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DETECTION OF OXOVANADIUM(IV) AND CHARACTERIZATION OF ITS LIGAND ENVIRONMENT IN SUBCELLULAR FRACTIONS OF THE LIVER OF RATS TREATED WITH PENTAVALENT VANADIUM(V)

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Summary. Tetravalent oxovanadium(IV) was detected in subcellular fractions of liver by ESR spectroscopy after i.p. injection of pentavalent vanadium(V) as sodium vanadate into rats for three days. This indicates that the metal ion was reduced from the pentavalent state to oxovanadium(IV). The ligand environment around this oxovanadium center was characterized using ESR parameters(g_0 and A_0) and the empirical bonding coefficients calculated from the ESR parameters. These values indicate that most of the ligand atoms around the oxovanadium(IV) are oxygens and that the metal may exist in a protein-bound form.

Vanadium is extremely widely distributed in nature (1,2) and vanadium ions, which may act as bi-, tri-, tetra- or penta-valent ions, are considered to be very toxic to animals (3,4). On the contrary, some evidence implies that vanadium may be physiologically essential to rats, chicks and humans (5,6).

In timed-distribution studies on selected organs and liver subcellular fractions of rats after intravenous injection of trace amounts of vanadium-48, radioactivity was detected in the liver supernatant, microsomes, mitchondria and nuclear fraction (7). In addition, the vanadium in liver and kidney displayed an ESR signal, indicating that it had been reduced from the penta-valent state to vanadium(IV) (8). However, in this study no subcellular fractionation was carried out and the nature of the vanadium-binding sites was not characterized.

In view of the biochemical importance of the toxic and beneficial actions of vanadium ion, we attempted to obtain further information at a molecular level about the nature of vanadium ion in animals. During investigations on these problems we detected tetravalent oxovanadium(IV) in subcellular fractions of rat liver by ESR spectroscopy after i.p. administration of pentavalent vanadium

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for three days. This paper reports studies on the characterization of this oxovanadium(IV) from the ESR parameters, showing that most of the ligand atoms around the oxovanadium(IV) are oxygens.

Materials and Methods

Vanadium(V) was administered to male Wistar rats, weighing 160g, by intraperitoneal injection as sodium metavanadate at a dose of 0.625mg V/Kg/day for three days. Liver subcellular fractions (mitochondria, centrifuged twice for 10 min at 12000g, microsomes, centrifuged 1 hour at 105000g, and mocrosomal supernatant) were obtained by differential centrifugation by the procedure of Hogeboom (9). ESR spectra of tetravalent vanadium(IV) were measured with a JEOL JES ME X-band spectrometer with a 100 KHz modulation. The magnetic field was calibrated with Mn(II)-dopoed magnesium oxide powder. Measurement were carried out at 298K in solution and at 77K in liquid nitrogen in the frozen state. Vanadium ions (IV and V) were standarized complexometrically.

Results and Discussion

The common valence states of vanadium ion are II, III, IV and V. The V state is diamagnetic and the III state, although paramagnetic, is usually not observable by ESR due to an internal electric field effect. Both the II and IV state can, however, be detected at room temperature. Vanadium(II) is oxidatively unstable, especially in living organisms, and easily yields the oxonium ion, VO^{2+}(IV). Vanadium IV exists almost exclusively as the oxovanadium(IV) ion in organic complexes. Furthermore, the IV state requires a noncubic field for observation by ESR and this is found in square pyramidal oxovanadium(IV)-type complexes (10).

The ESR spectra of rat liver subcellular fractions, mitochondria and microsomal supernatant fraction, have been found to show typical spectra of square pyramidal oxovanadium(IV) ion or complexes at 77K. Eight lines characteristic of isotropic oxovanadium(IV) with a spin od 7/2, were found in rats given 0.625 mg V/Kg/day of sodium metavanadate by intraperitoneal injection for three days (Fig. 1)(10,11). This clearly indicates that vanadate(V) is reduced to oxovanadium(IV) in rat liver, confirming the observation by Johnson et al (8).

