A Kinetic Study of the Complexation of Cysteine and Related Compounds with Aqueous Vanadium(II) and Vanadium(III) at Approximately Neutral pH; the Mediating Role of Sulphur Compounds in Electron Transfer†

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Vanadium(II) and vanadium(III) form with cysteine (cys) and other sulphhydryl compounds intensely yellow complexes soluble in neutral and weakly alkaline solutions. Some of the vanadium(II) complexes are powerful reductants. Thus, V^{II}–cys reduces water to dihydrogen under mild conditions. The formation of the reducing species, which is $[V^{II}(cysOS)_3]^{4-}$ [cysOS = cysteinate(2–)] proceeds in two stages, *i.e.* a stage corresponding to a jump in the absorbance at zero time and a second stage, which was followed kinetically in a stopped-flow instrument. The complexation of cysteine with vanadium(III) also proceeds in two stages, but leads to the formation of a species containing two cysteines instead of three (at pH values around neutral). The observed activation energy (E_a) and pre-exponential factor (A) for the stages that were followed kinetically are as follows: $E_a = 41 \pm 4$ kJ mol^{-1}, $A = 1.4 \times 10^7$ (pH 8.2) for V^{II} and $E_a = 41 \pm 4$ kJ mol^{-1}, $A = 1.4 \times 10^{10}$ (pH 8.8) for V^{III}. The reduction of water to dihydrogen by V^{II}–cys proceeds with a rate first order in $[V^{II}]_{total}$ and in the pH range 7.5—8.5 it is independent of hydrogen-ion concentration. The activation parameters are: $E_a = 54 \pm 2$ kJ mol^{-1}, $A = 5 \times 10^6$. Dihydrogen is also obtained with V^{II}–cysa (cysa = cysteamine) and V^{II}–cysme (cysme = cysteine methyl ester). The corresponding reaction of V^{II}–ser (ser = serine) is *ca.* one thousand times slower compared to the reaction of V^{II}–cys, even though the polarographic half-wave potentials have comparable values.

Recently[1] we reported on dihydrogen evolution from aqueous vanadium(II)–cysteine solutions in the pH range 6.0—9.5. Now we present a detailed account of the preceding complexation reactions, and expand the investigation to related compounds.

When discussing the participation of cysteine (cys) in redox processes, what naturally comes first to mind is its facile oxidation to cystine. Other compounds containing low-valent sulphur undergo similar reactions. Recent studies have been reviewed.[2–4] Here we take a closer look in a different direction, *i.e.* on how cysteine catalyzes electron transfer from a reductant to the solvent or to a dissolved oxidant.

Experimental

Materials and Preparations.—In the pH range investigated precipitation of hydrolyzed vanadium species is avoided by using a large excess of the organic ligand. Under these conditions even small amounts of impurities may cause extensive oxidation of vanadium(II) and lead to faulty results.

L-Cysteine hydrochloride was supplied by Merck (pro analyse) and further purified by recrystallization under an inert atmosphere. By using the chloride salt the cysteine solutions contain an equivalent amount of chloride. Mercaptoacetic acid (Riedel de Häen) was purified by triple fractional distillation under vacuum.[5] Its solutions must be fresh and air free. Cysteamine hydrochloride (Sigma) was freed from oxidative impurities by first adding to its solutions an equivalent (to the impurities) amount of required vanadium(II) before the desired quantity of vanadium(II).

L-Cysteine methyl ester hydrochloride (Sigma), thiolactic acid (Aldrich 95%), 3-mercaptopropionic acid (Merck), ethanethiol (Aldrich), *S*-methyl-L-cysteine (Aldrich), DL-*N*-acetylcysteine (Aldrich), DL-serine (Sigma) and *N*-acetylcysteine (Sigma) were used without purification.

Aqueous vanadium(II) solutions were prepared electrolytically as usually[6] on a mercury cathode. Reaction mixtures were prepared by adding the acidic solutions of $V_{aq.}^{2+}$ and $V_{aq.}^{3+}$ to the alkaline solutions of the organic ligand, under an atmosphere of argon and with vigorous stirring. In the absence of air, the alkaline solutions of the ligands investigated are stable. The pH was estimated from the acid and base contents of the separate solutions and it was also measured after the completion of the reaction. High ligand concentrations have a buffering effect, and the pH remains constant throughout the reaction.

The cysteine methyl ester–V^{II} complex was prepared by mixing in a cooled vessel 1 cm^3 of a solution containing 0.146 mol dm^{-3} V^{II} and 0.2 mol dm^{-3} HCl, with 10 cm^3 of a second solution containing 0.5 mol dm^{-3} ester and 5.0 mol dm^{-3} KBr, and with 5.5 cm^3 of a third solution containing 1.0 mol dm^{-3} NaOH. KBr was added in order to coagulate the colloidal precipitate. The precipitate was filtered off under an argon atmosphere, washed with air-free water, and dried in vacuum.

Spectra and Kinetics.—Electronic spectra were recorded with a Cary 14 spectrophotometer. Fast kinetics were followed using a stopped-flow spectrophotometer (Applied Photophysics), slow kinetics were followed either spectrophotometrically or by measuring the volume of the gas produced under constant pressure and temperature. Raman spectra were taken with a Ramanor HG-25 instrument, excitation at 454.5 nm with an argon laser and at 632.8 nm with a helium–neon laser.

pH *Measurements and Polarography.*—pH was measured with a Metrohm AG Herisau Potentiograph. Polarographic

† *Supplementary data available* (No. SUP 56325, 12 pp.): Tables of ε for V^{II}–cys and V^{III}–cys, $k_{obs.}$ for V^{II}–cys and V^{III}–cys, plots of log $k_{obs.}$ *vs.* log [cys] for V^{II}–cys, Arrhenius plot for V^{II}–cys, log $k'_{obs.}$ (pseudo-first-order rate constant) *vs.* log [cys] for V^{III}–cys, Arrhenius plot for V^{III}–cys, Arrhenius plot for redox reaction of V^{II}–cys. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1985, Issue 1, pp. xvii—xix.

