Anaerobic Oxidation of Cysteine to Cystine by Iron(III). Part 1. The Reaction in Acidic Solution

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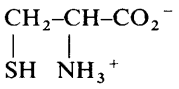
The anaerobic oxidation of cysteine (H_2L) by iron(III) has been followed in acidic media by use of a stopped-flow high-speed spectrophotometric method. It is suggested that the species responsible is $[FeL]^+$ which is observable as a transient blue colour on the addition of iron(III) solutions to acidic cysteine solutions. This species exhibits an absorption maximum at 614 nm with a molar absorption coefficient of 1 030 $dm^3\ mol^{-1}\ cm^{-1}$. The second-order rate constant for its formation from $[Fe(OH)]^{2+}$ and H_2L was found to be $1.14 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$ and the value of log K^{FeOH} {for the protonation of $[Fe(OH)]^{2+}$} required to fit the data is 2.82. Decomposition to iron(II) and subsequently cystine follows protonation, with an apparent third-order rate constant (including an unknown stability constant) of $1.63 \times 10^{11}\ dm^6\ mol^{-2}\ s^{-1}$. All measurements were carried out at 25 °C in solutions of ionic strength 0.10 mol dm^{-3} (KCl).

The reaction of cysteine (H_2L) with iron(III) in the absence of



H_2L
(I)

oxygen has been the subject of several investigations[1-3] but two difficulties remain. First the rate law studies carried out at pH <9 suggested that the redox reaction proceeded by a route other than that established at higher pH. Secondly, the identity and role, if any, of the light blue complex observed transiently in acidic solutions[4] was not known. We decided, therefore, as part of an ongoing study of metal–ligand internal redox reactions and the associated metal-ion-catalysed oxidation of bound ligands by molecular oxygen, to re-examine this system at both high and low pH. The use of a high-speed spectrophotometric stopped-flow method enabled not only the kinetics to be established with a much higher degree of confidence than previously, but also to obtain spectral evidence as to the nature and number of the species involved in the reaction. This paper discusses the results obtained over the range pH 2.7—3.9.

Results

A typical example of a stopped-flow run is shown in Figure 1 and the component spectra were shown to consist of a single broad band with a maximum at 614 nm. In order to work at a reduced pseudo-order, cysteine was always present in large excess, and the shape of the kinetic curve suggested that we were following the formation of an intermediate, B, in a reaction scheme of the type (1). A value of the rate constant, $k_2^{obs.}$, at each