The nature of the square pyramidal oxovanadium(IV) in each fraction responsible for the characteristic spectrum can be determined in more detail using the isotropic g_0(=($g_{/\!/}$ +2$g_{\perp}$)/3)-value and the hyperfine coupling constant, A_0(=($A_{/\!/}$

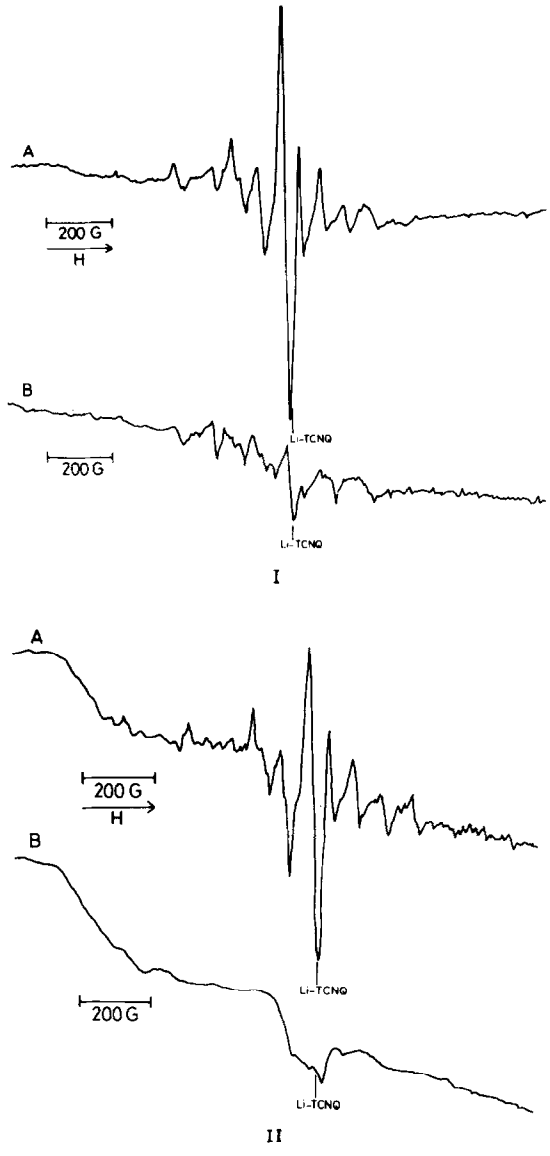

Figure 1. ESR Spectra at 77K of the Mitochondrial (I) and Microsomal Supernatant (II) Fractions of Liver of Rats Treated with Sodium Vanadate. Instrument coditions : modulation amplitude, 10 G; microwave power, 40 mW; microwave frequency, 9.15 GHz. See Table I for ESR parameters.

$+2A_{\perp}/3$), calculated from the ESR spectrum (10). Since the nature of the coordinating ligand atoms to oxovanadium(IV) has the greatest effect (electron delocalization) on g_0, and A_0 is related to the strength of the ligand field around the vanadium ion, we can deduce the type of ligand atom coordinated to the vanadium ion with the help of these values, g_0 and A_0. The ESR parameters of rat li-

Table I. ESR Parameters of Liver Subcellular Fractions containing Oxovanadium (IV)

Fraction	g_o	$g_{/\!/}$	$g_\perp$	A_o	$A_{/\!/}$	$A_\perp$
				(gauss)		
Microsomal Supernatant	1.975	1.942	1.991	107.9	190.1	66.9
Mitochondria	1.981	1.950	1.997	108.5	188.4	68.5

The g_o- and A_o-values were calculated with the relations, $g_o=(g_{/\!/}+2g_\perp)/3$ and $A_o=(A_{/\!/}+2A_\perp)/3$, using set of $g_{/\!/}$- and $g_\perp$-values and $A_{/\!/}$- and $A_\perp$-values, respectively, obtained from the ESR spectrum in the frozen state measured at 77K.

