Nitrogen Coordination in VO^{2+}-Substituted Imidazole Glycerol Phosphate Dehydratase Studied by Electron Spin−Echo Envelope Modulation Spectroscopy

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Imidazole glycerol phosphate dehydratase (IGPD, EC 4.2.1.19) catalyses the dehydration of imidazole glycerol phosphate to imidazole acetol phosphate which is an important step in the biosynthesis of histidine.[1−3] In the presence of certain divalent metals *apo*-IGPD assembles to the catalytically active form consisting of a 24mer of identical subunits (total $M_r = 573$ kDa).[2] We have previously demonstrated that vanadyl is able to assemble the enzyme into the high molecular weight form.[4] The EPR spectra of the VO^{2+} derivative of IGPD have shown that at least three inequivalent coordination environments exist for the enzyme at neutral pH. The EPR spin Hamiltonian parameters indicated the presence of multiple inner-sphere ^{14}N nuclei. Moreover electron nuclear double resonance spectra showed resonances attributed to interactions with ^{14}N nuclei which were consistent with metal-coordinated histidine imidazoles.[4] Electron spin−echo envelope modulation (ESEEM) spectroscopy in conjunction with VO^{2+} as a spin probe ($S = {}^1/_2$ in the vanadyl $3d_{xy}$ orbital) has been successfully used for a number of enzymes in which histidine imidazole is coordinating the metal[5−9] and in vanadyl model systems.[10,11] Herein we have used ESEEM spectroscopy to characterize further the VO^{2+} coordination environment of IGPD. IGPD has provided a unique environment within which to advance the interpretation of vanadyl ESEEM spectra.

IGPD from *Saccharomyces cerevisiae* was cloned, expressed, and purified, and EPR/ESEEM samples were prepared as described earlier.[4] Frozen solution ESEEM spectra have been recorded at X-band frequencies with a Bruker ESP380E spectrometer equipped with an Oxford Instruments CF395 liquid helium gas-flow cryostat. The τ-values used were optimized from 2-dimensional recordings of the ESEEM spectra and time-domain ESEEM data were approximated using a fourth-order polynomial. The $^{\pi}/_2$ pulse width was 16 ns with pulse-repetition rates of 200 Hz. Powder-type ESEEM simulations have been carried out using the program MAGRES described by Keijzers et al.[12]

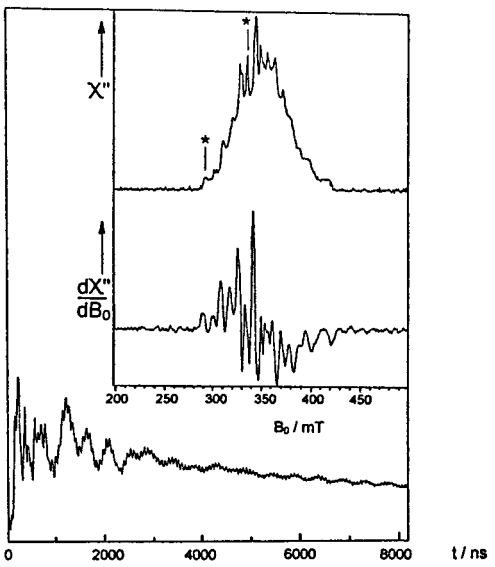

Figure 1. Stimulated echo ESEEM time-domain trace of VO^{2+}−IGPD in frozen aqueous solutions (pH 7.5) recorded at $B_0 = 335.1$ mT. The insert shows the field swept pulsed EPR spectrum of VO^{2+}−IGPD and the first derivative thereof. The two excitation field settings used for the ESEEM spectra are indicated by the asterisks representing the $-{}^7/_2$ parallel and $-{}^3/_2$ perpendicular vanadyl hf-lines. Experimental conditions: $T =$ 4.2 K; $\nu_{MW} = 9.685$ GHz; $\tau = 176$ ns.

Figure 1 depicts the stimulated-echo ESEEM time-domain trace for VO^{2+}-substituted IGPD recorded for a field setting at about the $M_I = -{}^3/_2$ perpendicular hyperfine (hf)-line of the vanadyl EPR spectrum. Modulations originating from couplings to different nuclei are observed. The echo-detected field-swept vanadyl EPR spectrum is shown in the insert together with the corresponding first derivative spectrum.

