Metal-ion Catalysis in Some Reactions of Hexacyanoferrate(III) Ions. Part II.[1] The Oxidation of Cysteine and Related Thiols in the Presence of Ethylenediaminetetra-acetic Acid

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The rates and mechanism of the reaction of hexacyanoferrate(III) ions with cysteine, *N*-acetylcysteine, and 3-mercaptopropanoic acid have been examined in the presence of ethylenediaminetetra-acetic acid (EDTA). Copper catalysis, which is dominant in the absence of EDTA, persists, but with changes in behaviour which appear to reflect the change in the reduction potential of copper(II) and the differential lability of complexes of the II and I oxidation states. The results suggest that care is necessary if valid conclusions are to be drawn from experiments in which species like EDTA are used to eliminate reactions of metal cations.

THE oxidation by hexacyanoferrate(III) ions of some thiols has been studied previously.[1] These reactions, under simple conditions, are due entirely to catalytic paths involving copper ions, present in less than 1 p.p.m. in the solutions, originating in the reagents and in the solvent. In the present paper we examine the reaction path when the copper is 'masked' by co-ordination with ethylenediaminetetra-acetic acid (EDTA). The equilibrium constant for formation of the copper complex is well known[2] (*ca.* 5×10^{18}), and this species has been used often to retard metal-ion catalysed paths[3] in the decomposition of peroxo-complexes. There is scattered but ample evidence that reactions of various thiols with copper(II) ions have high stability constants. There is thus reason to anticipate that 1 : 1 : 1 mixed complexes involving copper, EDTA, and thiols can be formed. The possibility exists that two or even three co-ordination sites may be available for the thiol.

It has been found[4,5] that rates of reaction of thiols with hexacyanoferrate(III) ions are lowered on addition of EDTA, and hence suggested that the reactions involve catalysis by metal ions, probably iron or copper. Some semiquantitative kinetic experiments have also been made. In this study with cysteine, *N*-acetylcysteine, and 3-mercaptopropanoic acid, the rates of reaction in the presence of EDTA were much lower than those in the absence of EDTA.[1] Most work therefore involved the most reactive compound, cysteine; use of the other compounds served to confirm the patterns of behaviour.

EXPERIMENTAL

Reagents and procedures used have been described previously.[1] Disodium ethylenediaminetetra-acetate was recrystallised[6] before use. The low concentrations required were attained by dilution of stock solutions, stored in Polythene bottles.

A full re-examination of the stoicheiometry of the reaction was not carried out. The hexacyanoferrate isosbestic point at 280·8 nm was accurately maintained throughout the reaction with cysteine. As previously, the disulphide compounds precipitated during reactions at the higher concentrations used for investigation of product formation. There was no evidence for sulphur-containing products other than the disulphide compounds.

Linear dependences are given as equations fitted by linear regression, with 95% confidence limits.

RESULTS AND DISCUSSION

Dependences of the Rate of Reaction.—Concentration of EDTA. The dependence of the initial rates of reduction of hexacyanoferrate(III) ions on the concentration of EDTA is illustrated in Figure 1 for cysteine and *N*-acetylcysteine. 3-Mercaptopropanoic acid behaved similarly to *N*-acetylcysteine. The linear decrease in the rate of reaction at low [EDTA] suggests that catalytically active copper ion becomes almost inactive when complexed. The extrapolation of this linear portion to zero rate gave as intercept EDTA concentrations which were very similar to the concentration of copper ions in the solutions.[1]

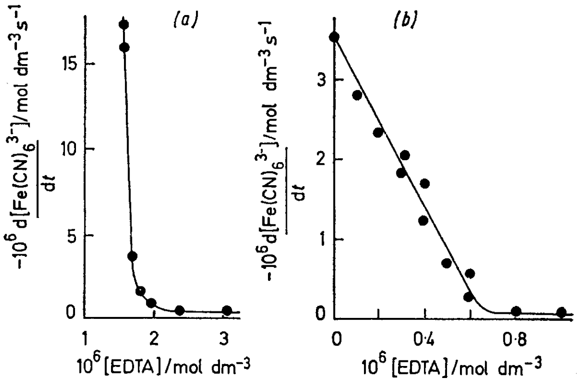

FIGURE 1 Change of initial rate of hexacyanoferrate(III) ion reduction with concentration of EDTA at $[Fe(CN)_6^{3-}] = 4{\cdot}0 \times 10^{-4}$ mol dm^{-3} and pH 4·05: (*a*) cysteine ($0{\cdot}40 \times 10^{-3}$ mol dm^{-3}); (*b*) *N*-acetylcysteine ($4{\cdot}0 \times 10^{-3}$ mol dm^{-3})

Ions which have larger equilibrium constants for reaction with EDTA than copper, added in or close to the linear region, may lead to an increase in the rate of

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[1] Part I, G. J. Bridgart, M. W. Fuller, and I. R. Wilson, preceding paper.

