# The Preparation and Electrochemistry of Cysteine–Ester Oxovanadium(IV) Complexes

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(Received May 18, 1987)

## Abstract

An improved synthesis of $VO(Cys–OCH_3)_2$, ($Cys–OCH_3$ = the anion of cysteine methyl ester), is reported, as is an analogous preparation of $VO(Cys–OCH_2CH_3)_2$, ($Cys–OCH_2CH_3$ = the anion of cysteine ethyl ester). These are the first two examples of isolated vanadium–cysteine compounds. The oxidation of $VO(Cys–OCH_3)_2$ in DMSO is a reversible one electron change at 0.24 V *versus* SCE followed by a rapid chemical reaction which produces a stable vanadium(V) species. This species is reduced back to the vanadium(IV) complex at −1.30 V. The electrochemistry of $VO(Cys–OCH_2CH_3)_2$ is nearly identical to that of the methyl ester compound.

## Introduction

In recent years studies of the bioinorganic chemistry of vanadium have focused on its inhibitory effect on $Na^+$, $K^+$ (ATPase) [1]. This is important since naturally occurring tissue levels of vanadium, when oxidized to vanadium(V), can significantly inhibit the enzyme and may lead to hypertension. We envision a mechanism where the metal cycles between the IV (inactive) and V (active) oxidation states. Our interest is thus in characterizing the electrochemistry of appropriate vanadium(IV) and (V) model complexes.

It has been suggested that glutathione is the reducing agent for vanadium(V) in this system [2]. Also, glutathione has been shown to form complexes with vanadium(IV), as in rat adipocytes [3, 4]. In general, we believe that vanadium complexes with biological thiols are important in this system. We have chosen cysteine as a simple model thiol ligand since it is the middle amino acid in glutathione and is expected to show similar bonding.

Although the aqueous solution chemistry of many metal amino acid complexes has been reported, few compounds have been isolated. Only two papers have appeared concerning an isolated complex of vanadium with a cysteine–ester ligand. Sakurai and coworkers isolated a cysteine methyl ester complex of oxovanadium(IV) [5] (Fig. 1) and suggested that an analogous complex (not isolated) forms with the acid form of the ligand [6].

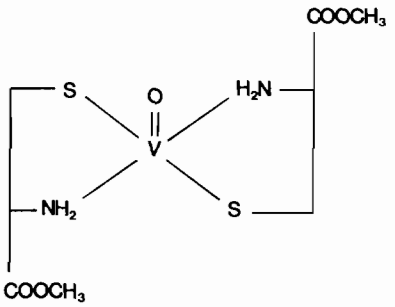


Fig. 1. The structure of the vanadium cysteine methyl ester complex as proposed by Sakurai and coworkers.

We report here (i) an improved synthesis of the cysteine methyl ester complex, (ii) the analogous preparation of a cysteine ethyl ester complex and (iii) the electrochemical characterization of these two complexes.

## Experimental

Cyclic voltammetric measurements were made with a Princeton Applied Research Model 173 three electrode potentiostat and a Model 175 Universal Programmer. The voltammograms were recorded on a Houston Instruments Model 2000 Omnigraphic X–Y recorder. Controlled-potential electrolysis was carried out with the above potentiostat and a Princeton Applied Research Model 179 digital coulometer.

The working electrode used for cyclic voltammetry was a Beckman platinum-inlay electrode. A platinum-mesh electrode was used for controlled-potential electrolysis. The auxiliary electrode was a small piece of platinum foil separated from the cell solution by a fine-porosity frit. The reference electrode consisted of a Ag/AgCl electrode in aqueous

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0020-1693/88/$3.50 

tetramethylammonium chloride (Aldrich) with the concentration adjusted to make the electrode potential 0.000 V *versus* SCE. The reference electrode junction was a platinum wire sealed in a pyrex tube. The electrode was positioned in a Luggin capillary in the cell assembly.