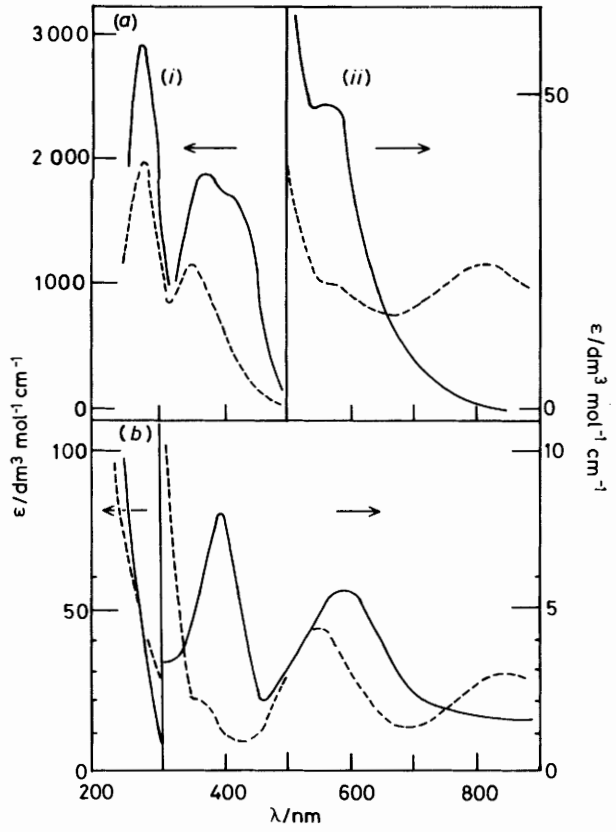


Figure 1. (*a*) Absorption spectra for V^{II}–cys (– – –) and V^{III}–cys (——) at pH 8.0 (*i*) and 8.2 (*ii*); T = 24 °C, $[V^{II}] = [V^{III}] = 8.0 \times 10^{-3}$, and [cys] = 0.68 mol dm^{-3}. (*b*) Absorption spectra of $[V(H_2O)_6]^{2+}$ (– – –) and $[V(H_2O)_6]^{3+}$ (——), in hydrochloric acid solutions, for comparison

measurements were done with a Metrohm model E261 polarograph at 23 °C using a dropping mercury electrode and a saturated Ag/AgCl electrode in air-free solutions.

Results

Equilibria and Kinetics of Vanadium(II) *Complexation: Spectra.*—Kinetically, two stages in the V^{II}–cys complexation reaction are observed. The first is too fast for stopped-flow; it appears as a jump in the absorbance at zero time, amounting to *ca.* 40% of the final value (Table A, SUP 56325).

The data, not unexpectedly for our stopped-flow instrument, are scattered, but it can be stated that in the range of cysteine and hydrogen-ion concentrations used, if there is any regular trend, this trend is small, and within experimental error, both the initial absorbance and the absorbance after complexation are constant. The concentration of cysteine was varied from 0.10 to 0.67 mol dm^{-3} and that of hydrogen ion from 0.79×10^{-7} to 0.4×10^{-9} mol dm^{-3}. The average absorption coefficients for the 32 experiments performed were $\bar{\varepsilon}_o = 552 \pm 100$ and $\bar{\varepsilon}_c = 1\,315 \pm 135$ dm^3 mol^{-1} cm^{-1} for the initial jump and after complexation, but before V^{II} to V^{III} conversion, respectively.

More accurate spectral data, but only for ε_c, were obtained with the Cary spectrophotometer (Figure 1). Note that the spectrum for the V^{II}–cys complex differs considerably from that of $[V(H_2O)_6]^{2+}$; the absorption coefficients in particular for the former are much larger. The spectrum of V^{II}–cys also differs from those of vanadium(II) hydroxides observed in our laboratory at different pH's.

The second stage of the complexation reaction is first order in $[V^{II}]_{total}$ and first order in [cys] (Figure A and Table B, SUP 56325). Kinetic data for this stage were collected in the temperature range 24—54 °C, with $[V^{II}]_{total} = 3 \times 10^{-4}$ and [cys] = 0.10—0.64 mol dm^{-3}. The second-order rate constant, k_2, depends on pH; in the pH range 7.5—9.0 it reaches a maximum value of 1.00 ± 0.05 dm^3 mol^{-1} s^{-1} at pH 8.25 and 24 °C (Figure 2). The points in Figure 2 are the averages of many measurements. The activation energy (E_a) for the reaction (at pH 8.2) was 41 ± 4 kJ mol^{-1} and the pre-exponential factor (A) 1.4×10^7 (Figure B, SUP 56325).

In an effort to determine the composition of the species formed we applied the variation of Bjerrum's titration method developed by Calvin and co-workers.[7] Typical results are given in Figure 3. An interesting feature regarding the redox reaction (see later) is that there is a flat maximum at pH *ca.* 8 and $n \simeq 6$

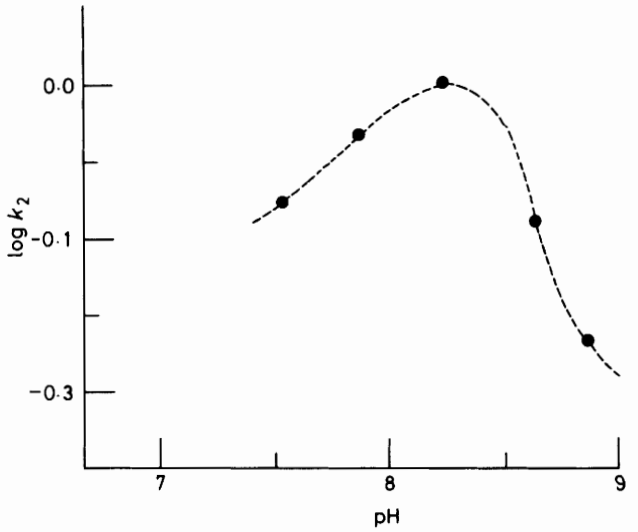


Figure 2. pH Dependence of the second-order rate constant, k_2, for the complexation reaction between vanadium(II) and cysteine. Each point represents an average of many measurements; $[V^{II}] = 3.0 \times 10^{-4}$, [cys] = 0.10 mol dm^{-3}, T = 24 °C

