{14}N dq transitions.[12] The small anisotropic component agrees with T = 0.4−0.5 MHz in other cases of VO^{2+}−nitrogen direct coordination.[6,13] The results for the remote nitrogen confirm our previous estimates.[8]

Turning now to the histidine complexes, the three-pulse spectra in Figure 1 show two strong lines at 4.2 and 7.5 MHz

† Pacific Northwest Laboratory.
‡ Institute of Chemical Kinetics and Combustion.
§ Gonzaga University.

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(11) The contour line shape (Dikanov, S. A.; Bowman, M. K. *J. Magn. Reson., Ser. A*, in press) in the 2D spectrum from I = 1/2 nuclei is $\nu_{\alpha(\beta)} = \{Q_{\alpha(\beta)}\nu^2_{\beta(\alpha)} + G_{\alpha(\beta)}\}^{1/2}$, where $Q_{\alpha(\beta)} = (T + 2a \mp 4\nu_I)/(T + 2a \pm 4\nu_I)$ and $G_{\alpha(\beta)} = \pm 2\nu_I(4\nu_I^2 - a^2 + 2T^2 - aT)/(T + 2a \pm 4\nu_I)$. Using any two points on the arc, one can find two sets of isotropic (a) and perpendicular anisotropic (T) hyperfine constants correponding to $|\nu_{\perp\alpha(\beta)}| > |\nu_{\parallel\alpha(\beta)}|$ and vise versa. The following sets (recalculated for ^{14}N) were found for the coordinated nitrogen, $a = \pm 5.9$ MHz, $T = \mp 0.38$ MHz or $a = \pm 5.52$ MHz, $T = \pm 0.38$ MHz, and the remote nitrogen: $a = \pm 0.37$ MHz, $T = \mp 0.17$ MHz or $a = \pm 0.28$ MHz and $T = \pm 0.17$ MHz.

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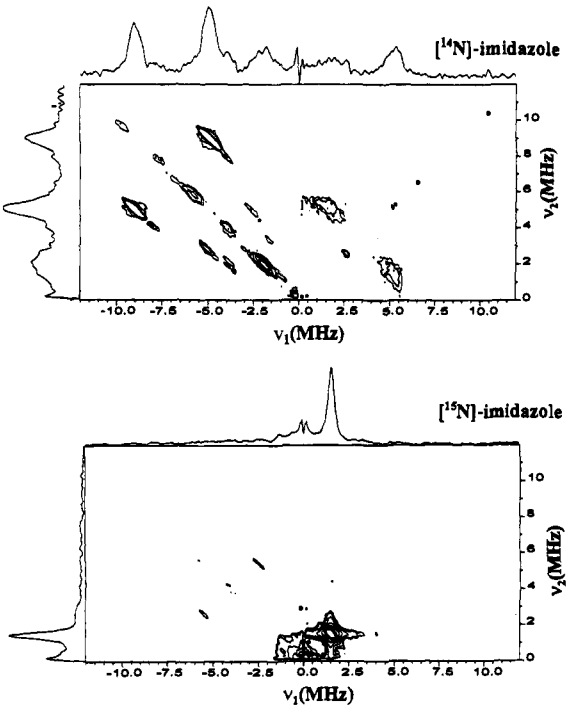

Figure 2. HYSCORE spectra of VO^{2+}-([^{14}N]imidazole)$_4$ (3436 G, τ = 256 ns) and VO^{2+}-([^{15}N]imidazole)$_4$ (3400 G, τ = 256 ns) recorded on the extra absorption ($m_I^V = -1/2$) and perpendicular ($m_I^V = -3/2$) components of the EPR spectrum, respectively.

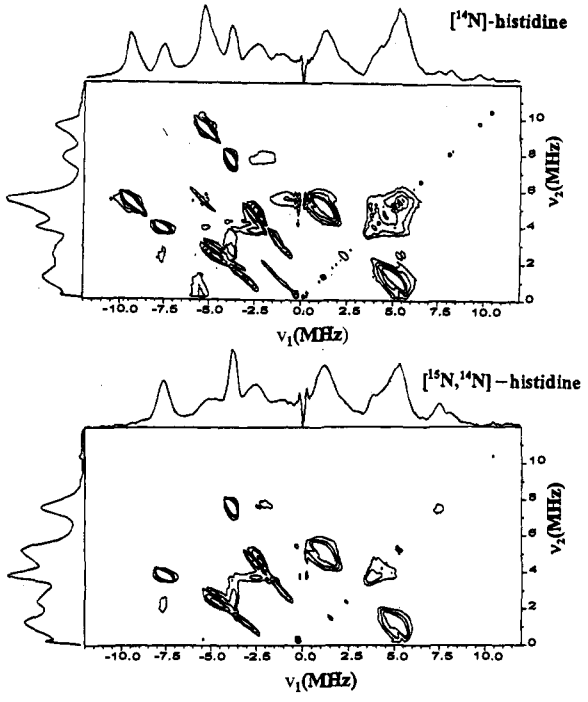

Figure 3. HYSCORE spectra of VO^{2+}-([^{14}N]histidine)$_2$ (3468 G, τ = 256 ns) and VO^{2+}-([^{15}N]histidine)$_2$ (3460 G, τ = 256 ns) recorded on the extra absorption peak ($m_I^V = -1/2$) of the EPR spectrum.