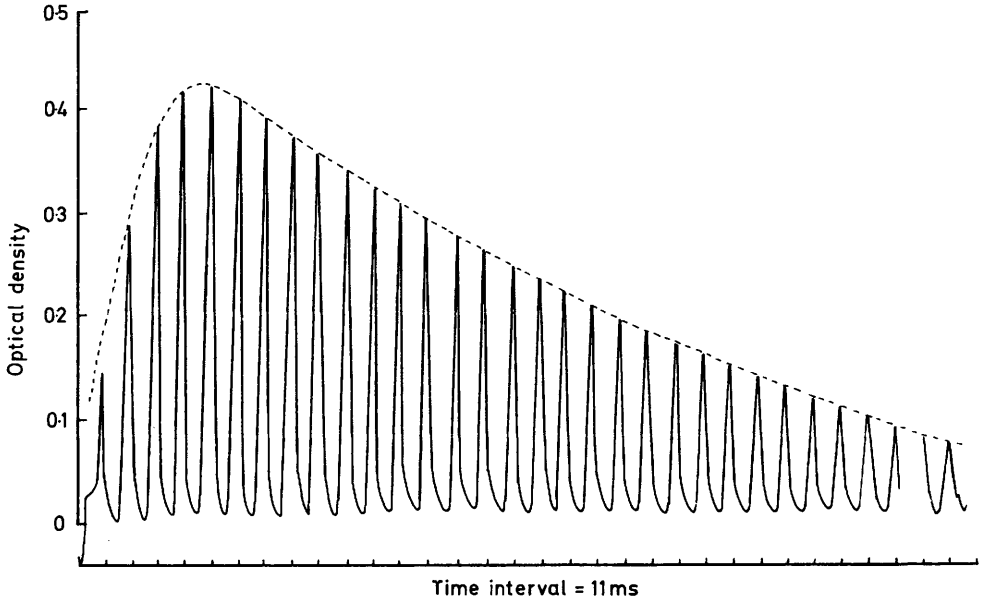


Figure 1. Typical kinetic run. Spectral scan from 405 to 960 nm. $[Cys]_0 = 0.01$, $[Fe]_0 = 0.000\ 25$ mol dm^{-3}, pH 2.99. Envelope calculated from the rate constants in the Table

Table. Typical values of first-order rate constants obtained at 25.0 °C and at an ionic strength of 0.10 mol dm^{-3} for reaction mixtures containing initial concentrations $[Fe]_0 = 0.000\,25$ mol dm^{-3} and $[Cys]_0 = 0.01$ mol dm^{-3}

pH	$10^5[H^+]$/ mol dm^{-3}	$k_2^{obs.}$/s^{-1}	$k_1^{obs.}$/s^{-1}	$10^4[Cys]_0/k_1^{obs.}$ mol dm^{-3} s	$10^3[Cys]_0/k_2^{obs.}$ mol dm^{-3} s
2.71	235	3.07	44.8	2.23	3.26
2.78	199	3.39	49.3	2.03	2.95
2.86	165	3.73	56.9	1.76	2.68
2.99	122	4.29	62.9	1.59	2.33
3.28	61.9	5.33	81.1	1.23	1.88
3.56	32.1	6.39	93.4	1.07	1.57
3.90	14.5	7.09	103.5	0.97	1.41
4.24*	6.63	10.25	96.2	1.04	0.98
4.52*	3.49	9.33	87.3	1.15	1.07
4.86*	1.58	14.11			

* The spectrum changed during the course of the kinetic run.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \quad (1)$$

pH was obtained directly from the decay part of each curve, and typical values are listed in the Table. It can be shown (see, for example, Schmid and Sapunov[5]) that the concentration of the intermediate, B, varies with time as in equation (2) in which

$$[B] = [A]_0 k_1 (k_2 - k_1)^{-1}(e^{-k_1 t} - e^{-k_2 t}) \quad (2)$$

$[A]_0$ is the initial concentration of A. By assuming that this was the initial concentration of iron(III) in the solutions, $[Fe]_0$ (see below), the values of $k_1^{obs.}$ at each pH listed in the Table were calculated.

Variation of the Observed Rate Constants with $[Fe]_T$ *and* $[Cys]_T$.—Over the whole of the pH range (2.71—4.86) the observed rate constants were independent of the initial iron(III) concentrations, $[Fe]_0$, and strictly first order with respect to the initial cysteine concentrations, $[Cys]_0$. In other words, neither rate depends upon the speciation, but only on the total amounts of iron(III), $[Fe]_T$, and cysteine, $[Cys]_T$. The basic experimental rate laws at constant pH are therefore as in equations (3) and (4).

$$d[\text{coloured complex}]/dt = (k_1^{obs.}[Cys]_0^{-1})[Fe]_T[Cys]_T \quad (3)$$

$$-d[\text{coloured complex}]/dt = (k_2^{obs.}[Cys]_0^{-1})[Fe]_T[Cys]_T \quad (4)$$

Dependence of $k_1^{obs.}$ *on* $[H^+]$.—The hydrogen-ion concentrations in the Table were calculated from the relationship (5)

$$[H^+] = 10^{-[(pH-0.131)/0.982]} \quad (5)$$

obtained by titrating HCl with KOH.[6] In Figure 2 $[Cys]_0/k_1^{obs.}$ is plotted against $[H^+]$ in accord with expression (6).