Some experiments were carried out in a Vacuum Atmospheres Company Model HE-43-2 glove box with an HE 493 Dri-Train, under a dry-nitrogen atmosphere. A simple electrochemical cell open to the box atmosphere was used. Other experiments were performed in an all glass electrochemical cell fabricated in our laboratory which was flushed with prepurified nitrogen before and during its use.

Spectrophotometric measurements were made on a Hewlett Packard Model 8450A UV–Vis spectrophotometer or on a Varian DMS 90 UV–Vis spectrophotometer and recorded on a Linear Model 1200 strip chart recorder. IR spectra were recorded on a Perkin-Elmer Model 683 spectrophotometer with data station.

*Reagents*

High purity dimethylsulfoxide (DMSO) (0.017% water), was obtained from Burdick and Jackson Laboratories and deoxygenated before use. Tetraethylammonium perchlorate (TEAP) was prepared from tetraethylammonium bromide (Aldrich) and perchloric acid as previously described [7] and used as the supporting electrolyte.

Tetraethylammonium hydroxide (TEAOH) was obtained from Aldrich Chemical Company as a 20 wt.% solution in water. Spectral quality methanol (0.05% water) and ethanol (0.05% water) was obtained from Fisher Scientific. Vanadium oxydichloride ($VOCl_2$) was obtained from ICN Biomedical and both L-cysteine methyl ester hydrochloride and L-cysteine ethyl ester hydrochloride were obtained from Sigma Chemical Company.

*Synthesis*

*Bis(methoxycysteinato)oxovanadium(IV), [VO(Cys–OCH$_3$)$_2$]*

In this procedure 1.64 g (0.02 mol, 100% excess) $CH_3COONa$ was dissolved in 50.0 ml dry methanol. This was added dropwise to a beaker containing 1.23 g (0.005 mol) $VOCl_2$ which was stirred and chilled yielding a white NaCl precipitate. This precipitate was filtered out using a medium porosity sintered glass funnel leaving a green solution of $VO(CH_3COO)_2$. The ligand solution was prepared by adding 4.30 g (0.025 mol, 5:1 mol ratio) L-cysteine methyl ester hydrochloride to 50.0 ml, pH 10.5 borate buffer which was degassed with prepurified nitrogen. The $VO(CH_3COO)_2$ solution was then added dropwise to the ligand solution while degassing continued forming a purple precipitate in a few minutes. The purple precipitate was filtered out using a medium porosity funnel and washed (in air) with cold methanol. The purple precipitate was briefly air dried, then put on a vacuum line overnight. *Anal.* Calc. for $C_8H_{16}N_2O_5S_2V$: C, 28.66; H, 4.81; S, 19.12. Found: C, 28.76; H, 4.83; S, 19.10%.

*Bis(ethoxycysteinato)oxovanadium(IV), VO(Cys–OCH$_2$CH$_3$)$_2$*

This complex was formed by a procedure analogous to that for the methoxycysteinato complex described above. The ligand solution was prepared by dissolving 4.64 g (0.025 mol, 5:1 mol ratio) cysteine ethyl ester hydrochloride in 60.0 ml, pH 10.5 borate buffer which was degassed with nitrogen. The $VO(CH_3COO)_2$ solution was added dropwise to the ligand solution, as described above, producing a green precipitate. The precipitate was washed with cold, dry ethanol and dried on a vacuum line overnight yielding a green powder. *Anal.* Calc. for $C_{10}H_{20}N_2O_5S_2V$: C, 33.06; H, 5.55; S, 17.65. Found: C, 33.15; H, 5.55; S, 17.62%.