[2] 'Stability Constants of Metal-ion Complexes,' Chem. Soc. Special Publ. No. 17, 1964.

[3] For example, S. B. Brown, P. Jones, and A. Suggett in 'Inorganic Reaction Mechanisms,' ed. J. O. Edwards (*Progr. Inorg. Chem.*, vol. 13, ed. S. J. Lippard), Interscience, New York, 1970, p. 159.

[4] W. Tysarowski and A. Konecka, *Acta Biochim. Polon.*, 1965, XII, 281.

[5] G. Gorin and W. E. Godwin, *J. Catalysis*, 1966, **5**, 279.

[6] A. I. Vogel, 'A Textbook of Quantitative Inorganic Analysis,' 3rd edn., Longmans, London, 1962, p. 433.

reaction due to ' demasking ' of the copper ions. This was observed, for example, on addition of iron(II) ions. The reported [5] catalysis by iron and other metal ions in reaction mixtures containing EDTA may have arisen in this way. We sought evidence for catalysis by iron(II) and the complex [Fe^{II}(edta)], but found none.

There was catalysis by the complex [Cu^{II}(edta)]. In solutions with $10^3[Fe(CN)_6{}^{3-}] = 0{\cdot}50$, 10^3[Cysteine] = 5·0, 10^3[EDTA] = 0·010 mol dm^{-3}, at pH 4·05 and 25 °C, we found, for added [Cu^{II}(edta)] between 0 and 8 × 10^{-7} mol dm^{-3}, that equation (1) was applicable.

$$-d[Fe(CN)_6{}^{3-}]/dt = (0{\cdot}58 \pm 0{\cdot}06)[Cu^{II}(edta)] + (1{\cdot}7 \pm 0{\cdot}3) \times 10^{-7}\ \text{mol dm}^{-3}\ \text{s}^{-1} \quad (1)$$

The constant term would be explained if 2·9 × 10^{-7} mol dm^{-3} copper were present as impurity; the known (minimum) was 1·1 × 10^{-7} mol dm^{-3}.

Thiol concentration for cysteine. The rates of reaction were very sensitive to the thiol concentration. Initial rates, with $10^3[Fe(CN)_6{}^{3-}] = 1{\cdot}0$, 10^5[EDTA] = 0·80 mol dm^{-3}, and pH 4·05, are correlated by equation (2) for 6 < 10^3[Cysteine] < 16.

$$-d[Fe(CN)_6{}^{3-}]/dt = (1{\cdot}48 \pm 0{\cdot}24) \times 10^{-3}[\text{Cysteine}]^2 + (0{\cdot}148 \pm 0{\cdot}026)[\text{Cysteine}]^3 \quad (2)$$

Thus, in experiments conducted with cysteine concentrations about 10^{-2} mol dm^{-3}, the two terms in the equation will contribute about equally, the second increasing in importance as [Thiol] rises. It is also to be noted that this rate law is one order higher than that (first plus second order) observed in the absence of EDTA.

When a substantial amount of hexacyanoferrate(II) ions was present, the thiol dependence became simpler, and the third-order term was no longer in evidence. For example, with $10^3[Fe(CN)_6{}^{3-}] = 0{\cdot}50$, $10^3[Fe(CN)_6{}^{4-}] = 0{\cdot}80$, 10^5[EDTA] = 0·80 mol dm^{-3}, and pH 4·05, for 2·5 < 10^3[Cysteine] < 7 equation (3) applies. Under these conditions, rates of reaction were higher than those in the absence of $Fe(CN)_6{}^{4-}$.

$$-d[Fe(CN)_6{}^{3-}]/dt = (1{\cdot}021 \pm 0{\cdot}084) \times 10^{-2}[\text{Cysteine}]^2 \quad (3)$$

Concentrations of hexacyanoferrate-(III) *and* -(II). Initial rates of reaction, studied with varying concentrations of hexacyanoferrate(III) ions [hexacyanoferrate(II) ions not added], were independent of the oxidant concentration, at least over a five-fold variation. This was observed at two cysteine concentrations, and both terms of equation (2) are therefore independent of hexacyanoferrate(III)-ion concentration.

