A Mechanistic Study of the Reduction of Cystine by Vanadium(II) in the pH Range from 7.5 to 12

G. KALATZIS, D. KATAKIS, E. VRACHNOU and J. KONSTANTATOS*

Research Center Demokritos, Aghia Paraskevi Attikis, Athens, and Inorganic Chemistry Laboratory, Athens 106 80, Greece

(Received July 6, 1987)

Abstract

The reduction of cystine by aqueous vanadium(II) was investigated in the pH range from 7.5 to 12. The product ratio $[V^{IV}]/[V^{III}]$ reaches a maximum at pH *ca.* 9 and depends linearly on the excess concentration of cystine. It is also affected by cysteine, but not by initially added vanadium(III). The rate of the oxidation is first order in total vanadium(II) and also depends on cystine and on added cysteine or mercaptoacetic acid. The data are consistent with a mechanism involving two parallel paths leading to vanadium(III) and vanadium(IV), with precursors differing by one cystine ligand. In either case, the net result is scission of the S–S bond.

Introduction

In a recent paper [1] we investigated the mechanism of electron transfer to a sulfur ligand, *i.e.* from vanadium(II) to mercaptoacetic acid at pH values of around neutral.

$$2V^{II} + \begin{matrix}2\,CH_2COOH\\ |\\ SH\end{matrix} + 2H^+ \longrightarrow 2V^{III} + \begin{matrix}CH_2COOH\\ |\\ CH_2COOH\end{matrix} + 2H_2S$$

The reaction involves the reductive cleavage of carbon–sulfur bonds and the formation of carbon–carbon bonds.

Here we report on the reaction of vanadium(II) with another sulfur ligand, cystine [abbreviated RSSR, R = $-CH_2CH(NH_2)COOH$]. In this case it is the S–S bond that breaks and the product is a mixture of vanadium(III), vanadium(IV) and cysteine [abbreviated RSH]

$$V^{II} + RSSR \xrightarrow{H_2O} V^{IV} + 2RSH$$

$$2V^{II} + RSSR \xrightarrow{H_2O} 2V^{III} + 2RSH$$

The reaction was investigated using excess cystine, in the pH range from 7.5 to 12. At pH < 2 redox reactions are slow; at 2 < pH < 7 there are solubility problems.

The disulfide bond in proteins is reduced by many organic or inorganic reductants [2]. An early investigation of the reduction by chromium(II) and vanadium(II) in 0.2–1.0 M hydrochloric acid solutions was made by Preisler [3], as suggested by Conant [4]. Recently, the reduction of organic disulfides by chromium(II) [5], cobalt(II) [6] and iron(II) [7] has been used for the synthesis of the corresponding metal(III) thiol complexes.

In the aprotic reductive cleavage of the disulfide bonds in bovine serum albumin and lysozyme by hydride ions, it has been suggested [8] that the first step is formally a two-electron transfer

$$PSSP + H^- \longrightarrow PSH + PS^-$$

Disulfides are also reduced with lithium aluminum hydride [9] and in liquid ammonia by metallic sodium [10]. With Hg_2^+ the disulfide bond is simultaneously reduced and mercurated [11]. The polarographic reduction of cystine also involves reductive cleavage by metallic mercury [12].

Experimental

Aqueous vanadium(II) solutions were prepared electrolytically [13]. Cystine (Merck) was used without further purification. Air-free reaction mixtures were prepared by adding the acid (HCl) solution of vanadium(II) to the alkaline solution of cystine. The final mixtures contained *ca.* 10^{-2} M chloride ions.

The pH was measured with a Metrohm A.G. potentiograph after the completion of the reaction. Spectra were recorded with a Cary 14 spectrometer. Fast kinetics were followed with an Applied Physics stopped-flow spectrophotometer, at various wavelengths above 340 nm, where both V(IV) and V(III), in the presence of cystine, have considerable absorption (Fig. 1).

Vanadium(III) and vanadium(IV) were determined after acidifying ($[H^+] = 0.3$ M) spectrophotometri-

*Author to whom correspondence should be addressed.

