Electrochemical Studies of Vanadium–Riboflavin Interactions in Dimethyl Sulfoxide

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The electrochemistry and complexation of VO-$(ClO_4)_2$, VCl_3, and VOF_3 with singly-reduced riboflavin have been studied by cyclic voltammetry and controlled-potential coulometry in dimethyl sulfoxide at a platinum electrode. The UV, visible, and near-IR spectra of solutions of these species have also been examined. Vanadium(IV) forms the complex $VO(Rib)_2$ (Rib^- = singly reduced riboflavin) which is reduced to $VO(Rib)_2^{2-}$ at −0.74 V vs. SCE. $VO(Rib)_2$ is oxidized at −0.15 V producing free VO^{2+} and neutral riboflavin. Reduced riboflavin forms a complex with VCl_3 having an oxidation potential of −0.12 V, which is similar to that for the vanadium(IV) complex. A mixture of reduced riboflavin and VOF_3 results in the spontaneous reduction of the metal and oxidation of the riboflavin anion to neutral riboflavin.

Introduction

Although it has been known for a decade that vanadium is an essential dietary trace metal in higher life forms [1] little is known about the specific sites of vanadium interaction in biological systems. For example, a vanadium deficient diet results in growth retardation of rats and chickens [2, 3] but the specific molecular effects are unknown. High levels of vanadium are toxic, yet low level diet supplements are beneficial as in lowering cholesterol levels [4, 5]. In two cases, organisms are known to selectively accumulate high concentrations of vanadium. The metal exists as a vanadium(III) hexaaquo complex in tunicates [6] but its function in the blood stream of these animals is unknown. Similarly, the function of the vanadium(IV) complex, Amavadine, in the mushroom *Amanita muscaria* [7] is not clear. Interest in biological vanadium has been renewed recently by the discovery that vanadium(V) is a naturally occuring inhibitor of (Na^+, K^+)-ATPase [8, 9]. Vanadate functions from the cytoplasmic side of the plasma membrane [10] but when reduced to vanadium(IV) no longer inhibits the enzyme.

These examples suggest, as pointed out by Macara [11], that the (III), (IV), and (V) oxidation states of vanadium are the most important in biological systems. Furthermore, the changes in oxidation state observed in some systems suggest a redox function for vanadium. Based on similar inorganic chemistry, the bioinorganic chemistry of vanadium may be similar to that of molybdenum. Molybdenum occurs naturally in several redox enzymes including nitrate reductase, xanthine oxidase, and aldehyde oxidase. In each of these cases FAD (flavin adenine dinucleotide) is a necessary cofactor [12] and may be directly associated with the metal. The electrochemistry of molybdenum complexes with riboflavin and other model systems has been studied [13] while vanadium has been investigated only with simpler model ligands [14].

In this paper we present a study of the complexation and electrochemistry of vanadium(III), (IV), and (V) with singly reduced riboflavin. As there is increasing evidence that the active sites of many metalloenzymes lie in hydrophobic regions of the proteins [15] we have carried out our study in a non-aqueous solvent.

Experimental

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

A Beckman platinum-inlay electrode served as the working electrode for cyclic voltammetry, while a platinum mesh electrode was used for electrolysis. The auxiliary electrode, fashioned from a small piece of platinum foil, was separated from the cell

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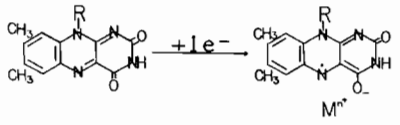

Fig. 1. The structures of neutral riboflavin and riboflavin radical anion with a complexing metal ion.

solution by a fine porosity frit. The reference electrode consisted of a Ag/AgCl electrode in aqueous tetramethylammonium chloride (Aldrich) with the concentration adjusted so that a potential of 0.000 V *vs.* SCE was obtained. A small soft-glass, cracked-bead sealed into a Pyrex tube served as the junction for the reference electrode [16] and this electrode was positioned in a luggin capillary in the cell assembly. The cell consisted of a 100 ml electrolytic beaker with a circular rubber cap. The cap had holes drilled through it for placement of the electrodes and also for the addition of reagents and the removal of sample aliquots.

The electrochemical experiments were performed in a Vacuum Atmospheres Co. Model HE 43-2 glove box with an HE 493 Dri-train, under a nitrogen atmosphere. Spectrophotometric measurements were made on a Cary Model 14 UV-Visible spectrometer using air-tight cells.

