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Reduction of Vanadate(V) to Oxovanadium(IV) by Cysteine and Mechanism and Structure of the Oxovanadium(IV)-cysteine Complex Subsequently Formed

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Since vanadium ion has been thought for some time to be an essential element in both animals and plants [1], there have been many reports on the physiological effects of exogenous vanadate(V) on tissues and animals [2–8] and on the inhibition of $(Na^+ + K^+)$ATPase by vanadate(V) [9–13]. In recent ESR spectrometric studies we detected vanadium ion as the reduced form oxovanadium(IV) in subcellular fractions of the liver after administration of pentavalent vanadium (vanadate(V)) to rats for three days [14]. However, we obtained no information on how vanadate(V) is reduced to oxovanadium(IV).

During further studies we found that vanadate(V) is readily reduced to oxovanadium(IV) by various types of compounds. This communication concerns the reduction of vanadate(V) and the subsequent formation of a complex of oxovanadium(IV) with cysteine in aqueous solution, and describes possible mechanisms of the reaction.

In connection with our findings, Macara *et al.* recently reported the very interesting findings that reduction of vanadate(V) to oxovanadium(IV) is the rate-limiting step in uptake of vanadate (V) by erythrocytes and that the reduction is driven by cytoplasmic glutathione [15].

When a mixture of sodium vanadate(V) (10 m*M*) and cysteine (100 m*M*) was added to 0.2 *M* borate buffer, pH 6.8, at 25 °C under air, a purple color developed instantaneously and the visible spectrum exhibited absorption maxima at 538 and 725 nm (Fig. 1, upper a), which correspond well to those of the oxovanadium(IV)-cysteine complex prepared in the same conditions (Fig. 1, lower a). The purple complex has a CD spectrum which is negative at 525 nm and positive at 710 nm. A blue, rather than

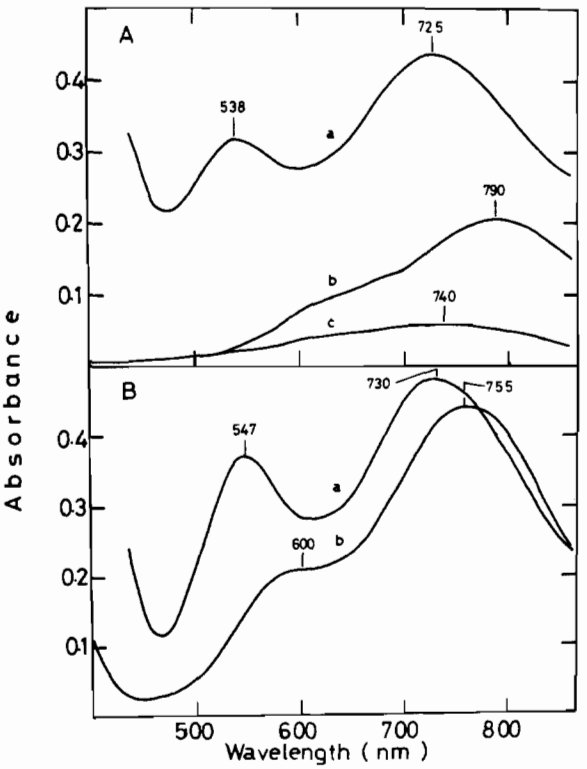

Fig. 1. Absorption Spectra of the Oxovanadium(IV)-Cysteine Complex Prepared from Vanadate(V) (A) or Oxovanadium(IV) (B) and Cysteine at Various pH-Values. A: The concentrations of $NaVO_3$ and cysteine HCl were 10 m*M* and 100 m*M*, respectively. pH: a; 6.8; b, 6.0, c; 3.0. B: The concentrations of $VOSO_4$ and cysteine HCl were 20 m*M* and 100 m*M*, respectively. pH: a; 6.8, b; 6.0.

a purple, complex was formed in the phosphate buffer, pH 6.0, and acetate buffer, pH 3.0 (Fig. 1, upper b, c and lower b). These observations suggest that vanadate(V) is reduced by cysteine irrespective of the pH value and that at pH 6.8 reduction is followed by formation of a purple complex. This purple complex is very similar to the biscysteine methyl ester-oxovanadium(IV) complex reported previously [16].