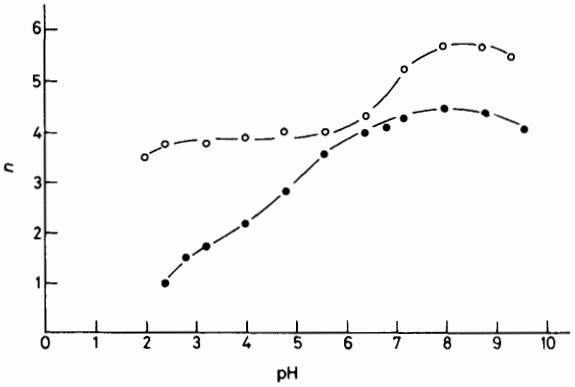


Figure 3. Number of protons (n) released by V^{II} (○) or V^{III} (●) due to complexation with cysteine. This number is calculated from the additional base (0.25 mol dm^{-3} NaOH) consumed in the presence of the metal ion at any pH. Composition of the reference solution for V^{II}, [cys] = 0.18 mol dm^{-3} (chloride salt), [HCl] = 0.06 mol dm^{-3}; composition of the metal-ion solution the same as reference solution, plus $[V^{II}] = 0.01$ mol dm^{-3}. Composition of the reference solution for V^{III}, [cys] = 0.35 mol dm^{-3} (chloride salt), [HCl] = 0.09 mol dm^{-3}; composition of the metal ion solution the same as reference solution, plus $[V^{III}] = 0.017$ mol dm^{-3}

Table 1. Absorption coefficients ($\bar{\varepsilon}_c$)[a] of V^{III}–cys solutions after complexation, at 375 nm and 24 °C; $[V^{III}]_0 = 0.47 \times 10^{-3}$ mol dm^{-3}

10^3 [cys]$_0$/mol dm^{-3}	pH	$\bar{\varepsilon}_c$/dm^3 mol^{-1} cm^{-1}
0.10	5.0	500
0.10	6.6	1 750
0.04	7.4	1 800
0.20	7.4	1 830
0.67	7.4	1 800
0.10	8.0	1 850
0.10	8.7	1 820
0.10	9.5	1 720
0.02	7.5	1 600[b]
0.20	7.5	1 600[b]

[a] Obtained using a Cary spectrophotometer. [b] At 415 nm.

where the dependence on pH is small. At this point the composition corresponds to a complex $[V^{II}(cysOS)_3]^{4-}$ [cysOS = cysteinate(2–)] in which the cysteines are bound through both sulphur and nitrogen. Above pH ≃ 3 the carboxyl group is completely dissociated and proton release from it is not measured by the differential titration. Similarly, the decrease in the observed number of protons released above pH ≃ 9 must at least be due partly to the fact that in the reference cysteine solution, cysteine is partly dissociated.

Equilibria and Kinetics of Vanadium(III) *Complexation: Spectra.*—The qualitative features of the complexation of $V_{aq.}^{3+}$ with cysteine resemble those of vanadium(II). The spectrum of the V^{III}–cys complex is included in Figure 1. Initial and final (after complete complexation) absorption coefficients are given in Table C (SUP 56325). The averages over the 12 measurements were $\bar{\varepsilon}_o = 518 \pm 66$ and $\bar{\varepsilon}_c = 1\,762 \pm 262$ dm^3 mol^{-1} cm^{-1}, but again there is a lot of scattering, and no dependence on pH or [cys] can be seen. In this case, however, complexation is not complicated by a subsequent redox reaction and $\bar{\varepsilon}_c$ can be measured accurately using the Cary spectrophotometer (Figure 1 and Table 1). The absorption coefficients are generally higher than those of V^{II}–cys. Four peaks are observed in the absorption spectrum, one of low intensity around 580 nm, and three of high intensity, at *ca.* 415, 375, and 280 nm. There is no dependence on [cys] but there is a dependence on pH (Table 1). For pH values higher than 8.7 the peak at 375 nm starts to shift towards shorter wavelengths, while that at 415 nm shifts to a longer wavelength and its intensity decreases. The intensity of the peak at 375 nm starts to decrease above pH 9.5. For pH values higher than *ca.* 10 the yellow colour vanishes, indicating that formation of V^{III}–cys complexes is not favoured over this value. For pH values lower than 7.0, however, the absorbance decreases with pH.

Raman spectra for a solution (pH 7.0) of 5×10^{-3} mol dm^{-3} V^{III} and 0.50 mol dm^{-3} cysteine show a new peak at *ca.* 350 cm^{-1}. This peak, which can be assigned to ν_{sym}(V—Cl), is detectable even under conditions of low metal-ion concentration and is seen with excitation at 454.5 nm, but not with excitation at 632.8 nm. These observations indicate resonance Raman.

The kinetics of the second stage of the complexation reaction were also studied in the stopped-flow instrument. They were found to be first order in $[V^{III}]_{total}$ and first order in [cys] (Figure C and Table D, SUP 56325). Figure 4 shows the dependence of the second-order rate constant for vanadium(III), k_2', on pH. As the pH rises above *ca.* 8.2, k_2' decreases. pH values lower than 8 could not be used because high absolute cysteine concentrations are necessary to achieve sufficient buffering capacity and then the reaction is too fast to be followed.

The Arrhenius activation energy is 41 ± 4 kJ mol^{-1} and the pre-exponential factor, 1.4×10^{10} (at pH 8.8) (Figure D, SUP 56325). Complexation of V^{III} is faster than complexation of V^{II} by three orders of magnitude (same pH). Enthalpies of activation are comparable but the entropies differ. Typical results of the differential titration experiments are included in Figure 3. The maximum number of cysteine ligands is two (four protons released) rather than three, and there is no plateau at lower pH values.

Table 2. Kinetic and stoicheiometric data for dihydrogen formation in the reaction between V^{II}–cys and water

[cys]/ mol dm^{-3}	$[V^{II}]_0$/ mol dm^{-3}	pH	T/°C	$10^3 k_1$/ s^{-1}	Moles H_2*
0.66	0.100	8.0	20.0	2.30	0.048
1.47	0.038	8.0	22.0	2.51	0.017
0.89	0.044	8.0	25.0	3.73	0.021
1.07	0.050	7.5	25.5	3.69	0.024
0.67	0.027	7.9	21.0	2.31	0.013
0.62	0.021	7.8	23.0	2.95	—
1.01	0.050	8.5	25.0	3.65	0.023
0.66	0.097	8.0	51.0	19.00	0.047
0.66	0.097	8.0	40.0	10.80	0.046
0.66	0.097	8.0	62.0	34.60	0.048

* Moles of dihydrogen produced per dm^3 of reaction solution; corrected for the amount of dihydrogen remaining in solution.