Reagents

High purity dimethyl sulfoxide (DMSO) was obtained from Burdick and Jackson Laboratories with lot analyses for water ranging from 0.009% to 0.019%. To further minimize water content, activated molecular sieve of 3 Å porosity (Fisher) was added to the pint bottles of solvent.

Riboflavin (98%, Aldrich) was recrystallized three times from 1 *M* acetic acid before use. Vanadyl perchlorate, $VO(ClO_4)_2 \cdot 5DMSO$, was prepared from $VOSO_4$ (Fisher) and barium perchlorate by the method of Michlmayr and Gutmann [17]. Vanadyl chloride was obtained from ICN Pharmaceuticals. Tetraethylammonium perchlorate (TEAP) was prepared by the stoichiometric addition of perchloric acid to tetraethylammonium bromide (Aldrich) The white crystalline product was collected, washed and recrystallized from water. The compounds VOF_3 and VCl_3 were obtained from Alfa Products.

Results and Discussion

Vanadium IV

Although neutral riboflavin does not bond to metals other than strong electron back-donors, *i.e.*, Ag^+, singly-reduced riboflavin has been shown to have strong affinity for transition metals with available d-orbitals [18]. Spectral and electrochemical studies have been reported for several such flavin-

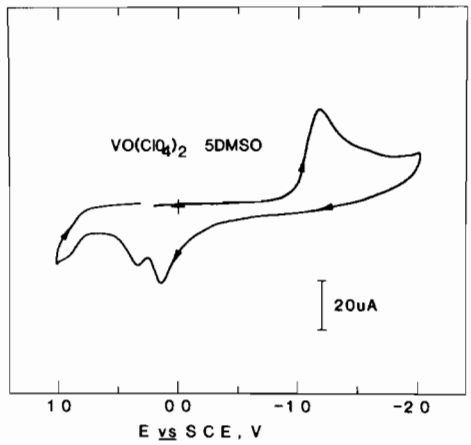

Fig. 2. Cyclic voltammogram in 0.1 *M* TEAP–DMSO solution of 1 m*M* $VO(ClO_4)_2 \cdot 5DMSO$. Scan rate 0.1 V/sec.

metal model compounds [13, 19, 20]. In each case, reduced riboflavin is believed to form a bidentate, (−1) charged ligand, bonding to the metal through oxygen and nitrogen atoms as shown in Fig. 1.

Sawyer and McCreery [20] have reported the cyclic voltammograms for neutral and singly-reduced riboflavin. The voltammogram for neutral riboflavin in DMSO is characterized by a quasi-reversible one electron couple with reduction and oxidation peaks at −0.82 and −0.56 V *vs.* SCE, respectively. Two additional broad reduction peaks at −1.30 and −1.56 V represent irreversible processes. Controlled-potential electrolysis at −0.95 V results in production of singly-reduced riboflavin radical anion (Rib^-). Indicative of reduced riboflavin is an initial anodic peak at −0.56 V. This species is stable for several hours under an inert atmosphere.

The cyclic voltammogram of 1.0 m*M* $VO(ClO_4)_2$ in DMSO is shown in Fig. 2. An initial cathodic scan shows a single reduction peak at −1.18 V and subsequent oxidation peaks at 0.14 and 0.35 V *vs.* SCE. Comparison of this voltammogram to that for $VOCl_2$* suggests that the same species exists in both solutions, probably as solvated VO^{2+}. This species is stable in solution.

A mole ratio study was undertaken by mixing aliquots of a pre-reduced riboflavin solution with a standard solution of $VO(ClO_4)_2$. The cyclic voltammograms for 0.5 1 and 1 1 mole ratios of Rib^- (riboflavin radical anion) to VO^{2+} show a new oxidation peak at −0.15 V *vs.* SCE. Also, a new reduction peak is observed at −0.74 V. The vanadium reduction peak at −1.18 V is present and indicates that an excess of metal exists at these low ligand to metal ratios.

*No further work was conducted with $VOCl_2$ due to the difficulty of preparing standard solutions

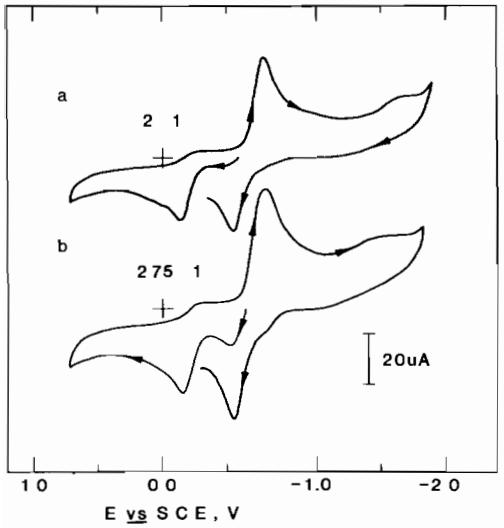

Fig. 3. Cyclic voltammograms in 0.1 *M* TEAP–DMSO solution of Rib^- VO^{2+} mole ratios of (a) 2·1 and (b) 2.75:1. Scan rate 0.2 V/sec.