The reduction of vanadate(V) to oxovanadium(IV) was confirmed by ESR spectrometry. An ESR signal characteristic of bound oxovanadium(IV) was observed at pH 6.8 (g_o; 1.986, $g_\parallel$; 1.963, $g_\perp$; 1.998, A_o; 84.4 gauss, $A_\parallel$; 159.0 gauss, $A_\perp$; 47.1 gauss) (Fig. 2), indicating formation of a sulfur-oxovanadium(IV) linkage [16].

The Fermi contact term (K-value) relating to the amount of unpaired electron density at the vanadium nucleus and the $(\beta_1^*)^2$-value of the in-plane σ-bonding coefficient for the oxovanadium (IV) complex

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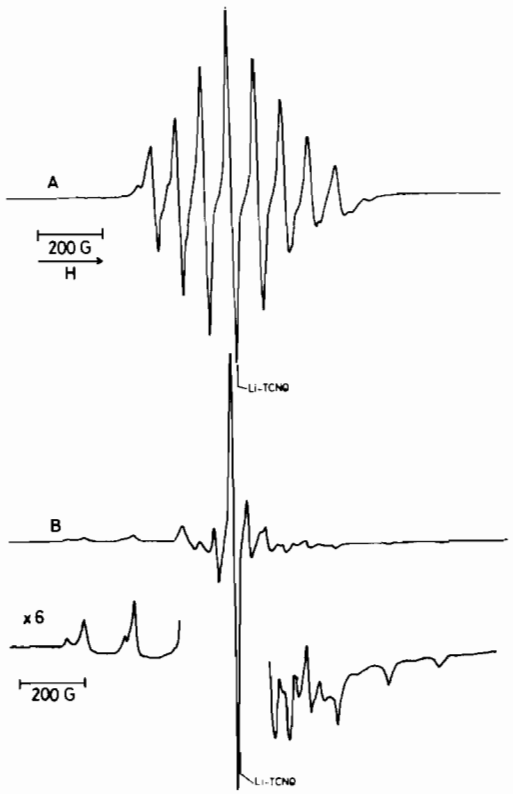


Fig. 2. ESR Spectra of the Oxovanadium(IV)-Cysteine Complex Prepared from Vanadate(V) and Cysteine at pH 6.8. The complex was prepared from 10 m*M* $NaVO_3$ and 100 m*M* cysteine HCl in 0.2 *M* borate buffer, pH 6.8. Temperature: 293 K for A and 77 K for B.

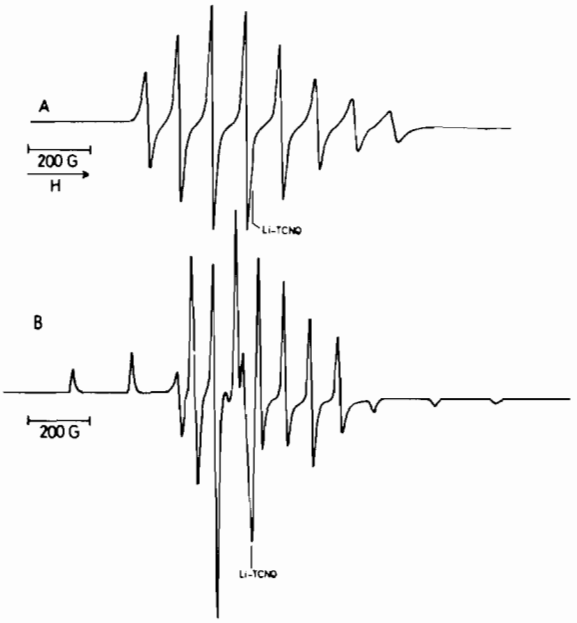


Fig. 3. ESR Spectra of the Oxovanadium(IV)-Cysteine Complex Prepared from Vanadate(V) and Cysteine at pH 3.0. The complex was prepared from 10 m*M* $NaVO_3$ and 100 m*M* cysteine HCl in 0.1 *M* acetate buffer, pH 3.0. Temperature: 293 K for A and 77 K for B.