$$[Cys]_0/k_1^{obs.} = \alpha[H^+] + \beta \quad (6)$$

Dependence of $k_2^{obs.}$ *on* $[H^+]$.—Again, in order to show clearly the dependence of $k_2^{obs.}$ on $[H^+]$ the function $[Cys]_0/k_2^{obs.}$ has been plotted against $[H^+]$ in Figure 3 [see equation (7)]. The following values were extracted:

$$[Cys]_0/k_2^{obs.} = \gamma[H^+] + \delta \quad (7)$$

$\alpha = 5.80 \times 10^{-2}$ s, $\beta = 8.77 \times 10^{-5}$ mol dm^{-3} s, $\gamma = 0.846$ s, and $\delta = 1.28 \times 10^{-3}$ mol dm^{-3} s.

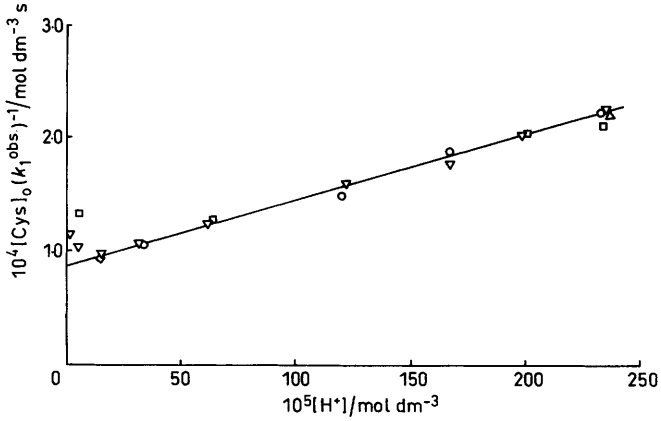


Figure 2. Dependence of $k_1^{obs.}$ on $[H^+]$. (*a*) For $[Cys]_0 = 0.01$, $[Fe]_0 = 0.001$ (○), 0.0005 (△), and 0.000 25 mol dm^{-3} (▽), (*b*) for $[Cys]_0 = 0.005$, $[Fe]_0 = 0.001$ (□) and 0.0005 mol dm^{-3} (◇)

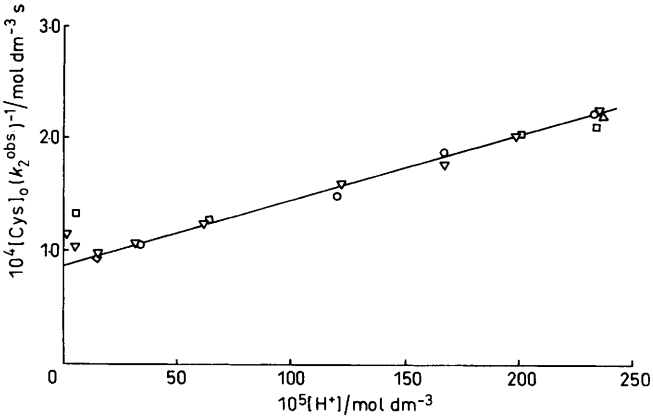


Figure 3. Dependence of $k_2^{obs.}$ on $[H^+]$. Details as in Figure 2

Interpretation of the Rate Laws.—(*i*) *Formation of the coloured complex.* It now remains to show that the empirical constants α and β in equation (6) are explicable in terms of a plausible reaction mechanism. We begin by postulating that over the pH range under investigation all of the cysteine present is in the form shown in (**I**), H_2L [equation (8)], and the iron(III)

$$[Cys]_T = [H_2L] \quad (8)$$

exists almost entirely as the aquated species Fe^{3+} and $[Fe(OH)]^{2+}$ [equations (9)—(11)].