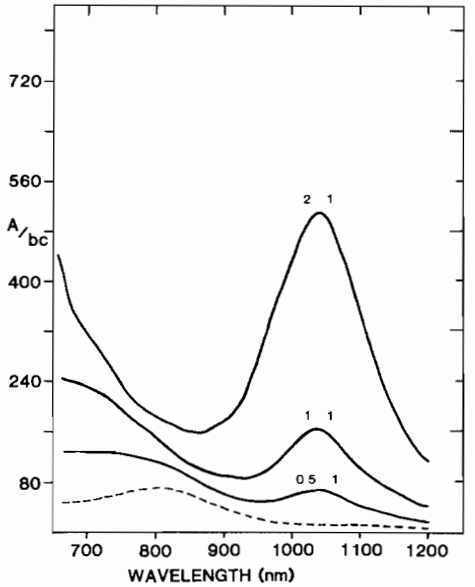

Fig. 4. Near-IR absorption spectra in DMSO of various Rib^-: VO^{2+} mole ratios. The dashed line represents the near-IR spectrum of VO^{2+} alone.

However, as the mole ratio is increased, the height of the vanadium reduction peak decreases while the height of the new oxidation peak at –0.15 V increases. The cyclic voltammogram of a 2:1 mole ratio solution (Fig. 3a) shows that the reduction peak for vanadium is no longer present indicating that all of the metal has reacted or been complexed. An increase in the mole ratio to 2.75:1 (Fig. 3b) results in an oxidation peak at –0.56 V which corresponds to the oxidation of reduced riboflavin. This indicates that free reduced riboflavin is present and that the stoichiometric point has been surpassed. Thus, VO^{2+} and Rib^- appear to form a complex oxidizable at –0.15 V and reducible at –0.74 V.

Absorption spectroscopy was performed on neutral riboflavin, reduced riboflavin, and the various mole ratio solutions. Characteristic of neutral riboflavin in DMSO are two absorption bands at 345 and 450 nm, the latter being stronger. The spectrum of reduced riboflavin shows a maximum absorbance at 375 nm with shoulders at 400, 450, and 475 nm. While the two riboflavin species give distinctly different UV-Visible spectra, the 2:1 mole ratio solution absorbs at 345 nm and 450 nm as does neutral riboflavin. The absence of a unique UV-Visible absorption band for the mole ratio solutions precludes the use of this spectral region for quantitative identification of a complex.

In a study of flavin-transition metal interactions in nonaqueous solvents, Muller and coworkers [21] reported that an absorption band characteristic of flavosemiquinone metal chelates is present in the near-IR region. Spectra of the near-IR region of our riboflavin-vanadium mixtures are shown in Fig. 4.

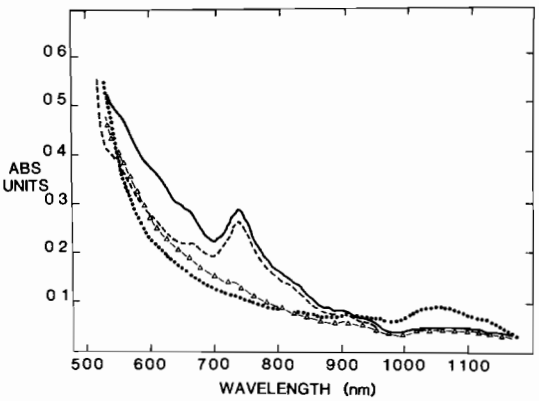

Fig. 5. Near-IR absorption spectra in DMSO of high mole ratios of $Rib^-.VO^{2+}$; —— 8:1, – – – 6:1, –△–△– 5 1, ... 4·1.

With no reduced riboflavin present, an absorption band belonging to vanadyl ion is evident at 840 nm. Addition of reduced riboflavin (which does not absorb in the near-IR) to the metal solution, results in a new absorption band at 1040 nm. The intensity of this band increases with the increasing mole ratio of reduced riboflavin to vanadium and reaches a maximum at about 2.5:1.