Addition of hexacyanoferrate(II) ions, in amounts comparable to those of hexacyanoferrate(III), led to a first-order dependence on $[Fe(CN)_6{}^{3-}]$. Thus, with $10^3[Fe(CN)_6{}^{4-}] = 1{\cdot}0$, 10^3[Cysteine] = 8·0, 10^5[EDTA] = 1·0 mol dm^{-3}, and pH 4·05, for 2 < $10^4[Fe(CN)_6{}^{3-}]$ < 9 equation (4) applies.

$$-d[Fe(CN)_6{}^{3-}]/dt = (0{\cdot}418 \pm 0{\cdot}036) \times 10^{-2}[Fe(CN)_6{}^{3-}] \quad (4)$$

With increasing amounts of hexacyanoferrate(II) ions (Figure 2), the initial rates of reaction showed a progressive (not a catastrophic) increase. The initial value (1·9 × 10^{-7} mol dm^{-3} s^{-1}) was similar to that (1·7 × 10^{-7} mol dm^{-3} s^{-1}) calculated from equation (2), and the higher values were comparable to those calculated from equation (4). The magnitude of these changes shows that minor contamination of the hexacyanoferrate(III) ion solutions with hexacyanoferrate(II) ions is not the sole cause of the effect on the rates of reaction observed in alleged absence of the latter. In particular, the rates of reaction which were found to be independent of hexacyanoferrate(III)-ion concentration were, in fact, measured in the effective absence of hexacyanoferrate(II) ions. The measurement of initial rates was made more difficult by this autocatalysis which is also evident in curves previously published.[5] The complex kinetics preclude use of a simple integrated form; in particular, $[Fe(CN)_6{}^{3-}]^{-1}$ is not linear with time.

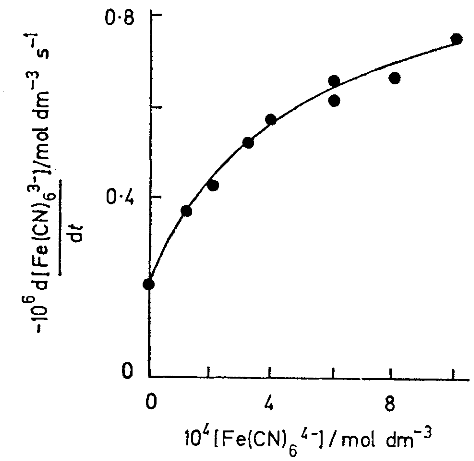

FIGURE 2 Variation of initial reaction rate with hexacyanoferrate(II)-ion concentration: $[Fe(CN)_6{}^{3-}] = 4{\cdot}0 \times 10^{-4}$; [Cysteine] = 8·0 × 10^{-3}; [EDTA] = 1·0 × 10^{-5} mol dm^{-3}

The increase of rate with increase of hexacyanoferrate(II)-ion concentration was most unexpected. One confirmed consequence was that two series of initial-rate experiments with increasing concentration of EDTA, (*a*) in which hexacyanoferrate(II) ions were absent and (*b*) in which hexacyanoferrate(II) ions were present at some constant concentration, showed a transition from rate (*a*) > rate (*b*) to rate (*a*) < rate (*b*). This behaviour is illustrated in Figure 3. Rates of reaction in such series became equal at concentrations near estimates of the concentration of copper ion in the solutions.

pH. The rates of reaction of cysteine and of *N*-acetylcysteine were examined between pH 3·8 and 4·6 in acetate buffers, in the absence of hexacyanoferrate(II) ions. Both reactions showed a linear dependence of the

$$-d[Fe(CN)_6{}^{3-}]/dt = l[H_3O^+]^{-1} + m \quad (5)$$

rate on $[H_3O^+]^{-1}$ [equation (5)] and the following values were found.