## Results and Discussion

*Synthesis*

The synthetic pathways for the methyl and ethyl ester compounds are analogous and are outlined below:

$$2CH_3COONa + VOCl_2 \xrightarrow{CH_3OH} VO(CH_3COO)_2 + 2NaCl(ppt)$$

$$VO(CH_3COO)_2 + 2NH_2CH(COOR)CH_2SH \xrightarrow[N_2]{pH = 10.5,\ borate\ buffer} VO[NH_2CH(COOR)CH_2S]_2 + 2CH_3COOH$$

$R = -CH_3, -CH_2CH_3$

An earlier synthesis of the vanadium cysteine methyl ester complex by Sakurai and coworkers [5] produced a strong acid by-product, $H_2SO_4$, and took 5–6 h for full precipitation. Our procedure yields a weak acid by-product, $CH_3COOH$, and allows for almost immediate precipitation and isolation of the vanadium complex. When a $VO(CH_3COO)_2$ solution is added dropwise to the ligand solution precipitation is rapid because the reaction is driven by formation of the weak acid. The synthesis was carried out under inert conditions (prepurified, dry nitrogen) rather than air [5] to prevent the oxidation of oxovanadium(IV) to vanadate(V).

The vanadium cysteine ethyl ester complex has been synthesized and isolated for the first time. This procedure is much the same as that for the

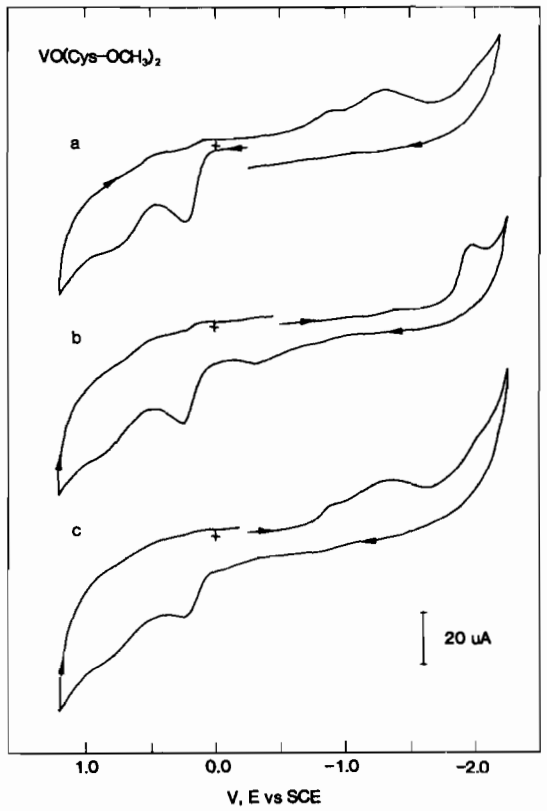


Fig. 2. Cyclic voltammograms of the vanadium cysteine methyl ester complex in 0.1 M TEAP/DMSO. Scan rate, 0.2 V/s. (a, b) 1.0 mM $VO(Cys–OCH_3)_2$, anodic and cathodic scans respectively; (c) the solution above after oxidation at 0.5 V *vs.* SCE.

methyl ester complex except for the initial ligand used and the washing of the final precipitate with dry ethanol rather than dry methanol. The vanadium cysteine ethyl ester precipitate was a green powder as compared to the microcrystalline purple methyl ester complex. Both of these compounds are relatively air stable but could not be oven dried or exposed to air for an extended period of time without oxidation occurring.

The IR spectrum of the vanadium cysteine methyl ester product exhibited two strong absorption bands. The band at 959 $cm^{-1}$ can be attributed to the vanadyl group (V=O) and corresponds well to the findings of Sakurai *et al.* [5] (956 $cm^{-1}$). The other absorption band is at 1734 $cm^{-1}$ and is indicative of an ester carbonyl (C=O) stretch.

