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Redox Chemistry of $[Fe_2(CN)_{10}]^{4-}$. Part 4.† Reaction with L-Cysteine‡

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L-Cysteine reduces $[Fe_2(CN)_{10}]^{4-}$ to $[Fe_2(CN)_{10}]^{6-}$ in a two stage process, a rapid reduction to $[Fe_2(CN)_{10}]^{5-}$ followed by a slower second order reaction involving $HSCH_2CH(NH_3^+)CO_2^-$ and a conjugate base.

The binuclear $Fe^{III}Fe^{III}$ complex $[Fe_2(CN)_{10}]^{4-}$ is a moderate oxidising agent with successive one electron reduction potentials[1] (against NHE) of 0.55 and 0.36 V respectively. It is an inert, diamagnetic complex and reacts with many reducing agents; with iodide reaction proceeds[2] to the $Fe^{III}Fe^{II}$ compound, but with ascorbic acid the additional stage to form $Fe^{II}Fe^{II}$ has also been observed.[3] When the reductant contains a nucleophilic sulfur centre as in $S_2O_3^{2-}$ and $(NH_2)_2CS$ then the binuclear complex may be cleaved and the iron(III) species $[Fe(CN)_5(S_{nuc})]^{n-}$ where $n = 4$ for thiosulfate[4] and $n = 2$ for thiourea can be observed in the products. These species are readily recognised by a strong absorption in the 550–650 nm range, giving rise to a pronounced blue colour. The present paper describes a study of the reaction with L-cysteine, which is a powerful reducing agent ($E° = -0.34$ V at pH 7) and also contains a nucleophilic sulfur.

Reaction was followed by monitoring the decrease in absorbance at 560 nm, the wavelength of the visible maximum for $[Fe_2(CN)_{10}]^{4-}$. Preliminary work showed that there were two steps, an initial fast reaction which was too rapid to follow under our conditions in which the $[Fe_2(CN)_{10}]^{4-}$ peak at 560 nm disappeared and was replaced by the near infra-red peak of $[Fe_2(CN)_{10}]^{5-}$ at 1200 nm. This was followed by a much slower process forming a pale yellow/green species with the spectrum[5] of $[Fe_2(CN)_{10}]^{6-}$. This change could readily be followed by stopped flow. The reaction was studied over the ranges 25–35 °C, pH 3.6–6.4. Individual runs for this second process gave good first order plots, yielding k_{obs}/s^{-1} values that are directly proportional to [L-cysteine] as shown in Table 1. The k_{obs} values varied with pH as shown in Table 2. At the higher acidities studied, pH 3.63–4.63, the change of k_{obs} with pH is very slow, but at higher pH values the rate constant rises rapidly. Measurements made at 29.7 and 35 °C showed similar behaviour.

In view of the observations of Wilson *et al.*[6] that trace amounts of copper catalyse the cysteine–$[Fe(CN)_6]^{3-}$ reaction we extended our original measurements to see if copper catalysis is important here also. Wilson comments on the lack of reproducibility in earlier work on the ferricyanide oxidation of thiols, and inspection of the data in Table 1 does show some scatter. We have analysed our reactants, buffers and distilled water for copper by AAS and conclude that the copper concentration is less than 20 ppb (our estimated detection limit); this corresponds to 3.15×10^{-7} mol dm^{-3}. In an attempt to complex copper (or other trace metal ions) we added $Na_2[EDTAH_2]$ over the concentration range $2–130 \times 10^{-7}$ mol dm^{-3}, but did not observe any effect on k_{obs}. Added copper ($CuSO_4$) did produce a marked catalytic effect, plots of k_{obs} *vs.* $[Cu^{2+}]$ being linear, with a well defined intercept on the $[Cu^{2+}] = 0$ axis. Two other differences from the work of Wilson *et al.* may be noted. For the reaction of $[Fe(CN)_6]^{3-}$ with cysteine pseudo second order kinetics {w.r.t. $[Fe(CN)_6]^{3-}$} were observed whereas in our case all runs gave good first order kinetics. Also for the $[Fe(CN)_6]^{3-}$ oxidations with cysteine, *N*-acetylcysteine and 3-sulfanylpropionic acid Wilson found an apparent order in [thiol] that was greater than one.

Table 1 Values of k_{obs} for the reaction of L-cysteine with $[Fe_2(CN)_{10}]^{5-}$. $[Fe_2(CN)_{10}^{5-}] = 1.7 \times 10^{-4}$ mol dm^{-3}; I = 1.0 mol dm^{-3} ($NaClO_4$); pH = 5.41 (acetate buffers); θ = 30.0 °C

10^3[Cysteine]/mol dm^{-3}	k_{obs}/s^{-1}	k_{obs}/[cysteine]
10	3.21	3.21
12	4.36	3.63
14	4.59	3.28
18	6.09	3.38
22	7.18	3.06
24	8.66	3.61
26	9.46	3.64

$$\frac{-d[Fe(CN)_6{}^{3-}]/dt}{[thiol]} = k_a + k_b[thiol] \qquad (1)$$

Our data in Table 1, though scattered, show no sign of any systematic trend in k_{obs}/[cysteine] with [cysteine]. We conclude that catalysis by adventitious copper has only a minor effect in our system, though with the *addition* of significant amounts of copper there is undoubtedly some catalysis.