0020-1693/88/$3.50

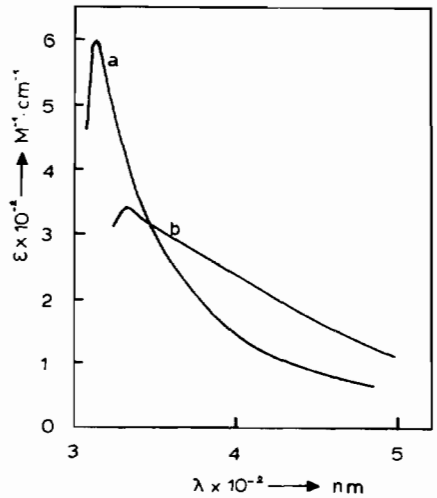
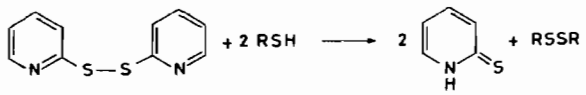


Fig. 1. Spectra of (a) VO^{2+} and (b) V^{3+} in the presence of 0.014 M cystine at 23 °C and pH 9.

cally. Vanadium(III) absorbs at 400 and 580 nm with absorptivities 8.12 and 5.65 M^{-1} cm^{-1}, respectively, and vanadium(IV) at 760 nm with an absorptivity of 16.1 M^{-1} cm^{-1}. Cysteine was also determined spectrophotometrically with 2,2′-dithiopyridine [14].



The absorptivities of 2,2′-dithiopyridone are $\epsilon_{233} = 1.39 \times 10^4$, and $\epsilon_{281} = 9.73 \times 10^3$, and those of 2-thiopyridone $\epsilon_{271} = 1.04 \times 10^4$ and $\epsilon_{343} = 7.06 \times 10^3$ M^{-1} cm^{-1}.

In a typical stoichiometric experiment the initial amount of V(II) was 0.316 mmol and after the reaction the amount of cysteine was found equal to 0.38 mmol, of V(III) equal to 0.221 mmol and of V(IV) equal to 0.074 mmol, *i.e.* the sum of the equivalents of the oxidized products equals 0.369 mmol. The agreement with the overall reactions given in the Introduction is considered satisfactory.

Results and Discussion

The development of the transmittance indicates that there are four stages in the reaction (Fig. 2). The first corresponds to a non-measurable (with our instrument) initial jump, attributed to complexation. The second is the redox step investigated. The third, which involves a slight increase in transmittance, and the fourth stages are attributed to reorganization of the V(III) and V(IV) products and/or their hydrolytic polymerization. If we start directly with a mixture of V(III), V(IV) and cystine we only observe a zero time

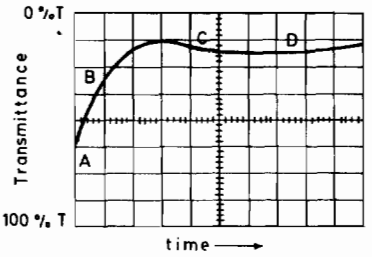


Fig. 2. Transmittance *vs.* time for the reaction between vanadium(II) and cystine. Time scale for stages A, B, and C, 20 ms per division; for stage D, 5 s per division.

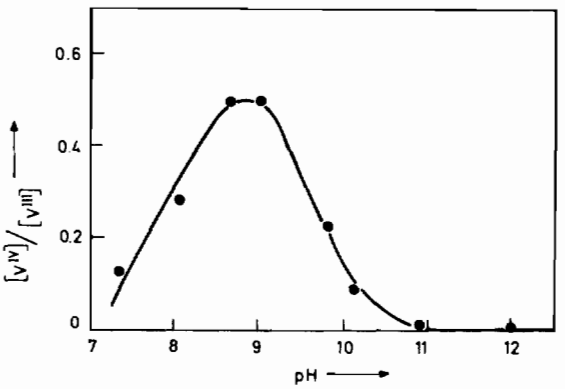


Fig. 3. Typical plot of the dependence of the product ratio [V(IV)]/[V(III)] on pH, $[RSSR]_0 = 0.046$ M, $[V(II)]_0 = 0.0048$ M, 20 °C.

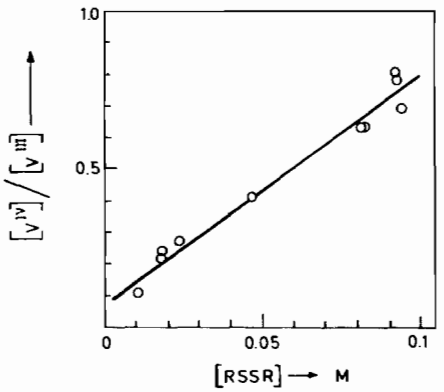


Fig. 4. Dependence of the product ratio [V(IV)]/[V(III)] on the concentration of cystine. $[V(II)]_0 = 0.0048$ M, pH 9, 20 °C.

complexation and then the reorganization/hydrolysis stages.