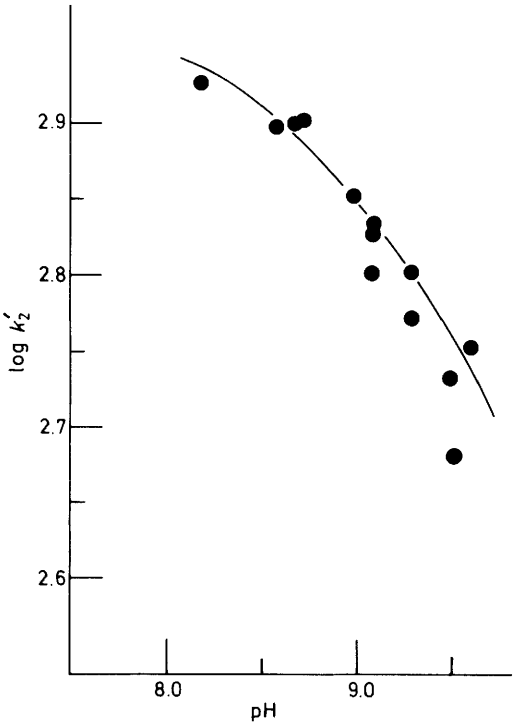


Figure 4. pH Dependence of k_2' (V^{III}–cys complexation reaction, second step); $[V^{II}] = 4.7 \times 10^{-4}$ mol dm^{-3}, wavelength 400 nm, T = 23 °C

Reduction of Water to Dihydrogen.—Stoicheiometric and kinetic data for dihydrogen production in V^{II}–cys solutions are given in Table 2. The stoicheiometry of V^{II}–cys:H_2 is 2:1; the overall reaction can be expressed by equation (1). This equation

$$V^{II}\text{–cys} + H_2O \longrightarrow V^{III}\text{–cys} + \tfrac{1}{2}H_2 + OH^- \quad (1)$$

was verified by measuring the amount of dihydrogen and V^{III} formed. Products of oxidation or reduction of cysteine itself

Table 3. Qualitative comparison[a] of cysteine to related ligands; $[V^{II}]_0 = 2 \times 10^{-3}$ mol dm^{-3}, $[ligand]_0 = 0.1$ mol dm^{-3}, room temperature

Ligand	Formula	pH range	$V^{II} \rightarrow V^{III}$	Gaseous products	Comments
Mercaptoacetic acid	$HSCH_2CO_2H$	3.5—9.5	Yes	H_2S	Formation of yellow soluble V^{III} complex. Organic product $(CH_2COH)_2$
Thiolactic acid	$HSCH(Me)CO_2H$	*ca.* 9	Yes	H_2S	Formation of yellow soluble V^{III} complex. Organic product $[CH(Me)CO_2H]_2$
3-Mercaptopropionic acid	$HS(CH_2)_2CO_2H$	5.0—9.5	No	—	Formation of insoluble V^{II} complex
Ethanethiol	$HSCH_2CH_3$	6.0—8.0	No	—	No complex formation
Cysteamine	$HS(CH_2)_2NH_2$	8.0—10	Yes	H_2	Heterogeneous oxidation of V^{II}
Cysteine	$HSCH_2CH(NH_2)CO_2H$	5.0—9.5	Yes	H_2	Formation of soluble yellow V^{III} complex. H_2S or NH_3 were not detected
S-Methylcysteine	$MeSCH_2CH(NH_2)CO_2H$	6.0—9.0	No	—	Formation of soluble pink complex of V^{II}. Above pH 8.5 colour changes to pale yellow and hydroxo-complexes precipitate
N-Acetylcysteine	$HSCH_2CH(NHCOMe)CO_2H$	5.0—9.0	No	—	Formation of soluble V^{II} complex
Cysteine methyl ester	$HSCH_2CH(NH_2)CO_2Me$	6.0—9.0	Yes	—	Heterogeneous oxidation of V^{II} (see text)
Serine	$HOCH_2CH(NH_2)CO_2H$	6.0—9.0	No	—	Formation of pink V^{II} complex

[a] Oxidation of V^{II} and gaseous product formation within a period of *ca.* 2 h. For longer times even solid V^{II} hydroxides give H_2.

were not detected. In the presence of air V^{III}–cys is further oxidized to $[VO]^{2+}$, which in turn catalyzes air oxidation of cysteine to cystine. The catalytic activity of $[VO]^{2+}$ was confirmed by passing oxygen through a 0.5 mol dm^{-3} solution of cysteine at pH 6.5 containing 2.5×10^{-3} mol dm^{-3} $[VO]^{2+}$; insoluble cystine appeared within two hours! Without $[VO]^{2+}$ no precipitate is formed, even after several days. In air-free V^{III}–cys or V^{II}–cys solutions there is no indication of formation of $[VO]^{2+}$ or of V^{II}, V^{IV} or V^{III}, V^{III} dimers. The kinetics of the reaction were followed by measuring the volume (at constant pressure) of dihydrogen produced *versus* time.

The data in Table 2 also show that dihydrogen production is first *order in* [V^{II}–cys]. By V^{II}–cys we denote any V^{II} species present, as measured by their average absorbance or estimated from the amount of dihydrogen produced and the known stoicheiometry. The value of the first-order rate constant is 2.3×10^{-3} s^{-1} (21 °C).

The rate of dihydrogen formation is independent of pH (for a 10-fold change in hydrogen-ion concentration around pH 8). Under the conditions used there is no dependence on the concentration of free cysteine either.

In order to obtain sufficient amounts of dihydrogen relatively high concentrations of vanadium(II) were used resulting in a low [cys]:[V^{II}] ratio for solubility reasons; this meant inadequate buffering. Hence experiments were not extended to pH values lower than 7.5. At pH values higher than 10, on the other hand, the V^{II}–cys complex is destroyed, and there is no hydrogen evolution.

The Arrhenius energy of activation of the redox reaction is 54 ± 2 kJ mol^{-1} and the pre-exponential factor is 5×10^6 (Figure E, SUP 56325).