$\frac{10^2[\mathrm{RSH}]}{\mathrm{mol\ dm^{-3}}}$	$\frac{10^3[\mathrm{Fe(CN)_6}^{3-}]}{\mathrm{mol\ dm^{-3}}}$	$\frac{10^5[\mathrm{EDTA}]}{\mathrm{mol\ dm^{-3}}}$	$\frac{10^{11}l}{\mathrm{mol^2\ dm^{-6}s^{-1}}}$	$\frac{10^7m}{\mathrm{mol\ dm^{-3}s^{-1}}}$
Cysteine				
1·2	0·50	0·60	0·60 ± 0·10	4·0 ± 0·2
N-Acetylcysteine				
1·2	0·40	0·20	0·34 ± 0·09	2·3 ± 0·3

Effects of EDTA *on Copper-catalysed Reactions*.—The rates of reaction observed in the present system do not arise from aquocopper(II) ions since their concentration is less than 10^{-18} mol dm^{-3}. The catalytic species is either [Cu^{II}(edta)] or a mixed copper–edta–thiol complex. The effect of complexing with EDTA on the electrode potential is relevant if the mechanism involves a Cu^{II}–Cu^{I} chain. The electrode potential does not appear to have been measured with any certainty, but an approximation can be made. The stability constant of the complex [Cu^{I}(edta)] may be similar to that for [Ag^{I}(edta)], *viz.* 2×10^7;[2] it has been independently suggested[7] that it is greater than or equal to 3×10^7. Hence, using other available data,[2] the equilibrium constant for process (6) may be calculated to be 10^{15}. The change

$$[\mathrm{Cu^{I}(edta)}] + [\mathrm{Fe(CN)_6}]^{3-} \rightleftharpoons [\mathrm{Cu^{II}(edta)}] + [\mathrm{Fe(CN)_6}]^{4-} \quad (6)$$

from the value for the aquocopper species (2×10^3) is dramatic. Although it has been claimed[8] that EDTA has little effect on the electrode potential, this is inconsistent with qualitative chemical evidence presented by the same authors. With changes in equilibrium constants as great as this (the better known case[9] of Fe^{III}–Fe^{II} is comparable), it is not surprising that complexing with a suitable reagent should retard one of the reactions of a catalytic chain to a point at which the chain becomes virtually ineffectual.

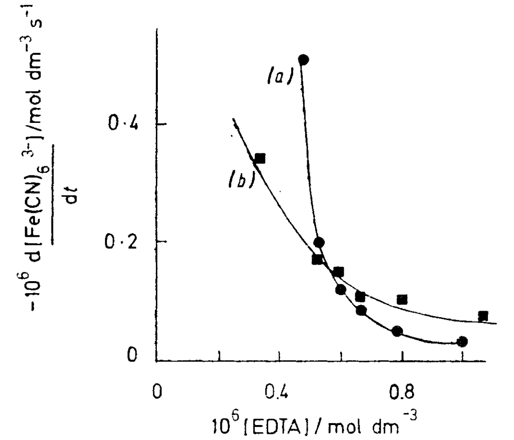

FIGURE 3 Variation of initial rates of reaction with concentration of EDTA for *N*-acetylcysteine ($1·0 \times 10^{-2}$ mol dm^{-3}) with $[\mathrm{Fe(CN)_6}^{3-}]_0 = 5·0 \times 10^{-4}$ mol dm^{-3} and [curve (*b*) only] $[\mathrm{Fe(CN)_6}^{4-}] = 5·0 \times 10^{-4}$ mol dm^{-3}

Evidence has been presented[4-7] to support the claim that the complex [Cu^{II}(edta)] has certainly one, probably two, and perhaps three co-ordination sites available to thiol molecules. The rates at which these sites are filled and vacated may be high. The equilibrium co-ordination number of copper(I) complexes of the present groups is probably 4 or 2.

A Tentative Mechanism.—The quantitative features of the present reactions are predicted by equations (7)—(16), where M represents the ethylenediaminetetra-acetatocopper complex and RSH the thiol. If it is

$$\mathrm{M^{II}} + \mathrm{RSH} \overset{K_1}{\rightleftharpoons} \mathrm{M^{II}(HSR)} \quad (7)$$

$$\mathrm{M^{II}(HSR)} + \mathrm{RSH} \overset{K_2}{\rightleftharpoons} \mathrm{M^{II}(HSR)_2} \quad (8)$$

$$\mathrm{M^{II}(HSR)_2} \xrightarrow{k_3} \mathrm{M^{I}} + \mathrm{RSSRH^{\bullet}} + \mathrm{H^+} \quad (9)$$

$$\mathrm{M^{II}(HSR)_2} + \mathrm{RSH} \xrightarrow{k_4} \mathrm{M^{I}(HSR)} + \mathrm{RSSRH^{\bullet}} + \mathrm{H^+} \quad (10)$$