In a typical plot of absorbance *vs.* mole ratio, if the ligand itself does not absorb at the wavelength of interest, then beyond the stoichiometric point the absorbance would be expected to level off to a constant value. Such a plot of the riboflavin-vanadium data at 1040 nm shows, however, a dramatic decrease in slope at ratios above 2.5:1. Figure 5

shows the visible and near-IR spectra of several high mole ratio solutions It is clear that at high mole ratios the 1040 nm absorbance band decreases in intensity, but that simultaneously a new band appears at 740 nm

An explanation for these spectral changes may be based on the chemistry of riboflavin radical anion in nonaqueous solvents. Ehrenberg and coworkers [22] described the formation of a biflavin-biradical by adding excess solid t-BuOK to a solution of neutral flavin in dimethyl formamide. Such dimerization is characteristic of activated methyl groups on electron-deficient aromatic compounds. This process is thought to be initiated by internal disproportionation of two flavin molecules. The newly formed biflavin-biradical reacts with neutral riboflavin thereby resulting in a mixture of reduced riboflavin and biflavin-radical. The absorption spectra of the biflavin-radical dimer exhibits a broad band (quintet) centered at 730 nm in dimethyl formamide.

Although the mechanism is probably different, the similar location and band shape of the 740 nm absorbance observed in our work, suggests that at high mole ratios of riboflavin radical anion to vanadium, the biflavin-radical is formed in preference to the riboflavin vanadium complex.*

As shown in Fig. 3, the mixture of vanadium and riboflavin radical anion also gives rise to a reduction peak at –0.74 V. This peak appears to be distinct from the riboflavin reduction peak at –0.82 V, since in some cyclic voltammograms both a peak at –0.74 V and a shoulder at –0.82 V are present. The close proximity and similar shape of these peaks is similar to that for riboflavin and a riboflavin-molybdenum complex reported by Sawyer and coworkers [13]. Thus, the reduction peak at –0.74 V probably corresponds to the $VO(Rib)_2$ complex.

Electrolytic reduction at –0.80 V of this 2·1 complex is accompanied by a color change of the reddish-orange solution to dark brown. The coulometric data indicate a two electron process per vanadium and the absorption band at 1040 nm is enhanced three to four fold in intensity. Although this result was unexpected, a similar enhancement was reported by Sawyer and coworkers [13]. Following the reduction of a molybdenum–riboflavin complex, they also observed an enhancement of a band in the near-IR region. They concluded that the data was indicative of greater electron density on the metal or that following reduction, the complex displays greater assymmetry. In the vanadium case, the identity of the reduction product is not clear, but the coulometric data does suggest that the riboflavin ligands may be doubly (fully) reduced at this point.

Further investigation of the 2.1 complex involved controlled potential oxidation at 0.05 V. The coulometric data indicate a two electron per vanadium process. The cyclic voltammogram of the oxidized solution (which is bright yellow) (Fig. 6) is identical to that obtained by adding $VO(ClO_4)_2$ directly to a solution of neutral riboflavin. The reduction peak at –0.82 V has been restored. The absorption band at 1040 nm is absent from the oxidized solution spectra, while the VO^{2+} band at 840 nm is observed. The UV–Vis spectra correspond to that of neutral riboflavin.

Table I summarizes the chemical and electrochemical reactions of riboflavin and vanadium(IV). Neutral riboflavin is reduced by one electron at –0.82 V and re-oxidized at –0.56 V (reactions 1 and 2). The reductions of $VO(ClO_4)_2$ and $VOCl_2$ occur at about –1.20 V and each require one electron. As indicated by reactions 3 and 4, the same vanadium species are produced (giving identical cyclic voltammograms)

TABLE I. Reactions of Riboflavin and VO^{2+} in DMSO.