Dihydrogen formation is inhibited by $[VO]^{2+}$ and by cystine, *e.g.* if an amount of $[VO]^{2+}$ equivalent to the amount of V^{II} is added to a solution (pH 8) of 0.1 mol dm^{-3} V^{II} and 1.5 mol dm^{-3} cysteine, dihydrogen formation is completely suppressed. Cystine has a similar effect. The transfer of an electron to these oxidants is obviously more facile than the transfer to the solvent which leads to dihydrogen formation.

Comparison to Related Compounds.—In order to understand the role of each polar group of cysteine in its reactions with V^{II} and V^{III} ions, a comparative study on the behaviour of these ions with the following related compounds was carried out: cysteamine, cysteine methyl ester, *S*-methylcysteine, mercaptoacetic acid, 3-mercaptopropanoic acid, thiolactic acid, ethanethiol, serine, and *N*-acetylcysteine. The observations made are summarized in Table 3. None of these compounds seems to react with either V^{II} or V^{III} in acidic solutions. For pH values higher than 4.0, however, complex formation was observed for a number of them. A critical examination of this Table shows that under the conditions of our experiments, only those compounds having a –SH group and at least one of the two groups $-NH_2$ or –COOH in adjacent positions give redox reactions within the time limit of 2 h. Replacement of the sulphur atoms by oxygen leads to a dramatic decrease in the rate [see results below for serine (ser)]. The only sulphur compound which does not seem to react, even though it has both –SH and –COOH groups, is 3-mercaptopropionic acid. It is also interesting that *S*-methylation or *N*-acetylation of cysteine renders it inactive. The inactivity of *S*-methylcysteine (for dihydrogen production) provides additional support for our hypothesis that the –SH group enhances the efficiency.

Complexes containing metal–sulphur bonds are known[8] to have a strong ligand-to-metal charge-transfer absorption at *ca.* 280 nm. V^{II}–cys and V^{III}–cys complexes have an additional charge-transfer band at 360 and 375 nm respectively (Figure 1). The spectrophotometric and polarographic data (see later) show that with a large excess of cysteine and at low pH values, the solutions contain mixtures of complex species. The two charge-transfer bands, however, are not assigned to different species. It is rather suggested that each M–S bond gives rise to two charge-transfer bands. Another diagnostically useful consequence of M–S bond formation is the dramatic increase in the absorptivity of the *d*–*d* transition of V^{III} at *ca.* 400 nm (Figure 1). This intensity *borrowing* is not simply due to the tail of the nearby charge-transfer band, but it is rather due to an admixture of sulphur orbital character into the *d* orbitals of the metal ion.

The Reactions of Aqueous Vanadium(II) *and Vanadium*(III) *Ions with Cysteamine and the Methyl Ester of Cysteine.*—Cysteamine (cysa) does not have a carboxyl group, and in the methyl ester of cysteine (cysme) this group is blocked. As a result the two compounds resemble each other in their behaviour towards V^{II}, while they show some characteristic differences from cysteine.

An amorphous green precipitate starts to form at pH 6, even with a large excess of the ester and at low vanadium(II)

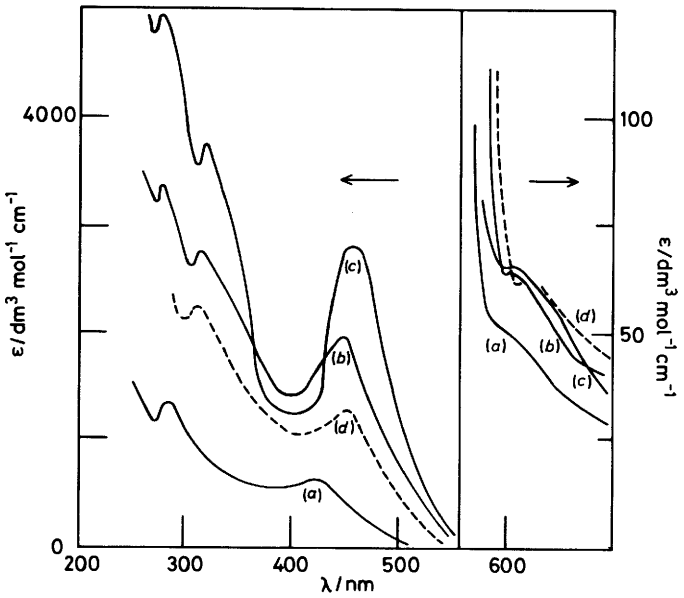


Figure 5. Absorption spectra for V^{III}–cysme at pH 5.4 (*a*), 6.9 (*b*), 8.1 (*c*), and 9.4 (*d*); $[V^{III}] = 7.0 \times 10^{-4}$ mol dm^{-3}, [cysme] = 0.30 mol dm^{-3}

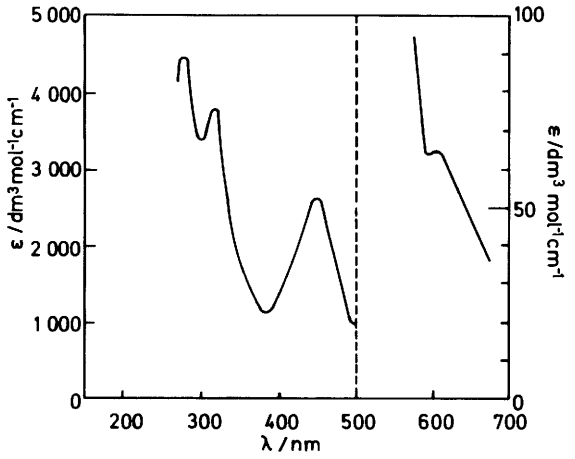


Figure 6. Absorption spectra for V^{III}–cysa; $[V^{III}] = 1.3 \times 10^{-3}$ mol dm^{-3}, [cysa] = 0.50 mol dm^{-3}, pH 9.0, T = 23 °C