*Electrochemistry*

An initial anodic scan of a 1 mM solution of $VO(Cys–OCH_3)_2$ in 0.1 M TEAP/DMSO gives the cyclic voltammogram shown in Fig. 2a. One large oxidation peak is observed at 0.24 V while a broad, less distinct oxidation occurs near 0.8 V. The subsequent cathodic part of the scan shows reduction

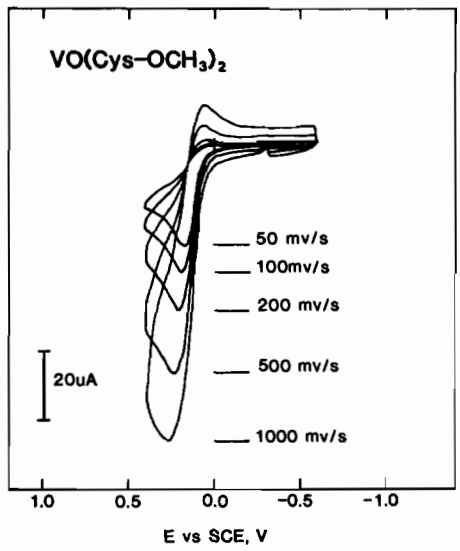


Fig. 3. Scan rate study of 1.0 mM $VO(Cys–OCH_3)_2$ in 0.1 M TEAP/DMSO.

peaks at −0.90 and −1.30 V. A slight shoulder is observed at −1.95 V. An initial cathodic scan of the same solution (Fig. 2b) shows only one reduction peak, at −1.95 V. A small oxidation wave is observed near −0.30 V in addition to the two other oxidations listed above. Since the reductions at −0.90 and −1.30 V are not present on the initial cathodic scan, they must be the result of the initial oxidation processes. Likewise, the oxidation at −0.30 V must be a result of the reduction at −1.95 V.

The oxidation process at 0.24 V was studied by controlled-potential coulometry at 0.5 V. The coulometric data indicates that the process is a one electron transfer but no significant color change of the solution was observed (see spectral changes in Fig. 6). The cyclic voltammogram of the resulting solution is shown in Fig. 2c. This cathodic scan shows that the species responsible for the reductions at −0.90 and −1.30 V are now present in the bulk solution.

To further characterize the relationship between the oxidation at 0.24 V and the reductions at −0.90 and −1.30 V, a scan rate study of the oxidation was carried out. Figure 3 shows cyclic voltammograms of a 1 mM solution of $VO(Cys–OCH_3)_2$ scanned aniodically past the 0.24 V oxidation at five different scan rates. As the scan rate increases the ratio of the cathodic peak current to the anodic peak current ($i_{pc}/i_{pa}$) increases and approaches unity. Although the ratio never reaches 1.0, it is clear that this represents a reversible process followed by a rapid chemical reaction (an EC mechanism). Thus, vanadium(IV) is reversibly oxidized to vanadium(V), but the initial product is not stable. The final vanadium(V) products are reduced at −0.90 and −1.30 V.