for both the ^{14}N and the ^{15}N,^{14}N complexes. This immediately indicates that these lines belong to the α-amino group of the histidine that had not been isotopically substituted. The HYSCORE spectra for both complexes (Figure 3) contain intense cross peaks with coordinates of 4.2 and 7.5 MHz. The field dependence identifies them as dq transitions of a nitrogen with hyperfine coupling of A = 5.0 MHz and a quadrupole coupling constant (qcc) of $K = e^2qQ/4h = 0.58 \pm 0.02$ MHz.[12] Two other peaks are seen only in the unlabeled complex at 5.7 and the 9.3 MHz in three-pulse and HYSCORE spectra. We assign these peaks to coordinated imine nitrogens, similar to VO^{2+}-(imidazole)$_4$ and having the couplings A = 6.3 MHz and $K = 1.02 \pm 0.07$ MHz. The ^{14}N HYSCORE spectra of histidine complex show an even better resolved set of cross lines, similar to those from the imidazole ligand, as well as additional dq−sq peaks in the (+,−) quadrant, e.g., near (−2.5, −8.0) MHz.

The 2D spectra of the [^{15}N,^{14}N]histidine complex do not show resolved lines from the directly coordinated imine nitrogen as in the case of the [^{15}N]imidazole complex. These lines lie too near the intense sq transitions of the amine nitrogen. The contribution from the ^{15}N remote nitrogen in the histidine imidazole ring appears as a weak peak on the diagonal at the ^{15}N Zeeman frequency.

The hyperfine and quadrupole couplings for amine and imine nitrogens are distinct from each other and consistent with those reported for model oxovanadium complexes and proteins. Hyperfine couplings for equatorially coordinated simple amines are consistently 1 MHz smaller than those of coordinated aza aromatic compounds.[9,14] The estimated qccs are quite different and agree with those reported for amine nitrogens coordinated with zinc or copper[15] complexes and amine[9] and imine[6] nitrogens in oxovanadium complexes.

A recent study[16] suggests that at pH ≈ 7, the predominate species in solution is VO^{2+}-(histidine)$_2$ with one bidentate and one tridentate histidine. The tridentate ligand, in addition to binding to the VO^{2+} equatorial sites via the imine and amine nitrogens, coordinates axially via a carboxylate oxygen, which is ESEEM silent. We have verified spectroscopically in our solvent the 1:2 stoichiometry for VO^{2+}/histidine. ESEEM results support the stoichiometry VO^{2+}-(histidine)$_2$ at pH ≈ 7, with the histidine coordinated equatorially to the oxovanadium with both imine and amine nitrogens.

The application of 2D ESEEM to the [^{15}N]imidazole complex shows, in the same spectrum, both the coordinated and the remote ring nitrogens with very different isotropic hyperfine couplings. In the case of histidine ligation, equatorial coordination by both imine and amine nitrogen is demonstrated. Neither conclusion was reached in the previous ENDOR investigation. The reproducible differences between both the hyperfine and the quadrupole constants of the amine and imine nitrogens are reflected in the shape and position of the lines in the 2D spectra and allow them to be distinguished in proteins. With both imine and amine coordination, the VO^{2+}/histidine complex cannot be considered an adequate model for coordination by the histidine side chain in proteins. The imidazole, as a monodentante ligand, is better for this role.

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(12) The coupling A and parameter $\kappa = K^2(3 + \eta^2)$ of ^{14}N nuclei were calculated from the dq frequencies $\nu_{dq\pm} = 2[(\nu_I \pm A/2)^2 + K^2(3 + \eta^2)]^{1/2}$, Dikanov, S. A.; Tsvetkov, Yu. D.; Bowman, M. K.; Astashkin, A. V. *Chem. Phys. Lett.* **1982,** *90,* 149. The frequencies 9 and 5 MHz (±0.3 MHz) for imime imidazole nitrogen, 9.3 and 5.7 MHz (±0.1 MHz) for imine, and 7.5 and 3.8 MHz (±0.05 MHz) for side chain amine histidine nitrogens are observed in ESEEM spectra recorded on the perpendicular, and the extra absorption EPR components. The uncertainty in qcc covers values of the asymmetry parameter $0 \le \eta \le 1$.

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