$$[Fe(OH)]^{2+} + H^+ \rightleftharpoons Fe^{3+} \quad (9)$$

$$K^{FeOH} = [Fe^{3+}]/[Fe(OH)^{2+}][H^+] \quad (10)$$

$$[Fe]_T = [Fe(OH)^{2+}](K^{FeOH}[H^+] + 1) \quad (11)$$

It is then highly probable that the coloured complex is formed, at least in the first instance, by reaction (12). Using equations (8) and (11), equation (13) becomes (14). Comparison of equations (3) and (14) then yields (15) the reciprocal of which

$$[Fe(OH)]^{2+} + H_2L \xrightarrow{k_1} [Fe(OH)(H_2L)]^{2+} \quad (12)$$

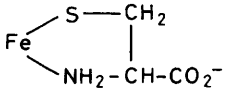
$$d[Fe(OH)(H_2L)^{2+}]/dt = k_1[Fe(OH)^{2+}][H_2L] \quad (13)$$

$$d[Fe(OH)(H_2L)^{2+}]/dt = k_1(K^{FeOH}[H^+] + 1)^{-1}[Fe]_T[Cys]_T \quad (14)$$

$$k_1^{obs.}[Cys]_0^{-1} = k_1(K^{FeOH}[H^+] + 1)^{-1} \quad (15)$$

is identical to (6) if we put $\alpha = K^{FeOH}/k_1$ and $\beta = k_1^{-1}$. Making use of the experimental values of α and β thus gives $k_1 = 1.14 \times 10^4$ dm^3 mol^{-1} s^{-1} and $K^{FeOH} = 6.61 \times 10^2$ dm^3 mol^{-1} ($\log K^{FeOH} = 2.82$). Note that nothing is implied as to the actual structure of the coloured complex since (*a*) the reacting species are arbitrarily defined in equation (12) and (*b*) there could well have been a rapid rearrangement of the initial species, say by rapid proton loss, afterwards.

(*ii*) *Decomposition of the coloured complex.* In order to show that the empirical constants γ and δ are indeed explicable in terms of a plausible rate mechanism it is necessary to begin by assigning a structure to the coloured complex in the light of the results in (*i*) above. We assume that the complex is $[FeL]^+$, *i.e.* that the initial interaction is followed by rapid proton loss from the co-ordination sites and protonation of the iron(III) hydroxyl group to give structure (**II**). If the internal redox



(**II**)

reaction is then kinetically controlled by protonation of the ligand amino group we then have equations (16) and (17). The rate law is then expressible as in equation (18).

$$[FeL]^+ + H^+ \xrightarrow{k_2} Fe^{2+} + HL^{\bullet} \quad (16)$$

$$HL^{\bullet} + HL^{\bullet} \xrightarrow{fast} \text{cystine} \quad (17)$$

$$-d[\text{coloured complex}]/dt = k_2[FeL^+][H^+] \quad (18)$$

In order to simplify equation (18) we define the equilibrium (19). Substituting equation (20) in (18) followed by (8) and (13)

$$[Fe(OH)]^{2+} + HL^- \rightleftharpoons [FeL]^+ + H_2O \quad (19)$$

$$K' = [FeL^+]/[Fe(OH)^{2+}][HL^-] \quad (20)$$

leads to equation (21) where K_2^H is the second protonation

$$-d[FeL^+]/dt = k_2K'(K_2^H)^{-1}(K^{FeOH}[H^+] + 1)^{-1}[Fe]_T[Cys]_T \quad (21)$$

constant for cysteine [equation (22)]. Comparison with (4) then

$$K_2^H = [H_2L]/[HL^-][H^+] \quad (22)$$

gives equation (23), the reciprocal of which is clearly identifiable

$$k_2^{obs.}[Cys]_0^{-1} = k_2K'(K_2^H)^{-1}(K^{FeOH}[H^+] + 1)^{-1} \quad (23)$$

with equation (7) when $\gamma = K^{FeOH}K_2^H/k_2K'$ and $\delta = K_2^H/k_2K'$. From the experimentally determined values of γ and δ, therefore, $k_2K' = 1.63 \times 10^{11}$ dm^6 mol^{-2} s^{-1} and $K^{FeOH} = 6.61 \times 10^2$ dm^3 mol^{-1} in which k_2K' is the apparent third-order rate constant for the reaction (K_2^H has been taken as 2.09×10^8 dm^3 mol^{-1}, a value obtained kinetically [7] for the reaction carried out at pH >9), and K^{FeOH} is identical with that obtained in (*i*) above.