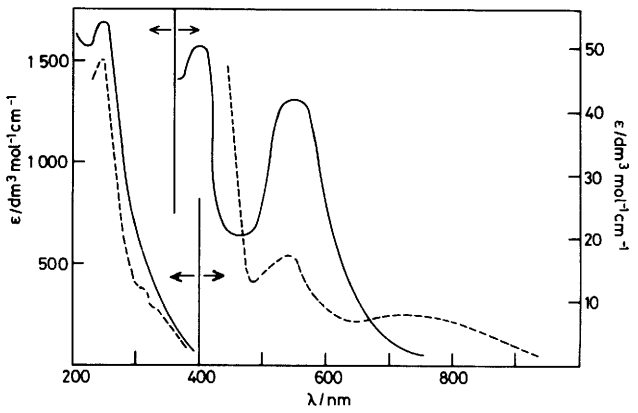


Figure 7. Absorption spectra for V^{II}–ser (– – –) and V^{III}–ser (——); $[V^{II}] = [V^{III}] = 2.7 \times 10^{-3}$ mol dm^{-3}, [ser] = 0.10 mol dm^{-3}, pH 8.5, T = 23 °C

concentrations (5×10^{-4} mol dm^{-3}). This insoluble complex is oxidized slowly (heterogeneously) to a yellow soluble vanadium(III) complex, having a pH-dependent spectrum (Figure 5). The oxidation is associated with hydrogen evolution. In a solution (pH 7.5) of 8×10^{-3} mol dm^{-3} V^{II} and 0.3 mol dm^{-3} cysme, after mixing for 11 d some 80% of the expected dihydrogen (equivalent to V^{II}) was collected, and there was still precipitate left. The precipitate was hygroscopic and gave the following analysis: C, 24.3; H, 4.1; N, 6.9; V, 11.5%. For a neutral 1:2 V^{II} complex with the formula $[V\{SCH_2CH(CO_2Me)NH_2\}_2(OH_2)]\cdot 5H_2O$ the expected percentages are: C, 24.3; H, 6.6; N, 6.9; V, 11.5%. It is interesting that a third ester does not enter the co-ordination sphere of V^{II}, in spite of the large excess used.

The behaviour of cysteamine is similar. Again, there is precipitation, followed by slow dihydrogen evolution and parallel oxidation to an intensely orange soluble V^{III} complex (Figure 6). In a solution (pH 8) of 1.7×10^{-2} mol dm^{-3} V^{II} and 1.4 mol dm^{-3} (impurity free) cysteamine, all the expected dihydrogen is collected within 9 h.

Aqueous Vanadium(II) *and Serine.*—V^{II}–ser complexes are formed above pH 3. They are also oxidized but at a much slower rate compared to V^{II}–cys. Typical spectra of V^{II}–ser and V^{III}–ser solutions are shown in Figure 7. With a large excess of serine there is no precipitation and the reaction is pseudo-first-order in $[V^{II}]$. For a mixture (pH 8.5) of 0.022 mol dm^{-3} V^{II} and 0.57 mol dm^{-3} serine, the observed rate constant is 2.06×10^{-6} s^{-1}. For a similar mixture but at pH 9.2 the value of the constant becomes 1.7×10^{-6} s^{-1}.

Polarographic Data.—Polarographic measurements were performed with solutions of 10^{-3} mol dm^{-3} V^{II} or V^{III}, 0.08 mol dm^{-3} ligand, and 0.5 mol dm^{-3} KCl (supporting electrolyte). The results are summarized in Table 4. The pH is gradually increased by adding 2 mol dm^{-3} NaOH.

V^{II}–cys gives an anodic current. With increasing pH the $E_{\frac{1}{2}}$ values become more negative. V^{III}–cys gives a cathodic current. With increasing pH the $E_{\frac{1}{2}}$ values also become more negative. In the pH range 3.5—9.4, with the concentrations used, the difference between the $V^{II} \rightarrow V^{III}$ anodic wave and the $V^{III} \rightarrow V^{II}$

Table 4. Polarographic data of various V^{II}- or V^{III}–ligand[a] mixtures: [V] = 0.001 mol dm^{-3}, [ligand] = 0.08 mol dm^{-3}, [KCl] = 0.5 mol dm^{-3}, 23 °C; $E_{\frac{1}{2}}$/V *vs.* saturated Ag/AgCl

pH	V^{III}–ser cathodic	V^{III}–ser anodic	V^{II}–ser anodic	V^{III}–cys cathodic	V^{II}–cys anodic	V^{III}–mac[b] cathodic	V^{III}–cysa[c] cathodic	V^{III}–cysme cathodic	V^{II}–cysme anodic
1.7	—	—	—	−0.58, −0.55, −1.05	−0.442, −0.49[d]	—	—	—	—
3.5	−0.702	—	−0.528	−0.595,[e] −0.920	−0.59[e]	—	—	—	—
6.0	−0.732	−0.378	—	—	—	—	—	—	—
7.1	−1.024[e]	−0.228	−0.116, −0.528, −0.968[e]	—	—	—	—	—	—
7.2	—	—	—	−1.15[e]	−1.12[e]	—	−0.904	—	—
7.4	—	—	—	−1.198[e]	—	−0.696, −1.068	—	—	—
8.0	—	—	—	—	—	−0.74, −1.028	−0.904	−1.200[e]	−1.200[e]
8.2	−1.144[e]	−0.248	−0.250, −1.132[e]	−1.19[e]	—	—	—	—	—
8.6	−1.180[e]	−0.248	—	−1.204[e]	−1.250[e]	—	—	—	—
8.8	—	—	—	−1.230[e]	—	—	−0.942	—	—
9.2	−1.260	−0.280	—	—	—	—	—	—	—
9.4	—	—	—	−1.245[e]	—	−1.412	—	—	—

[a] ser = Serine, mac = mercaptoacetic acid, cysa = cysteamine, cysme = cysteine methyl ester. [b] V^{III}–mac: up to pH *ca.* 8.0 two waves, both irreversible. At higher pH values one of these waves cannot be measured because of interference from the anodic wave of the ligand. V^{II}–mac cannot be measured, because of formation of H_2S. [c] V^{II}–cysa gives poor waves. [d] Aqueous vanadium(II) at pH 1.7 also gives an irreversible wave at −0.48 V, but at pH values higher than 4 a black precipitate forms. [e] The difference between $V^{II} \rightarrow V^{III}$ (anodic) and $V^{III} \rightarrow V^{II}$ (cathodic) is smaller than 0.06/*n* V.

cathodic wave is smaller than 0.06/*n* V (where *n* is the number of electrons taken up, equal to 1 in this case) which is a necessary condition for reversibility but not sufficient.