The ratio of the products of the redox step, [V(IV)]/[V(III)], depends on pH (Fig. 3): from pH 7 to pH *ca.* 9 it increases; at pH *ca.* 9 it reaches a maximum, and then there is a decrease again. This ratio also depends linearly on the constant (excess) concentration of cystine (Fig. 4). If cysteine is added initially, the fraction of V(IV) over V(III) decreases (Table I). In the presence of cysteine the electron of

TABLE I. Effect of Cysteine on the Ratio of the Products of the Redox Reaction between V(II) and Cystine

[V(II)] ($M \times 10^3$)	[RSH] (M)	[RSSR] (M)	pH	[V(IV)]/[V(III)]
7.5	0.16	0.055	8.5	0.03
7.2	0.15	0.056	9.0	0.05
2.3	0.15	0.056	8.8	0.06
9		0.047	8.8	0.36
9		0.047	8.5	0.25

TABLE II. The Ratio of Products V(IV)/V(III) with and without Initially Added Vanadium(III)[a]

[V(II)] ($M \times 10^3$)	[V(III)] ($M \times 10^3$)	[RSSR] (M)	pH	[V(IV)]/[V(III)]
3.7	3.9	0.08	9.15	0.60
3.5	3.7	0.076	8.2	0.44
3.5		0.076	9.1	0.58

[a]The initially added V(III) is not included in the calculation of the ratio.

vanadium(II) is transferred to the solvent giving dihydrogen [15], but this reaction is slow compared to the oxidation of cystine. Cystine is an effective interceptor of the electrons. In contrast to cysteine, vanadium(III) added initially has no effect (Table II).

It is also noted that at the pH range investigated vanadium(III) does not reduce cystine and vanadium(IV) is not reduced by cysteine nor is it oxidized to vanadium(V) by cystine. Thus, it seems safe to conclude that the formation of V(IV) does not go through V(III), and that the formation of V(III) does not go through V(IV). These two species are rather formed by parallel paths, with precursors differing by one cystine ligand, in accordance with the dependence of the product ratio on the cystine concentration.

All data (Suppl. 1) were obtained with cystine in excess. The kinetics of the redox step are first order in total V(II). The errors of the pseudo-first-order plots (Suppl. 2) are small. It follows that within each run the ratio [V(IV)]/[V(III)] remains constant and the apparent absorptivity does not change. At the wavelengths of the kinetic experiments, both V(IV) and V(III) contribute to the increased absorbance, and the measured rates are weighed averages of the formation of these two species.

Within experimental error, there is no effect of the ionic strength on the rate (up to 1 M NaCl), but there is a strong pH dependence (Fig. 5), consistent with the pH dependence of the analytically determined [V(IV)]/[V(III)] ratio. The activation parameters were determined (Suppl. 3) at pH 9.2–9.3 and have the values $E_a = 67 \pm 8$ kJ/mol, and $\log(A) = 12.3$.

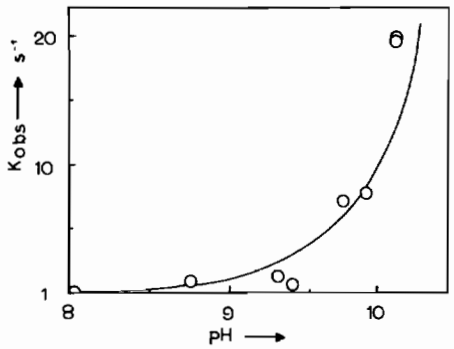


Fig. 5. pH dependence of the observed pseudo-first-order rate constant of the reaction of V(II) with excess cystine. $[RSSR]_0 = 0.02$ M, $[V(II)]_0 = 2.75 \times 10^{-3}$ M, 23 °C.

The data in Suppl. 1 also indicate a strong dependence on the (excess) concentration of cystine, but the exact functional form of this dependence cannot be determined accurately. The large scattering is attributed to pH changes during the reaction and to hydrolysis. In order to minimize these effects, we also performed the reaction in the presence of mercaptoacetic acid (mac) and cysteine (cys). These ligands, in excess, have a buffer capacity and they prevent hydroxide formation.

With 0.12 M mac the activation parameters are $E_a = 55.5 \pm 4.8$ kJ/mol and $\log(A) = 10$ (Suppl. 4).