Reaction	Potential, V *vs.* SCE	
	Epc	Epa
(1) $Rib + e^- \rightarrow Rib^-$	–0.82	
(2) $Rib^- \rightarrow Rib + e^-$		–0.56
(3) $VO(ClO_4)_2 + e^- \rightarrow V^{III}$	–1 18	
(4) $VOCl_2 + e^- \rightarrow V^{III}$	–1.20	
(5) $V^{III} \rightarrow VO^{2+} + e^-$		+0.14, +0 35
(6) $2Rib^- \rightleftharpoons Rib + Rib^{2-}$		
(7) $VO^{2+} + 2Rib^- \rightleftharpoons VO(Rib)_2$		
(8) $VO(Rib)_2 + 2e^- \rightarrow VO(Rib)_2^{2-}$	–0.74	
(9) $VO(Rib)_2 \rightarrow VO^{2+} + 2Rib + 2e^-$		–0.15

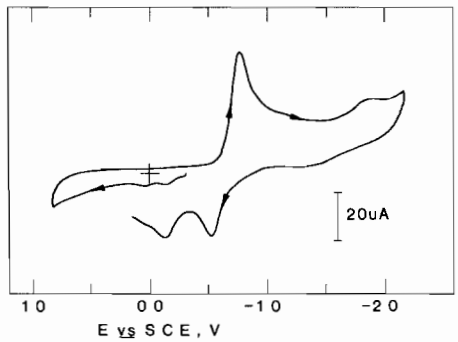

Fig. 6. Cyclic voltammogram in 0.1 *M* TEAP–DMSO solution of the product formed after oxidation at 0.05 V of a 2 1 $Rib^-.VO^{2+}$ solution

*Another possible explanation is that a complex of a higher ligand to metal ratio is formed. Based on the large size of the riboflavin ligand, such a complex is unlikely due to steric hindrance

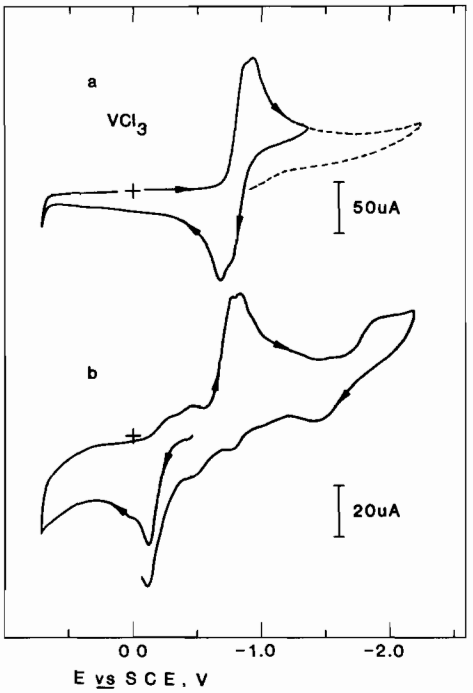

Fig 7. Cyclic voltammograms in 0.1 *M* TEAP–DMSO solution of (a) VCl_3 and (b) approximately 2.1 mole ratio of Rib^-:VCl_3. Scan rate 0.2 V/sec.

and two oxidation processes lead back to VO^{2+} (reaction 5).

The chemistry of flavin compounds in aqueous solution is complicated by several oxidation states and various protonated forms [23]. The use of a non-aqueous medium minimizes the number of species produced in electrochemical and complexation reactions. The most important flavin moieties in nonaqueous solvents are neutral riboflavin, singly reduced riboflavin and fully reduced riboflavin. The appearance of a shoulder (–0.82 V) next to the complex reduction peak (–0.74 V) indicates that a small amount of neutral riboflavin is present. This can be accounted for by the disproportionation of riboflavin radical anion. This process (reaction 6) is well documented [18] but occurs to only a slight extent in aprotic solvents.

Based on the electrochemical and spectroscopic data the most probable formula for the vanadium–riboflavin complex is $VO(Rib)_2$ (reaction 7). This complex is reduced by two electrons at –0.74 V. Since vanadyl ion requires a potential of about –1.18 V for reduction, most probably the reduction produces fully reduced riboflavin (Rib^{2-}) which may remain coordinated to vanadium as $VO(Rib)_2^{2-}$ (reaction 8). Although the location of the two added electrons is uncertain, these results parallel those reported by Sawyer and coworkers for the molybdenum–riboflavin complex [13].

The $VO(Rib)_2$ complex is oxidized at –0.15 V by two electrons as indicated in reaction 9. Cyclic voltammograms and spectra of the resulting solution indicate that the complex dissociates to neutral riboflavin and vanadyl ion.

Vanadium III

Since vanadium(III) has also been shown to be important in biological systems, the interactions of VCl_3 with reduced riboflavin were studied. Figure 7a shows a typical cyclic voltammogram of VCl_3 in DMSO. The overlapping reduction peaks at –0.86 and –0.94 V are close to the polarographic half wave potentials (–0.74 and –0.84 V) reported by Michlmayr and Gutmann [17]. The corresponding oxidation peaks at –0.70 and –0.76 V suggest two essentially reversible couples. Based on coulometric results, we agree with Michlmayr and Gutmann that these waves represent two pathways for the one-electron reduction of V(III) to V(II).