V^{II}–ser mixtures give an anodic wave, which is also shifted to more negative values with increasing pH. V^{III}–ser mixtures give a cathodic wave corresponding to reduction to V^{II}–ser, but also an anodic wave due to oxidation to $[VO]^{2+}$. For V^{III}–cys and the other thio-complexes the anodic waves overlap with the oxidation waves of the sulphur compound itself. At pH 8.0 the methyl ester of cysteine gives an anodic wave. Table 4 also includes data for V^{III}–mercaptoacetic acid, V^{III}–cysteamine and V^{II}–water (in the presence of Cl^-).

The behaviour of V^{II}–*S*-methylcysteine mixtures is unusual. A solution (pH 8.6) of 10^{-3} mol dm^{-3} V^{II} and 0.04 mol dm^{-3} in *S*-methylcysteine is pale yellow. At lower pH values it is light pink. The anodic current due to the oxidation $V^{II} \rightarrow V^{III}$ does not decrease with time, showing little, if any, oxidation. It is shifted, however, and in *ca.* 1 h a precipitate appears. At this stage the pH has become 7.8. It is also noted that *S*-methylcysteine itself (without metal ion) gives a cathodic current, which indicates an impurity.

Discussion

The systems were investigated kinetically from the moment of mixing, through complexation, until completion of the redox reaction. We will follow the reaction as closely as the data allow, focusing mainly on cysteine.

Water exchange between $[V(H_2O)_6]^{2+}$ and the solvent is relatively slow.[9] On the other hand, proton transfer reactions are fast and in the pH range of our experiments, they have been completed within the time of mixing. Moreover, it is well known from base hydrolysis (conjugate base mechanism) that hydroxide ligands are very labile, equations (2) and (3). The

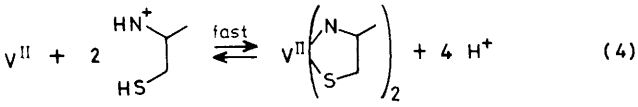
$$V^{II}(OH_2) \overset{\text{fast}}{\rightleftharpoons} V^{II}\text{–}OH^- + H^+ \quad (2)$$

$$V^{II}\text{–}OH^- + \text{cys} \overset{\text{fast}}{\rightleftharpoons} V^{II}\text{–cys} + OH^- \quad (3)$$

analogous reactions for vanadium(III) are also expected to be fast. In the systems studied, a maximum of three cysteine ligands enter the co-ordination sphere of V^{II} and two the co-ordination sphere of V^{III}. Hence, although several elementary steps are involved in each case we have measured the kinetics of one step only. Which of the elementary steps, therefore, is this slow step?

Here we support the idea that the measurable slow step is one of the elementary reactions in the complexation of the last cysteine, and that all the other complexation processes have been completed upon mixing (initial jump).

We will first discuss vanadium(II). The final product is $[V(cysOS)_3]^{4-}$ and the most likely choice for its precursor is $[V(cysOS)_2]^{2-}$, with the two other co-ordination sites taken up by two other ligands (*e.g.* Cl^- or OH^-). The formation of $[V(cysOS)_2]^{2-}$ involves release of four protons, as indicated by the plateau in Figure 3. The precursor of the final product at high pH is the stable dominant species at lower pH (3—6). The polarographic results render further support to this suggestion. There is only one polarographically detectable species at the lower pH range, and only one at the higher pH range, but they are different, corresponding to half-wave potentials −0.59 and −1.12 V, respectively. Thus the following reaction* can be postulated to be fast [equation (4)]. At low vanadium and high



(4)

cysteine concentrations in the complexation experiments, polynuclear vanadium species[10] are not formed.

An approximate value for the constant of the equilibrium (5), $K_5 = 3.5 \times 10^5$ dm^3 mol^{-1}, is obtained from the data in Figure

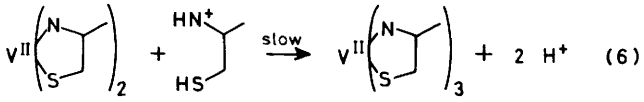
$$[V^{II}(cysOS)_2]^{2-} + cysOS^{2-} \rightleftharpoons [V^{II}(cysOS)_3]^{4-} \quad (5)$$

3 using Bjerrum's $\bar{n} - \frac{1}{2}$ method.[7] The concentration of the doubly charged anion, $cysOS^{2-}$, is estimated from the known[11] dissociation constants.

* Here and in equations (6)—(9) we have used the relevant skeletons only, showing the labile protons; charges are omitted.

Neither $[V(cysOS)_2]^{2-}$ nor $[V(cysOS)_3]^{4-}$ seems to involve co-ordination through the carboxyl group. It rather appears that the role of this group is merely to provide sufficient solubility. With cysteine methyl ester the charge on the carboxyl group is neutralized and there is an early precipitation of a species containing only two ester ligands. Similar remarks can be made for cysteamine.

The kinetically measurable slow reaction in the V^{II}–cys system is given by equation (6). This reaction is not elementary.



(6)

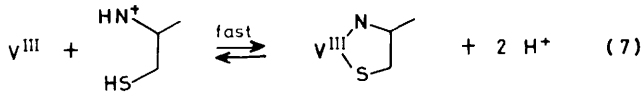
The rate-determining step can be the formation of the first bond or the closure of the ring,[12,13] and either of these processes can be analyzed further. Moreover, in equation (6) the entering cysteine has been symbolized as being protonated on N and on S, but the pH dependence of the rate (Figure 2) indicates that dissociated forms are also implicated.

The kinetics were found to be first order in [cys], in accordance with reaction (6); they were also found to be first order in $[V^{II}]_{total}$, which implies that in the pH range of interest, the formation of the precursor complex, $[V(cysOS)_2]^{2-}$, is practically quantitative and that $[V^{II}]_{total} \simeq [V(cysOS)_2{}^{2-}]_0$. The proposed scheme also requires that the initial jump in absorbance, which corresponds to the formation of the precursor, should be independent of pH and [cys], as was indeed observed.