The frequency domain ESEEM magnitude spectra recorded at the vanadyl $M_I = -{}^3/_2$ perpendicular and $-{}^7/_2$ parallel field settings are shown in Figure 2. At these positions, where almost single-crystal-like ESEEM spectra are obtained,[13] a number of well-resolved ESEEM lines can be identified for both excitation field settings despite possible contributions to the ESEEM spectra from superimposed VO^{2+}−IGPD EPR subspectra. The intense resonances at frequencies below 10 MHz, with lines observed at 0.75, 1.20, 2.15, 5.0, and 9.1 MHz for a field setting centered at the $-{}^3/_2$ perpendicular vanadyl hf-line are attributed to couplings to ^{14}N-nuclei in the inner-coordination sphere of VO^{2+}. The feature at 9.1 MHz with a slight shoulder at the high-frequency side is assigned, in agreement with the literature,[5−9] to the high-frequency double quantum transition ($\Delta M_I = 2$), $\nu_{dq}{}^+$, in one of the two $M_S = \pm{}^1/_2$ electron spin manifolds. The frequency of the $\Delta M_I = 2$ transition is similar to that measured by ENDOR.[4]

The frequencies of the double quantum transitions allow an estimate of the superhyperfine-coupling (shfc) components[8] of the coordinated ^{14}N nucleus. Using the same first-order expression as that used in the ENDOR study,[14] we determine from the frequency of $\nu_{dq}{}^+$ measured by ESEEM with the field set at the perpendicular hf-line,[13] that $|A^{\perp}|$ is ∼7.0 MHz. Similarly the shfc-component, $|A^{||}|$, can be determined to be ∼7.5 MHz, from $\nu_{dq}{}^+$

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(13) It should be noted that at the perpendicular field setting, contributions from a parallel orientation can underly the spectrum. However, these are neglected since they are of much lower intensity.
(14) The high frequency $\Delta M_I = 2$ transition was measured by ENDOR at a frequency of $\nu^+{}_{dq} = 8.7$ MHz with a field setting at the $-{}^3/_2$ perpendicular hf-line. From this frequency the hf-coupling component $|A^{\perp}|$ was estimated to be ∼7 MHz, using the first-order expression, $\nu_{dq}{}^+ = |A + 2\nu_n(^{14}N)|$.

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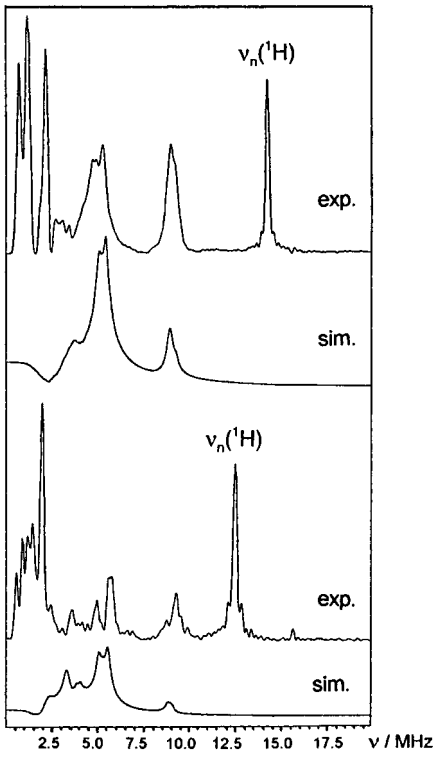

Figure 2. Stimulated echo ESEEM spectra of VO^{2+}−IGPD after Fourier transformation recorded at the two fields corresponding to the $-3/2$ perpendicular and $-7/2$ parallel hf-lines with field settings of $B_0 = 335.1$ mT (top spectrum labeled exp.) and 292.4 mT (bottom exp. spectrum), respectively. Resonances due to protons are indicated by $\nu_n(^1H)$. Experimental conditions were as for Figure 1 except that τ was 112 ns. The traces below the experimental spectra are the respective powder ESEEM simulations for some of the features in the experimental spectra below 10 MHz. Parameters for the simulations of the experimental spectra were ^{51}V **g**-tensor, $g^{\|} = 1.996$, $g^{\perp} = 1.948$; hf-tensor $|A_{iso}| = 305.8$ MHz, $|A^x_{dip}| = |A^y_{dip}| = 99.6$ MHz, $|A^z_{dip}| = 199.2$ MHz; ^{14}N shfc-tensor, $|A_{iso}| = 7.0$ MHz, $|A^x| = |A^y| = 6.86$ MHz, $|A^z| = 7.27$ MHz with the Euler angles, $\Psi = 20°$, $\theta = 120°$, $\phi = -35°$, and ^{14}N nqi-tensor, $e^2Qq/h = 1.75$ MHz, $\eta \cong 1$, and angles $\alpha = -35°$, $\beta = 90°$, $\gamma = -30°$.

at the parallel field setting. This resonance was not previously observed by ENDOR.