$$\mathrm{M^{II}(HSR)_2} + [\mathrm{Fe(CN)_6}]^{4-} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \mathrm{M^{I}(HSR)_2} + [\mathrm{Fe(CN)_6}]^{3-} \quad (11)$$

$$\mathrm{M^{I}(HSR)_2} + [\mathrm{Fe(CN)_6}]^{3-} \xrightarrow{k_6} \mathrm{M^{I}} + \mathrm{RSSRH^{\bullet}} + \mathrm{H^+} + [\mathrm{Fe(CN)_6}]^{4-} \quad (12)$$

$$\mathrm{RSSRH^{\bullet}} + [\mathrm{Fe(CN)_6}]^{3-} \xrightarrow{k_7} \mathrm{RSSR} + [\mathrm{Fe(CN)_6}]^{4-} + \mathrm{H^+} \quad (13)$$

$$\mathrm{RSSR^{\bullet}} + [\mathrm{Fe(CN)_6}]^{4-} \xrightarrow{k_8} \mathrm{RS^-} + [\mathrm{Fe(CN)_6}]^{3-} + \mathrm{RSH} \quad (14)$$

$$\mathrm{M^{I}} + [\mathrm{Fe(CN)_6}]^{3-} \xrightarrow{\text{fast}} \mathrm{M^{II}} + [\mathrm{Fe(CN)_6}]^{4-} \quad (15)$$

$$\mathrm{M^{I}(HSR)} + [\mathrm{Fe(CN)_6}]^{3-} \xrightarrow{\text{fast}} \mathrm{M^{II}(HSR)} + [\mathrm{Fe(CN)_6}]^{4-} \quad (16)$$

assumed that the copper present in the system is almost entirely in the form of the complex [Cu^{II}(edta)], and if the steady-state assumption is used for $\mathrm{RSSRH^{\bullet}}$ and $\mathrm{M^{I}(HSR)_2}$, the predicted rate law emerges as (17).

$$-\mathrm{d}[\mathrm{Fe(CN)_6}^{3-}]/\mathrm{d}t = 2\mathrm{d}[\mathrm{RSSR}]/\mathrm{d}t = \frac{2K_1K_2k_7[\mathrm{Fe(CN)_6}^{3-}][\mathrm{M}][\mathrm{RSH}]^2}{k_7[\mathrm{Fe(CN)_6}^{3-}] + k_8[\mathrm{Fe(CN)_6}^{4-}]}\left(k_3 + k_4[\mathrm{RSH}] + \frac{k_5k_6[\mathrm{Fe(CN)_6}^{4-}]}{k_{-5} + k_6}\right) \quad (17)$$

In writing the above mechanisms, the product-forming steps have been postulated to resemble those in the previous paper,[1] because of similarities in the rate laws. The first two terms correspond to spontaneous rearrangement and to thiol-assisted rearrangement of a copper(II)–edta–thiol complex. The most restrictive aspect of

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[7] K. Srinavasan and R. S. Subrahmanya, *J. Electroanalyt. Chem.*, 1971, **31**, 257.

[8] R. Belcher, D. Gibbons, and T. S. West, *Analyt. Chim. Acta*, 1955, **12**, 107.

[9] G. Schwarzenbach and J. Heller, *Helv. Chim. Acta*, **1951**, **34**, 576.

this mechanism is the requirement of dependences on reductant and oxidant concentrations. We now propose that formation of a copper(I) complex confers sufficient kinetic advantage for the oxidative rearrangement to be fast. In Part I,[1] attention was drawn to the fact that copper(I) thiol complexes are formed in the absence of hexacyanoferrate(III), ions and it is possible that multiple complexing (of the EDTA complex) by thiol facilitates reduction. The estimate given for the equilibrium constant of the $[Cu^I(edta)]$–$[Fe(CN)_6]^{3-}$ reaction shows that insignificant amounts of the $[Cu^I(edta)]$ complex are present at equilibrium.

From equation (2), it is possible to estimate that $k_3/k_4 = (1{\cdot}4 \times 10^{-3})/0{\cdot}15 = 10^{-2}$ for cysteine, and from the variation of reaction rate with $[Cu^{II}(edta)]$ concentrations, we estimate $K_1K_2k_3 = 8 \times 10^3$. From the variation of reaction rate with [Cysteine] in the presence of hexacyanoferrate(II) ions, k_8/k_7 and k_5/k_3 may be estimated as 1·0 and 6×10^7 respectively.

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