Our oxidant, $[Fe_2(CN)_{10}]^{4-}$, is a low spin, inert complex, and so we propose that the very fast initial reaction is due to an outer sphere electron transfer to form $[Fe_2(CN)_{10}]^{5-}$. This is followed by a slower outer sphere reaction to form $[Fe_2(CN)_{10}]^{6-}$. The reduction potential for $[Fe_2(CN)_{10}]^{4-}$ is 0.55 V, considerably higher than the 0.36 V for $[Fe_2(CN)_{10}]^{5-}$ thus providing much more driving force for the first reaction. While we conclude that there is little or no

Table 2 Values of k_{obs} for the reaction of L-cysteine with $[Fe_2(CN)_{10}]^{5-}$. [L-cysteine] = 0.020 mol dm^{-3}; $[Fe_2(CN)_{10}]^{5-} = 1.7 \times 10^{-4}$ mol dm^{-3}; I = 1.0 mol dm^{-3} ($NaClO_4$). θ = 25 °C

pH	k_{obs}/s^{-1}
3.63	0.59
4.14	0.52
4.36	0.66
4.53	0.69
4.72	0.88
4.93	1.27
5.10	1.98
5.32	3.28
5.54	4.95
5.63	5.38
5.94	8.73

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†Part 3: see ref. 3.

‡This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

$$\mathrm{Fe_2(CN)_{10}}^{4-} + \text{cysteine} \xrightarrow{\text{fast}} \mathrm{Fe_2(CN)_{10}}^{5-} + \text{cysteine}^{+\bullet}$$

$$\mathrm{Fe_2(CN)_{10}}^{5-} + \mathrm{A} \xrightarrow{k_1} \mathrm{Fe_2(CN)_{10}}^{6-} + \mathrm{A}^{+\bullet}$$

$$\mathrm{Fe_2(CN)_{10}}^{5-} + \mathrm{B} \xrightarrow{k_2} \mathrm{Fe_2(CN)_{10}}^{6-} + \mathrm{B}^{+\bullet}$$

$$\mathrm{Fe_2(CN)_{10}}^{5-} + \mathrm{C} \xrightarrow{k_3} \mathrm{Fe_2(CN)_{10}}^{6-} + \mathrm{C}^{+\bullet}$$

$$2\mathrm{A}^{+\bullet}\ (\text{or } \mathrm{B}^{+\bullet} \text{ or } \mathrm{C}^{+\bullet}) \longrightarrow \text{cystine}$$

Scheme 1

copper catalysis for the second reaction we cannot exclude the possibility that it may contribute to the first reaction, though the fact that the first reaction was still fast in the presence of added edta argues against it. In the absence of kinetic data we cannot go further in discussing the first reaction.

For the second reaction the variation in k_{obs} with pH must surely be due to the acid/base equilibria of L-cysteine. It has three ionisable groups, carboxyl (CO_2H), amino (NH_3^+) and sulfhydryl (SH). In aqueous solution L-cysteine can exist in five different forms depending on the pH of the solution [eqns. (2)–(6)]. The values for the respective dissociation constants[7] are $pK_1 = 2.0$; $pK_2 = 8.53$; $pK_3 = 8.86$; $pK_4 = 10.36$; $pK_5 = 10.03$.

$$\mathrm{HSCH_2CH(NH_3^+)CO_2H} \overset{K_1}{\rightleftharpoons} \mathrm{HSCH_2CH(NH_3^+)CO_2^-} + \mathrm{H^+} \quad (2)$$

$$\mathrm{HSCH_2CH(NH_3^+)CO_2^-} \overset{K_2}{\rightleftharpoons} \mathrm{^-SCH_2CH(NH_3^+)CO_2^-} + \mathrm{H^+} \quad (3)$$

$$\mathrm{HSCH_2CH(NH_3^+)CO_2^-} \overset{K_3}{\rightleftharpoons} \mathrm{HSCH_2CH(NH_2)CO_2^-} + \mathrm{H^+} \quad (4)$$

$$\mathrm{^-SCH_2CH(NH_3^+)CO_2^-} \overset{K_4}{\rightleftharpoons} \mathrm{^-SCH_2CH(NH_2)CO_2^-} + \mathrm{H^+} \quad (5)$$

$$\mathrm{HSCH_2CH(NH_2)CO_2^-} \overset{K_5}{\rightleftharpoons} \mathrm{^-SCH_2CH(NH_2)CO_2^-} + \mathrm{H^+} \quad (6)$$

Over our pH range it is clear from the pK_a values that the main component of cysteine, more than 98%, is $HSCH_2CH(NH_3^+)CO_2^-$ with only minor contributions from other species. The fact that k_{obs} is almost constant from pH 3.63 to 4.36 at 25 °C suggests that the bimolecular rate constant for $HSCH_2CH(NH_3^+)CO_2^-$ must be close to $0.59/0.02 = 29.5\ \mathrm{mol^{-1}\ dm^3\ s^{-1}}$ and that undissociated cysteine has negligible reactivity under our experimental conditions. The increase in k_{obs} with pH must be due to contributions from conjugate base species.