The frequencies of the two double quantum transitions should change with the magnetic field strength by $2|\Delta\nu_n|$, with ν_n being the ^{14}N nuclear Larmor frequency, shifting $\nu_{dq}{}^+$ and $\nu_{dq}{}^-$ in opposite directions (this corresponds to about 0.13 MHz at the two field settings used in the present study). However, in contrast to experiments with D-xylose isomerase,[7] the frequency of the $\nu_{dq}{}^+$ resonance shows little dependence on the field and even seems to shift to lower frequency when the field is raised. This is diagnostic of a small dipolar hf-contribution which approximately cancels the shift due to the different magnetic field values. It further corresponds to a situation where the $A^{\|}$ shfc component must be larger than $A^{\perp}$.

The simulations of the ESEEM spectra for both field settings are represented in Figure 2 by the traces below the experimental spectra. The best simulations were obtained with the parameters listed in the figure legend. The vanadyl **g**- and **A**-tensor components used for the simulations were those for the α-conformation.[4] Even though reasonable simulations have also been obtained for other sets of Euler angles relating nitrogen and vanadium principal axes orientations, the shfc components and nuclear quadrupole interaction (nqi) parameters varied only slightly (±0.2 MHz for A^i and ±0.3 MHz for e^2Qq/h depending on η).

The magnitude of the ^{14}N shfc of $|A| \sim 6-8$ MHz can be used to give some indication about the type of ligand. ^{14}N resonances attributed to interactions with a coordinated lysine residue have consistently shown shfc components that are about 1 to 2 MHz smaller than those found for histidine.[9,15,16] On this basis a lysine ligand can be ruled out, and we assign these resonances to the nitrogen of a coordinated histidine.

The $A^{\|}$ shfc component agrees well with lines generally found at 5.7 and 8.0 MHz for other VO^{2+}-substituted enzymes and model systems with histidine imidazole ligands.[7,8] The shfc for IGPD lies within the same range and is consistent with the observation that the shfc is primarily isotropic. Moreover, similar nqi parameters were observed for other VO^{2+}−enzyme complexes where they were attributed to a nitrogen coordinated in the equatorial plane of vanadyl. We likewise assume that VO^{2+}−IGPD possesses at least one equatorially coordinated nitrogen.

The experimental ESEEM spectra contain pronounced features below 2.5 MHz, whereas the simulations do not. Therefore, only the two ^{14}N features at the higher frequencies can be attributed to the nitrogen of equatorially coordinated histidines with coupling parameters similar to those found in other histidine-ligated systems. The remaining ^{14}N ESEEM resonances observed at frequencies below 2.5 MHz almost certainly arise from couplings to ^{14}N nuclei. These may originate from axially coordinated histidines as proposed for the α-conformation of VO^{2+}−IGPD.[4] Remote imidazole nitrogens[17] are unlikely candidates because their couplings are expected to be some 20 times smaller than those of the coordinating nitrogen in vanadyl systems.[18] A recently reported pronounced ESEEM resonance at ∼4.5 MHz was tentatively assigned to an axially coordinated nitrogen.[19] It is, of course, possible that the shoulder in the 5.0 MHz feature in Figure 2 is due to such a nitrogen although it is not necessary to invoke such an explanation in our system since this assumption was not used to produce the simulations. In addition, the features observed for IGPD at frequencies below 3.0 MHz are not reproduced in the other work.[19]

In summary, we demonstrate that ^{14}N ESEEM resonances arising from inner-sphere coordinated nitrogen ligands are detected for VO^{2+}−IGPD. The ^{14}N resonances are characteristic of VO^{2+} with coordinated histidine(s) and are similar to those found in other VO^{2+} complexes. We previously proposed, primarily on the basis of EPR studies, that the oxo−vanadium in IGPD was coordinated by at least one equatorial histidine. The ESEEM results reported here support this and have also allowed us to define the coordination environment in terms of the shfc components and nqi parameters.

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Supporting Information Available: Powder EPR simulations deconvoluted for the three identified sub-spectra and ESEEM simulations with the ^{14}N interaction parameters as reported for D-xylose isomerase (3 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(20) Note that ESEEM spectra at neutral pH are a superposition from separate vanadyl coordination environments, since insufficient discrimination of spectral contributions is achieved at the excitation field settings used. Thus, the number of ESEEM nqi lines and the line width of the double quantum transitions could at least partially be affected by contributions from different coordination environments. However, simulations using the parameters for the β-site have shown almost identical ESEEM spectra. This explains why the experimental spectra appeared to be little-broadened by the presence of different subspectra.