Scans to more negative potentials (dashed line in Fig. 7a) show no other reductions, but the reverse scan indicates that the initial products are not stable. In such voltammograms the oxidation peaks near –0.8 V are replaced by a broad oxidation peak at more positive potentials. The potential of this peak shifts more positively as the initial scan is carried out to more negative potentials.

Since the vanadium(III) species is stable in solution, additions of reduced riboflavin were made. The cyclic voltammogram of a 2:1, riboflavin to vanadium mixture is shown in Fig. 7b. Although no oxidation peaks appear on an initial positive scan of the VCl_3 solution, a large oxidation peak is present at –0.12 V for the mixture. This peak is similar to that for the $VO(Rib)_2$ complex; furthermore, a strong absorbance occurs at 1040 nm in the spectrum of this solution. The UV portion of the spectrum is different from that of $VO(Rib)_2$.

Two interpretations are possible. Vanadium may have been oxidized resulting in formation of $VO(Rib)_2$, but there is no obvious oxidizing agent present. More probably, complex formation occurs with vanadium(III) and the near IR spectra are similar since the absorbance wavelength is determined predominantly by the ligand, rather than by the metal [21]. Furthermore, an initial negative scan of this solution indicates that some free vanadium(III) remains. No reduction peak for vanadium(IV) is observed.

Vanadium V

The final aspect of this study involves the investigation of vanadium(V) and reduced riboflavin. Several compounds were screened as sources of vanadium(V). Most were quite unstable in DMSO except

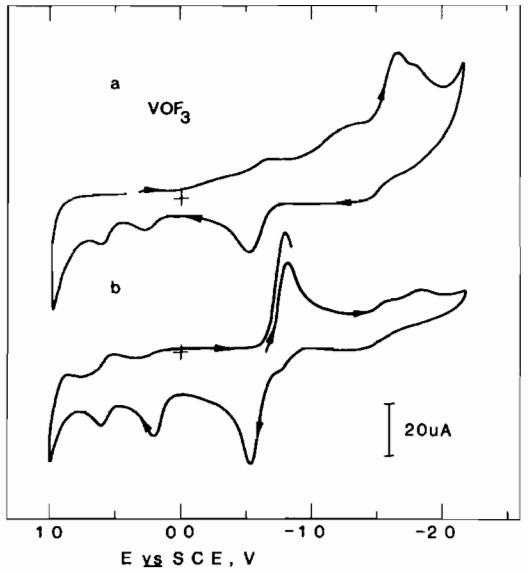

Fig. 8. Cyclic voltammograms in 0.1 *M* TEAP–DMSO solution of (a) 2 m*M* VOF_3 and (b) 2:1 mole ratio of Rib^{-}·VOF_3 Scan rate 0.2 V/sec.

for VOF_3*. The cyclic voltammogram of a 2 m*M* solution of VOF_3 in DMSO is shown in Fig. 8a. The voltammogram is complex, but remains unchanged for several hours. The most prominent reduction peak appears at about −1.65 V but no oxidation peaks are present on an initial positive scan.

When a solution of a 2:1 mole ratio of reduced riboflavin to vanadium(V) is prepared, the resulting cyclic voltammogram is as shown in Fig. 8b. Of major importance is a redox couple with peak potentials at −0.82 V (reduction) and −0.56 V (oxidation). Furthermore, the vanadium(V) reduction peak is essentiallly gone and there are no new peaks which might signify complex formation. Spectra of this solution are similar to those for neutral riboflavin and do not show a near IR absorption. These results indicate that VOF_3 is reduced, while riboflavin radical anion is oxidized. There is no evidence for complexation.

Conclusions

The electrochemistry of these vanadium-riboflavin systems is dominated by the electrochemical, disproportionation, and dimerization reactions of riboflavin. There is strong evidence for a complex of singly reduced riboflavin and VO^{2+} in a 2 1 ratio. Complex formation with vanadium(III) also appears likely, but vanadium(V) solutions spontaneously oxidize the ligand. Thus, if vanadium functions in biological systems with flavin cofactors, the (III) and (IV) oxidation states are most probable.

*The vanadium(V) compounds $VOCl_3$, $VO(OCH_3)_3$, $VO(OC_2H_5)_3$ and $VO(OC_3H_7)_3$ were all unstable in DMSO, perhaps reacting with residual water in the solvent

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