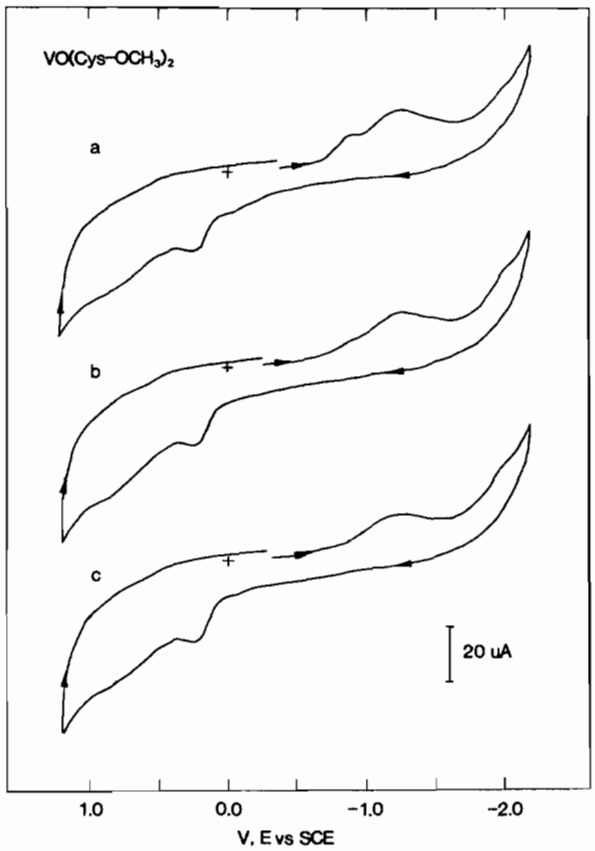


Fig. 4. Cyclic voltammograms of $VO(Cys–OCH_3)_2$ in 0.1 M TEAP/DMSO. Scan rate 0.2 V/s. (a) 1.0 mM $VO(Cys–OCH_3)_2$ after oxidation at 0.5 V *vs.* SCE; (b) solution (a) treated with 5.0 μl of 1.4 M TEAOH (0.22 equivalent of base); (c) solution (b) with second addition of 5.0 μl of 1.4 M TEAOH (0.44 equivalent of base).

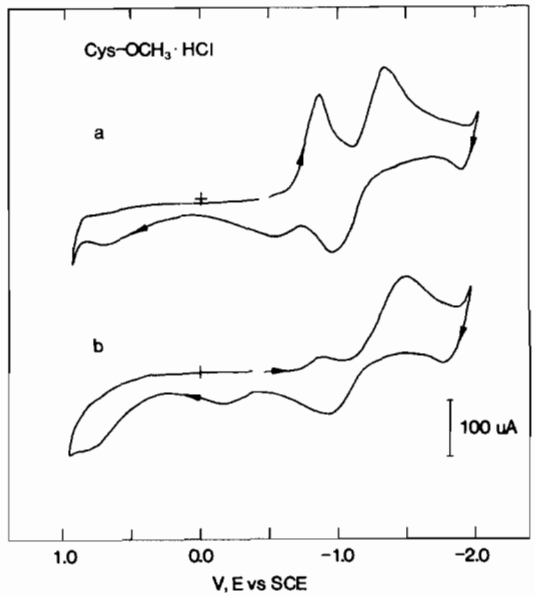


Fig. 5. Cyclic voltammograms in 0.1 M TEAP/DMSO. Scan rate 0.2 V/s. (a) 5.0 mM $Cys–OCH_3$·HCl; (b) solution (a) treated with 108 μl TEAOH (1 equivalent of base).

These vanadium(V) products were studied by adding TEAOH to a previously oxidized solution of $VO(Cys–OCH_3)_2$ (Fig. 4a). A 5 μl aliquot of TEAOH (1.40 M) (~0.22 equivalent of base) was added to the cell solution and resulted in almost completely removing the reduction peak at −0.90 V (Fig. 4b). Another 5 μl aliquot was added (Fig. 4c) and the peak at −0.90 V was completely removed while the reduction peak at −1.30 V was slightly diminished. This evidence suggests that the reduction peak at −0.90 V is due to protons.

The voltammogram of the vanadium(V) products was also compared to that of the ligand. An initial cathodic scan of a 5 mM solution of cysteine methyl ester, HCl adduct, gives the voltammogram shown in Fig. 5a. Two large reduction peaks are observed at −0.85 and −1.37 V. The anodic portion of this scan produces two oxidation peaks at −0.95 and −0.55 V with a small oxidation wave at 0.70 V. The result of adding 108 μl of 1.4 M TEAOH (1 equivalent of base) to this solution is shown in Fig. 5b. The reduction peak previously seen at −0.85 V is essentially removed and a slight shift of the other reduction peak to −1.50 V is observed. This data shows that the reduction peak at −0.85 V is due to protons, in this case from the HCl bound to the ligand as an adduct. A similar peak was observed in our earlier experiment involving TEAOH additions (Fig. 4) and in separate experiments where HCl was added to a solution of just TEAP/DMSO.

Comparing Figs. 4 and 5 leads to two conclusions. First, oxidation of the vanadium(IV) complex releases protons, apparently from partial breakdown of the ligand itself. (Based on elemental analysis there is no HCl moiety associated with the isolated $VO(Cys–OCH_3)_2$ compound.) Second, the reduction peak at −1.30 V (Fig. 4) is most probably due to the dominant, stable vanadium(V) species formed following oxidation. This is because this reduction peak does not coincide with reduction of the ligand itself at −1.50 V (Fig. 5b).