Finally, use can be made of equation (2) to calculate the molar absorption coefficient, ε, of the coloured complex, (**I**), and this was done using the maximum absorbance at 614 nm for each kinetic run; a mean value of $\varepsilon = 1\,030$ dm^3 mol^{-1} cm^{-1} was obtained.

Discussion

(*i*) *Formation of the Coloured Species.*—There seems little doubt that the species giving rise to the colour is $[FeL]^+$ as shown in (**I**) and that it is formed by the reaction of $[Fe(OH)]^{2+}$ with the ligand H_2L, followed by rapid deprotonation at the sulphur site. Its structure will be discussed below. Note that this is not possible to establish kinetically on the basis of its formation alone, but seems a safe assumption when the decomposition of the complex is taken into account. It is interesting that the rate constant, k_1, calculated on this basis is very similar to that obtained [8] for the reaction of $[Fe(OH)]^{2+}$ with SCN^- (1.0×10^4 dm^3 mol^{-1} s^{-1}).

The presence of chloride ions certainly helps to prevent precipitation in the low pH stock solutions of iron(III) (because of the formation of weak chloro-complexes) but these are not able to prevent precipitation in the absence of cysteine over the pH range studied kinetically. Although it seems unlikely to us, the existence of co-ordinated chloride ions in the iron–cysteine complexes cannot of course be ruled out without detailed study of how the chloride concentration affects the kinetics.

(*ii*) *Decomposition of the Coloured Species.*—The observed kinetics presented above lead one to propose, from the hydrogen-ion concentration dependence, that the iron(III) complex is formed by the loss of only one proton. This implies that the complex is as shown in (**I**) and that the internal redox reaction is then triggered by protonation of the amino group bound to the iron(III). In basic media not only is iron(III) co-ordinated to hydroxide in the complexes (which are purple and not blue) but the decomposition follows a completely different path.[7]

Addition of oxygen to the reaction mixture does not result in any observable return of the blue colour, and this might merely reflect the relative rates of the redox reactions of iron(II) with dioxygen and of iron(III) with cysteine.

An unresolved feature of the present kinetic study is the fate of the free radical formed by the one-electron transfer from the sulphur atom of cysteine to the iron atom. Since the final products of the reaction are cystine (see below) and iron(II), it

$$^{-}O_2C\text{–}\underset{\displaystyle NH_3^{+}}{\underset{|}{CH}}\text{–}CH_2\text{–}S\text{–}S\text{–}CH_2\text{–}\underset{\displaystyle NH_3^{+}}{\underset{|}{CH}}\text{–}CO_2^{-}$$

must be assumed that cystine is formed from the interaction of two free radicals as in equations (16) and (17).

Experimental

All kinetic measurements were carried out on a stopped-flow spectrograph supplied complete with multichannel analyser, *etc.* by Applied Photophysics Ltd., London. Cysteine solutions were made up with L-cysteine (pure) from Merck, and iron(III) solutions from iron(III) chloride hexahydrate (Pro Analyse) from Merck.

Solutions of the required final pH were made up from deoxygenated stock solutions of cysteine and of iron(III) that contained calculated amounts of HCl and sufficient KCl to maintain the final ionic strength at 0.10 mol dm^{-3}. The pH employed was that measured directly after each kinetic run. All preparative work was carried out in a nitrogen-filled glove-box, the reaction solutions being transferred to the stopped-flow apparatus in sealed syringes.

The pH-meter was a Schott C6818.

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