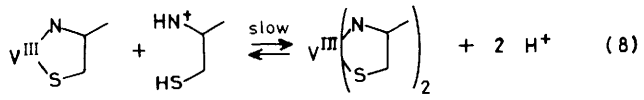
The increase in absorbance, both at zero time and after complexation, is mainly attributed to the formation of the V–S bond; the contribution of V–N bond formation is expected to be relatively small.

With V^{III} there is again only one dominant product in the complexation reaction (Figure 3, Table 4), but which differs in composition from that of the V^{II} reaction. The final V^{III}–cys species corresponds to two tridentate cysteines or more likely to two bidentate cysteines and two other ligands, *e.g.* chloride ions, as indicated by the detection in the Raman spectrum of the V–Cl bond. Another difference between V^{II} and V^{III} is that the former has a tendency to combine with more cysteine even at low pH (2), presumably because charge transfer (back bonding) from V^{II} is more effective.

The mechanism proposed for V^{III} can be summarized by equations (7) and (8). The values of K_7 and K_8 estimated by the



(7)



(8)

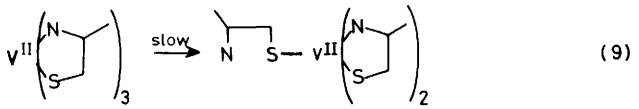
Bjerrum $\bar{n}-\frac{1}{2}$ method are 10^{13} and 10^{7}, respectively. Equilibrium (7) is responsible for the initial jump, the forward reaction of equilibrium (8) for the observed kinetics. It is also interesting that, in the pH range where the substitution experiments were performed, there is only one product polarographically detectable. At lower pH values, where the two equilibria compete, two polarographic waves appear.

Within experimental error, the enthalpy of activation calculated from the observed temperature dependence is the same for V^{II} and V^{III} (38.5 ± 5 and 38.0 ± 4 kJ mol^{-1}) but there is considerable difference in the observed entropy of activation (-117 and -60 J K^{-1} mol^{-1}, respectively). With an equilibrium preceding the rate-determining step, the observed activation parameters contain the thermodynamic entropy and enthalpy changes of this equilibrium. The fact, then, that the observed entropy of activation is more negative for V^{II} is attributed to a more negative change of the corresponding pre-equilibria.

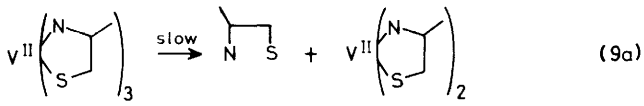
The decrease of $\bar{\varepsilon}_c$ and of the rate of substitution at high pH values indicate the formation of new, less reactive species, without V–S bonds.

Now turning our attention to dihydrogen production, we first note that complexation through sulphur is a prerequisite for an effective reaction. The observations summarized in Table 3 imply that at least one five-membered ring containing sulphur is necessary. Particularly instructive in this respect is the contrast in effectiveness between cysteine and serine.

Complexation is fast compared to electron transfer; all complex formation equilibria having been established. As stated in the pH range where the redox reaction was investigated, only one vanadium(II) and one vanadium(III) species dominate. In the pH range considered, the composition of the reactant and product complexes is practically independent of the concentration of the hydrogen ion. The rate of the overall redox reaction is also independent of this concentration (within experimental error). Therefore, the rate-determining step cannot involve addition of a proton nor a pre-equilibrium with proton participation. It should rather be first order in the active complex, and a reasonable suggestion is that it should involve complete or partial removal of one of the cysteine ligands, *e.g.* equation (9) or (9a). Reaction (9) [similarly for reaction (9a)] is



(9)



(9a)

followed by fast proton, and possibly also Cl^-, addition. Omitting the ligands and representing for convenience the co-ordinatively unsaturated intermediate as $V^{II}{}_{uns}$, we can write equations (10) and (11). Reaction (11) is analogous to the

$$V^{II}{}_{uns} + H^+ \xrightarrow{fast} V^{III} \cdots H \qquad (10)$$

$$2V^{III} \cdots H \xrightarrow{fast} H_2 + 2V^{III} \qquad (11)$$

reaction of two $[CoH(CN)_5]^{3-}$ ions to give dihydrogen and $2[Co(CN)_5]^{3-}$.[14]

Alternatively, if reaction (10) is regarded as oxidative proton addition, the following hydride mechanism can be proposed [equations (12) and (13)]. The analogous reaction to (13) for

$$H^+ + H^-\text{–}V^{IV} \xrightarrow{fast} H_2 + V^{IV} \qquad (12)$$

$$V^{II} + V^{IV} \xrightarrow{fast} 2V^{III} \qquad (13)$$

aqua ions proceeds *via* a detectable binuclear intermediate.[10] In our systems such an intermediate was not detected but this does not necessarily exclude the hydride mechanism. The rapid inhibition of H_2 formation by $[VO]^{2+}$ and cystine shows that reaction (13) is indeed fast. For the formation of H_2 from aqueous suspensions of $V(OH)_2$ and from homogeneous V^{II}–pyrocatechol solutions, Schrauzer and co-workers[15] favour a hydride mechanism.

If the last complexation equilibrium has the smaller equilibrium constant, and if the forward reaction of this equilibrium is the slowest, and the one that can be measured, it is reasonable to expect that the rate constant of the reverse reaction, which is postulated to control the rate of the redox process [reactions (9) and (9a)] should also be small and measurable.

It is well known [16] that cysteine occupies 'strategic' positions in biological redox systems such as cytochromes, ferredoxins *etc.*, being directly bound to the prosthetic group. In this respect V^{II}–cys resembles these systems.

It has also been reported [17] that some cysteinyl sulphurs of biological redox systems participate in redox processes.

The finding of this investigation that cysteine is not just like serine, but is much more effective in facilitating electron transfer, suggests that in biological systems too, cysteine is perhaps not just a passive link as any between the prosthetic group and the protein, but that it can also play a more active kinetic role in the redox process itself. This may very well be the reason why 'synthetic' FeMo–coenzyme does not reduce dinitrogen,[18] in contrast to the native enzyme. From this point of view then it would have been better to consider cysteine as part of the active centre (prosthetic group + cysteine).

It is also interesting that cysteine mediates effectively not only in electron transfer from V^{II} to added oxidants, but also to water itself. This is perhaps why in 'real life' the heme or the iron–sulphur clusters, together with their cysteines are surrounded by protective hydrophobic residues.[16]

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