*Spectroscopy*

The ultraviolet and visible spectra of a 1 mM solution of $VO(Cys–OCH_3)_2$ is shown in Fig. 6a. A broad absorption peak is observed at 517 nm which overlaps a second broad peak whose absorbance maximum is near 680 nm. This agrees with the data of Sakurai and coworkers [5], who reported absorbance maxima at 530 and 678 nm. The solution appeared light purple in color. This solution was oxidized holding the potential at 0.5 V and produced the spectrum shown in Fig. 6b. This spectrum shows a level absorption through the visible range and little color change was observed in the solution. The coulometric

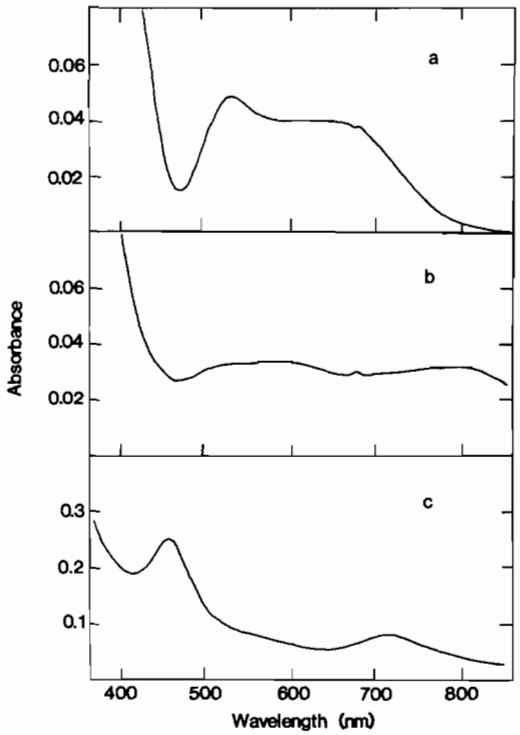


Fig. 6. UV–Vis spectra in 0.1 M TEAP/DMSO using a 1.0 cn cell. (a) 1.0 mM $VO(Cys–OCH_3)_2$; (b) solution (a) after oxidation at 0.5 V *vs.* SCE; (c) solution (b) after reduction at −1.9 V *vs.* SCE.

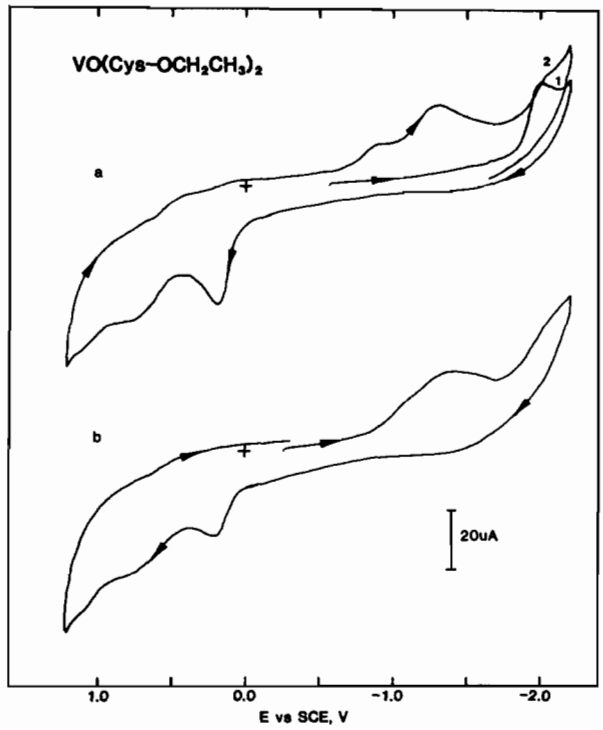


Fig. 7. Cyclic voltammograms of the vanadium cysteine ethyl ester complex in 0.1 M TEAP/DMSO. Scan rate 0.2 V/s. (a) 1.0 mM $VO(Cys–OCH_2CH_3)_2$; (b) solution (a) after oxidation at 0.6 V *vs.* SCE.