ver subcellular fractions , calculated from the spectrum, are shown in Table I. These values were not calculated for the microsomal fraction because its ESR signal attributable to oxovanadium(IV) was too small. In Fig. 2, g_o-value are plotted against A_o for a number of oxovanadium chelates having donor atom formulae of the type, $VO(S_2N_2$ or $S_2O_2)$, $VO(N_2O_2)$, $VO(O_4)$, $VO(O_2OH_2)$ and $VO(RSH_4)$(12), and adding the values reported by Boucher et al (10). It can readily be seen that the parameters are clustered in five domains, corresponding to different types of ligand environment. The parameters for the rat liver subcellular fractions are also plotted and fall primarily in the $VO(O_4)$ region, suggesting a ligand environment largely consisting of oxygen donors. Similar plots have been used to determine the oxovanadium(IV)-binding sites in oxovadium(IV)-substituted human transferrin (13) and to identify a nonporphyrin vanadium(IV) component in petroleum (14).

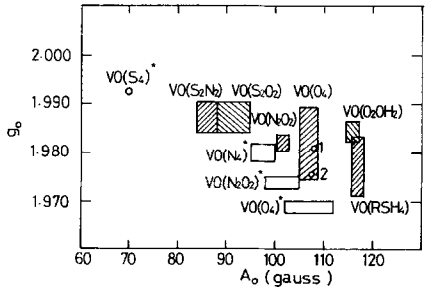

Figure 2. Plot of Isotropic g_o-values vs. Isotropic Nuclear Hyperfine Coupling Constants A_o-values for Various Types of Square Pyramidal Oxovanadium(IV) Complexes.
Values for model complexes are taken from references (10) for * and (12). Point, 1 and 2, is the value for microsomal supernatant and mitochondrial fraction containing oxovanadium(IV), respectively.

Table II. Isotropic Fermi Contact Term and Bonding Coefficient for Oxovanadium (IV) Complexes

Ligand to Oxovanadium(IV)	Type of Coordination	K	$(\beta_2^*)^2$
Mitochondrial Fraction		0.78	0.97
Microsomal Supernatant Fraction		0.77	0.99
Oxalic Acid[a]	$VO(O_4)$	0.76	1.02
L-Ascorbic Acid[a]		0.84	1.00
Pyrocatechol[a]		0.75	1.10
Acetylacetone[a]		0.78	0.96
Serine[a]	$VO(O_2N_2)$	0.73	0.95
Tetraphenylporphyrin[b]	$VO(N_4)$	0.69	0.94
Cysteine methyl ester[a]	$VO(S_2N_2)$	0.61	0.87
Vanadium Sulfate[a]		0.84	1.01

a and b ; see reference (12) and (10), respectively.

Our conclusion described above was corroborated by the results obtained by comparison of the empirical bonding coefficients, calculated from the ESR parameters. These values, listed in Table II, are relative values, including those of some model complexes. The K-value, the Fermi contact term, is related to the amount of unpaired electron density at the vanadium nucleus, and lowering of the $(\beta_2^*)^2$-value is caused by delocalization of the electron onto the ligand with increase in covalent bonding (10). The values for the microsomal supernatant and mitochondrial fraction fall within, or close to, the values of $VO(O_4)$-type complexes. Therefore, in the microsomal supernatant and mitochondrial fraction containing oxovanadium(IV), donor sites containing only the oxygen atom should be clearly distinguishable from other donor sites and free oxovanadium(IV) ion. It is possible, therefore, that oxovanadium(IV) is present as a protein-bound form in liver fractions containing vanadium as suggested by Johnson et al (8). It is interesting that ESR studies have suggested that in oxovanadium(IV)-labeled transferrin most of the vanadium ligands are oxygen donors, although imidazol groups are believed to be ligated to other metal ions (13).

Our conclusion presented here will be useful in clarifying the toxicological and physiological significance of vanadium ion in biological systems.

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