Scheme 1 shows the proposed mechanism, and from this rate law (7) may be written, where A, B and C are $HSCH_2CH(NH_3^+)CO_2^-$, $^-SCH_2CH(NH_3^+)CO_2^-$ and $HSCH_2CH(NH_2)CO_2^-$ respectively.

$$\text{Rate} = (k_1[\mathrm{A}] + k_2[\mathrm{B}] + k_3[\mathrm{C}])[\mathrm{Fe_2(CN)_{10}}^{5-}] \quad (7)$$

Now in our pH range $[\mathrm{A}] = [\mathrm{cys}]_T[\mathrm{H^+}]/([\mathrm{H^+}] + K_2 + K_3)$, $[\mathrm{B}] = [\mathrm{cys}]_T K_2/([\mathrm{H^+}] + K_2 + K_3)$ and $[\mathrm{C}] = [\mathrm{cys}]_T K_3/([\mathrm{H^+}] + K_2 + K_3)$ where $[\mathrm{cys}]_T$ is the total, stoichiometric concentration of L-cysteine. On substituting into eqn. (7) and rearranging eqn. (8) was obtained.

$$k_{obs}([\mathrm{H^+}] + K_2 + K_3)/[\mathrm{cys}]_T = k_1[\mathrm{H^+}] + k_2K_2 + k_3K_3 \quad (8)$$

When eqn. (8) is plotted using the data in Table 2 a straight line plot is obtained, and least squares analysis yields $k_1 = 27.0 \pm 0.9\ \mathrm{mol^{-1}\ dm^3\ s^{-1}}$ and $10^4(kK_2 + k_3K_3) = 4.4 \pm 1.5\ \mathrm{s^{-1}}$. Treatment of the data by non-linear regression analysis yields 26.8 ± 1.0 and 4.5 ± 1.0 respectively. As the concentrations of the tautomeric species B and C must always be in the constant ratio K_2/K_3 we cannot obtain separate values for k_2 and k_3 except by some ad hoc postulate such as assuming that one of the tautomers is very much more reactive than the other. Thus if $k_2 \gg k_3$ then k_2 is $1.5 \times 10^5\ \mathrm{mol^{-1}\ dm^3\ s^{-1}}$ at 25 °C. It should be noted that although we have assigned k_1 to $HSCH_2CH(NH_3^+)CO_2^-$ there must also be a small concentration of the tautomer $^-SCH_2CH(NH_3^+)CO_2H$. From the fact that the pK_a values for CO_2H and SH dissociation differ by some 6.5 log units suggests the concentration of this tautomer may be some $10^{6.5}$ times smaller than the main carbohydrate ionised tautomer. However this would still give a bimolecular rate constant well below the encounter limit. A similar argument applies to $HSCH_2(NH_2)CO_2H$. Further analysis of the data is not justified. With other reductants that contain a nucleophilic sulfur centre reaction with $[\mathrm{Fe_2(CN)_{10}}]^{4-}$ can give rise to coloured pentacyanoferrate(III) complexes with a sixth ligand bound to iron through sulfur. We do not see this with L-cysteine, possibly because over our pH range the sulfhydryl group is only slightly ionised, and reduction to $[\mathrm{Fe_2(CN)_{10}}]^{6-}$ is faster than nucleophilic substitution.

Experimental

Materials.—The complex, $[\mathrm{Fe_2(CN)_{10}}]^{4-}$, was prepared as described previously.[4] Solutions had λ_{max} 560 nm and concentrations were calculated using $\epsilon = 1600\ \mathrm{M^{-1}\ cm^{-1}}$. L-cysteine hydrochloride was supplied by Aldrich Chemical Co., USA and used without further purification.

Kinetic Studies.—Reactions were followed by stopped-flow spectrophotometry using a Hi-Tech Scientific SF-51 stopped-flow attached to a Hi-Tech Scientific SU-40 spectrophotometer unit. The machine was attached to a Haake GH constant temperature water bath fitted with a Haake D8 circulating pump. The pH was varied using acetate and disodium hydrogen orthophosphate–citric acid buffers, the pH being measured with an Orion Research Expandable Ion Analyzer EA 920 fitted with a Cole-Parmer combination electrode. Ionic strength was maintained at $1.0\ \mathrm{mol\ dm^{-3}}$ using $NaClO_4$. In all cases, the reaction was investigated under pseudo first order conditions with [cysteine] ≥ [complex].

Analysis for Copper.—This was carried out using a Perkin Elmer 2380 AAS instrument, calibrated with standard copper sulfate solution. A good Beer Lambert Law plot was obtained up to 10 ppm of copper. All reagents including reactants, buffers and distilled water were checked and found to contain less than 20 ppb, our detection limit.

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