data for four replicates gave an average of 1.1 electrons transferred per mole of compound. The subsequent reduction of this solution was carried out holding the potential at −1.9 V and produced the spectrum shown in Fig. 6c. It shows much larger absorbances with peaks at 453 and 717 nm and the solution color was yellow–brown. The coulometric data for the reduction at −1.9 V (2 replicates) was 1.1 electrons/mol. The anodic and cathodic cyclic voltammograms of this solution were virtually identical to those of the original solution shown in Fig. 2a and b, respectively. Thus, the cyclic voltammograms and the coulometric data indicate that $VO(Cys–OCH_3)_2$ can cycle between the IV and V oxidation states of vanadium. The form of the stable vanadium(V) species is not known, and the reduction of this species leads to at least one product in addition to $VO(Cys–OCH_3)_2$. This second product, although present in very low concentration based on the voltammetric data, dominates the spectra due to its high molar absorptivity.

### *$VO(Cys–OCH_2CH_3)_2$*

Only a survey of the electrochemistry of $VO(Cys–OCH_2CH_3)_2$ was made. The initial cathodic cyclic voltammogram of a 1 mM solution of $VO(Cys–OCH_2CH_3)_2$ is shown in Fig. 7a. A reduction peak at −2.0 V is observed which is similar to that seen in Fig. 2b for the methyl ester compound. The oxidation peaks and the reduction peaks of the second cycle are also very similar to those seen in the voltammogram of the vanadium methyl ester complex (Fig. 2a). The subsequent oxidation of this solution, holding the potential at 0.60 V, produced the cyclic voltammogram shown in Fig. 7b. There was no color change observed in the solution and the coulometric data resulted in a one electron transfer. The voltammogram shows the reduction peaks present on the first cycle. An initial anodic scan shows that the oxidation peak at 0.20 V is gone. The products appear to be very similar to the products observed after the oxidation of the vanadium methyl ester complex (Fig. 2c). Thus, the electrochemistry of these two complexes appears to be virtually identical.

## Conclusion

The interaction of vanadium with the sodium pump may involve the cycling of vanadium between the IV and V oxidation states. The cysteine ester complexes of vanadium studied here undergo similar electrochemistry. $VO(Cys–OCH_3)_2$ is reversibly oxidized, followed by a rapid chemical reaction (an EC mechanism). Thus, under certain conditions vanadium(V) may be stabilized in the presence of

a reducing ligand. This final vanadium(V) product can be reduced back to the initial vanadium(IV) species. We believe that in this way $VO(Cys–OCH_3)_2$ and its oxidation product serve as models for vanadium in the sodium pump reaction. Studies with other model thiol ligands and vanadium are underway.

## Acknowledgements

This work was supported by the National Institutes of Health under Grant No. 1-R15-HL37252-01 and the Faculty Research Committee of Miami University.

## References

1 L. C. Cantley, Jr., L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. Biol. Chem., 252*, 7421 (1977).
2 I. G. Macara, K. Kustin and L. C. Cantley, Jr., *Biochim. Biophys. Acta, 629*, 95 (1980).
3 H. Degani, M. Gochin, S. J. D. Karlish and Y. Shechter, *Biochemistry, 20*, 5795 (1981).
4 M. Delfini, E. Gaggelli, A. Lepri and G. Valensin, *Inorg. Chim. Acta, 107*, 87 (1985).
5 H. Sakurai, Y. Hamada, S. Shimomura, S. Yamashita and K. Ishizu, *Inorg. Chim. Acta, 46*, L119 (1980).
6 H. Sakurai, S. Shimomura and K. Ishizu, *Inorg. Chim. Acta, 55*, L67 (1981).
7 T. L. Riechel and D. T. Sawyer, *Inorg. Chem., 14*, 1869 (1975).