On the data with mercaptoacetic acid and cysteine (Suppl. 5), we tried two least-squares fits, a linear (A) and an inverse (B) relationship.

$$k_{obs} = k + k'[RSSR] \quad (A)$$

$$1/k_{obs} = k'' + k'''/[RSSR] \quad (B)$$

The inverse fit was done in order to test a fractional rate law of the form

$$\text{rate} = \frac{k[V(II)][RSSR]}{1 + k'[RSSR]}$$

postulated elsewhere [16]. The results are summarized in Figs. 6 and 7. Both fits can be characterized as good, but we base our interpretation on the linear form (A).

The linear dependence of k_{obs} on [RSSR] can be correlated with the linear dependence of the ratio of [V(IV)] over [V(III)] on [RSSR]. The k term can be attributed to V(III) formation and the k' term to V(IV) formation. Within this context it is also pointed out that if vanadium(II) forms a complex with the excess of cystine quantitatively and the rate-determining step is an intramolecular electron transfer within this complex, the rate should appear as zero order in [RSSR]. A first-order term in [RSSR] is also first order in the quantitatively formed precursor complex and first order in total vanadium(II).

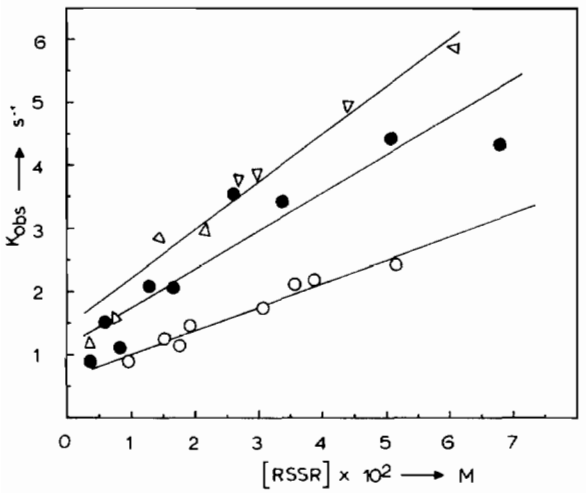


Fig. 6. Linear fit (eqn. A) of k_{obs} *vs.* [RSSR] in the reaction between V(II) and cystine in the presence of mercaptoacetic acid or cysteine (Suppl. 5): • $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [mac] = 0.2 M, pH 9.3–9.4, temperature 22 °C; ○ $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [mac] = 0.4 M, pH 9.1, temperature 23.5 °C; ▽ $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [cys] = 0.18 M, pH 9.2, temperature 23 °C.

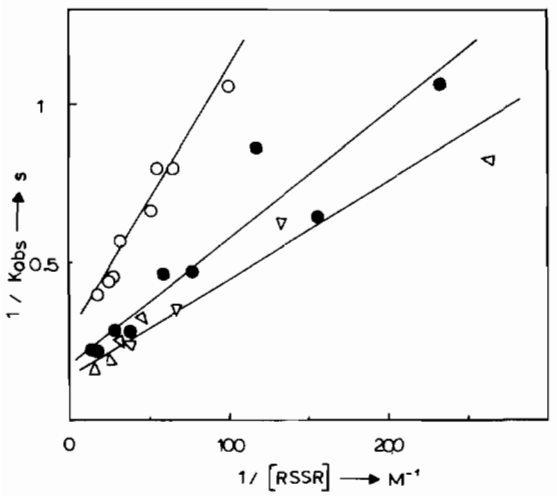


Fig. 7. Inverse fit (eqn. B) of $1/k_{obs}$ *vs.* 1/[RSSR] in the reaction between V(II) and cystine in the presence of mercaptoacetic acid or cysteine (Suppl. 5): • $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [mac] = 0.2 M, pH 9.3–9.4, temperature 22 °C; ○ $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [mac] = 0.4 M, pH 9.1, temperature 23.5 °C; ▽ $[V(II)]_0 = 0.59 \times 10^{-3}$ M, [cys] = 0.18 M, pH 9.2, temperature 23 °C.

This argument is consistent with the observed zero-time increase in absorbance and also with the fact that, within experimental error, there is no effect of the ionic strength on the rate.

Under the conditions in which the activation parameters were determined, the fraction of [V(IV)] over [V(III)] is small and the zero-order term dominates.