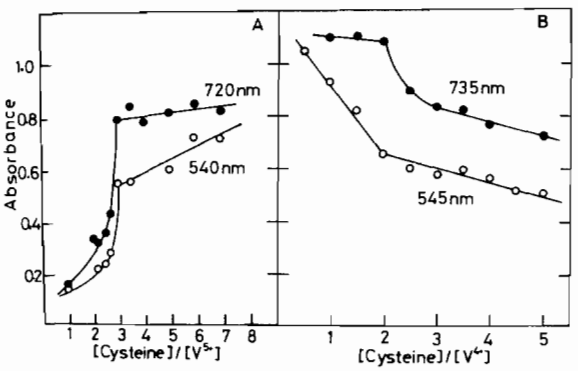


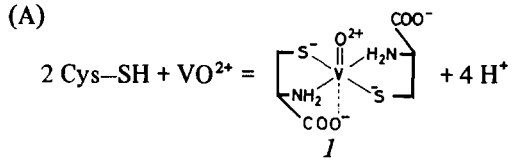
Fig. 4. Photometric Titrations of Vanadate(V) (A) or Oxovanadium(IV) (B) and Cysteine at pH 6.8. The concentration of $NaVO_3$ (A) and $VOSO_4$ (B) were 20 m*M* (constant) and 22 m*M* (constant), respectively.

prepared with vanadate(V) and cysteine were calculated from the ESR parameters at 0.60 and 0.56, respectively [17]. The purple complex prepared from oxovanadium(IV) and cysteine had the same values (g_o; 1.985, $g_\parallel$; 1.964; $g_\perp$; 1.996, A_o; 84.4 gauss, $A_\parallel$; 159.5 gauss, $A_\perp$; 46.9 gauss, K; 0.60, $(\beta_1^*)^2$; 0.56). Thus the purple complex has a sulfur-nitrogen doner set to oxovanadium (IV) as described previously [16]. A minor oxovanadium(IV) component was detectable in the ESR spectrum, suggesting the presence of *cis* and *trans* isomers. At pH 3 (Fig. 3) and 6, ESR signals characteristic of oxovanadium(IV) were detected but the oxovanadium-(IV) was not bound to cysteine. Kinetic studies now in progress indicate that the reduction of vanadate(V) to oxovanadium(IV) strongly depends on the pH value of the solution.

The stoichiometry of the reduction and complex formation in the reaction of vanadate(V) and cysteine was confirmed by spectrophotometric titration by monitoring the absorption maxima at 540 and 720 nm at pH 6.8, comparing with the reaction between oxovanadium (IV) and cysteine. Figure 4 shows that vanadate(V) and cysteine reacted in a molar ratio of 3:1, and oxovanadium(IV) and cysteine in one of 2:1. These results were supported by pH titration measurements.

From the present results, formation of a complex between oxovanadium(IV) or vanadate(V) and cysteine may occur by the mechanism shown in Scheme 1 (A and B). In the reaction between vanadate(V) and cysteine, reduction of vanadate(V) to oxovanadium(IV) is coupled with oxidation of

(A)



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(B)

$$Cys\text{–}SH + VO_3^- + 3\,H^+ =$$

$$= \tfrac{1}{2}\,Cys\text{–}S\text{–}S\text{–}Cys + VO^{2+} + 2H_2O$$

$$2Cys\text{-}SH + VO^{2+} = \textit{1} + 4H^+$$

$$3\,Cys\text{–}SH + VO_3^- =$$

$$= \textit{1} + \tfrac{1}{2}\,Cys\text{–}S\text{–}S\text{–}Cys + 2H_2O + H^+$$

Scheme 1. Possible Reaction Mechanisms of Oxovanadium(IV) (A) or Vanadate(V) (B) and Cysteine. *1* is a mixture of *cis* and *trans* isomers.

cysteine to cystine, which has been identified by TLC, and the stable complex *1* is formed between oxovanadium(IV) and cysteine added in excess. Addition of iodide did not alter the intensity of the absorption bands of complex *1* at 538 and 725 nm, indicating that the sixth position of oxovanadium-(IV) is occupied with either carboxylate-oxygen of two cysteine molecules.

The findings that cysteine reduces vanadate(V) to oxovanadium(IV) and that the latter subsequently forms stable purple complex at pH 6.8 seem important in understanding the uptake and reduction of vanadate(V) in living systems.

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