A mechanism consistent with the observations is the following:

$$V^{II} + RSSR \overset{K_1 \gg 1}{\rightleftharpoons} V^{II}(RSSR) \quad \text{fast} \quad (1)$$

$$V^{II}(RSSR) \xrightarrow{k_2} V^{III} + RS\cdot + RS^- \quad \text{slow} \quad (2)$$

$$V^{II}(RSSR) + RS\cdot \xrightarrow{k_3} V^{III}(RSSR) + RS^- \quad \text{fast} \quad (3)$$

$$V^{II}(RSSR) + RSSR \overset{K_4}{\rightleftharpoons} V^{II}(RSSR)_2 \quad \text{fast} \quad (4)$$

$$V^{II}(RSSR)_2 \xrightarrow{k_5} V^{IV}(RSSR) + 2RS^- \quad \text{slow} \quad (5)$$

Vanadium(IV) formation is postulated to take place by a concerted two-electron transfer, presumably via simultaneous overlap with the empty d orbitals of the neighboring sulfur atoms. Cysteine competes with cystine and decreases the concentration of the precursor of V(IV). Mercaptoacetic acid has a similar effect, as indicated by the data in Fig. 6. The formation of this precursor (eqn. 4) is not quantitative like the formation of the first complex (eqn. 1) and depends on pH (Figs. 3 and 5).

Supplementary Material

Suppl. 1. Kinetic data for the reaction of V(II) with cystine.

Suppl. 2. Typical first-order plot for the reaction between V(II) and cystine. $[V(II)]_0 = 0.75 \times 10^{-3}$ M, $[RSSR]_0 = 0.025$ M, pH = 9.8, 28 °C.

Suppl. 3. Arrhenius plot for the reaction of V(II) and cystine. pH 9.2–9.3, $[V(II)]_0 = 0.59 \times 10^{-3}$ M, $[RSSR]_0 = 0.057$ M.

Suppl. 4. Arrhenius plot for the reaction of V(II) and cystine in the presence of mercaptoacetic acid. pH 9.5–9.6, $[V(II)]_0 = 0.59 \times 10^{-3}$ M, $[RSSR]_0 = 0.011$ M, [mac] = 0.12 M.

Suppl. 5. Kinetic data for the reaction between V(II) and cystine in the presence of mercaptoacetic acid or cysteine.

References

1 J. Konstantatos, G. Kalatzis, E. Vrachnou-Astra and D. Katakis, *Inorg. Chem.*, *22*, 1924 (1983).
2 M. Friedman, 'The Chemistry and the Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins', Pergamon, Oxford, 1973.
3 P. W. Preisler, *J. Biol. Chem.*, *87*, 767 (1930).
4 J. B. Conant, *Chem. Rev.*, *3*, 1 (1926).
5 L. E. Asher and E. Deutsch, *Inorg. Chem.*, *11*, 2927 (1972); C. J. Weschler and E. Deutsch, *Inorg. Chem.*, *12*, 2682 (1973).

6 R. H. Lane and L. E. Bennett, *J. Am. Chem. Soc., 92,* 1089 (1970); L. E. Asher and E. Deutsch, *Inorg. Chem., 12,* 1774 (1973); G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc., 89,* 3607 (1967).
7 S. Koch, S. C. Tang, R. H. Holm and R. B. Frankel, *J. Am. Chem. Soc., 97,* 914 (1975).
8 L. H. Krull and M. Friedman, *Biochem. Biophys. Res. Commun., 29,* 373 (1967); *J. Polym. Sci., 5,* Part 1-A, 2535 (1967); *Biochim. Biophys. Acta, 207,* 361 (1970).
9 R. C. Arnold, A. P. Lien and R. M. Alm, *J. Am. Chem. Soc., 72,* 731 (1950).
10 V. du Vigneaud, L. F. Audrieth and H. S. Loring, *J. Am. Chem. Soc., 52,* 4500 (1930).
11 M. M. David, R. Sperling and I. Z. Steinberg, *Biochim. Biophys. Acta, 539,* 101 (1974).
12 I. R. Miller and J. Teva, *J. Electroanal. Chem., 36,* 157 (1972); W. Lee, *Biochem. J., 121,* 563 (1971); I. M. Kolthoff and C. Barnum, *J. Am. Chem. Soc., 63,* 520 (1941).
13 E. Vrachnou-Astra, P. Sakellaridis and D. Katakis, *J. Am. Chem. Soc., 92,* 811 (1970).
14 D. R. Grassetti and J. F. Murray, Jr., *Arch. Biochem. Biophys., 119,* 41 (1967).
15 J. Konstantatos, G. Kalatzis, E. Vrachnou-Astra and D. Katakis, *J. Chem., Soc. Dalton Trans.,* 2461 (1985); G. Kalatzis, J. Konstantatos, E. Vrachnou-Astra and D. Katakis, *J. Am. Chem. Soc., 105,* 2897 (1983).
16 L. E. Asher and E. Deutsch, *Inorg. Chem., 14,* 2799 (1975).