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Inhibition and Catalysis in the Oxidation of Cysteine and Other Mercaptans by Ferricyanide

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The oxidation of cysteine by ferricyanide in aqueous solution is strongly inhibited by ethylenedinitrilotetraacetate in a manner which indicates that the reaction is catalyzed by traces of metal ions usually present as impurities, probably iron and/or copper. Similar effects have been demonstrated in the reaction of ferricyanide with 3-mercaptopropionic acid and 1-octanethiol. Representative rate measurements are reported.

INTRODUCTION

Mercapto compounds usually react readily with oxygen or other oxidizing agents (*1*) and the reactions have important applications, especially in living organisms (*2*). The reaction of ferricyanide with cysteine in neutral medium has been utilized for the quantitative determination of the latter substance (*3, 4, 5*). In one of the investigations of this reaction, it was noted that copper(II) ions catalyze the reaction and that cyanide inhibits it, and it was suggested that the inhibition is due to reaction of the cyanide with catalytic impurities present in the reagents (*4*).

The probable role of catalysts in this reaction was brought to the attention of the writers when they added ethylenedinitrilotetraacetate [as the disodium salt, $NaH(O_2C)_2NCH_2CH_2N(CO_2)_2HNa$, henceforth symbolized by EDTA] to solutions of cysteine in order to protect them from air oxidation. It was found that, in addition to the effect desired, EDTA strongly inhibited the reaction with ferricyanide as well. The present paper reports an investigation of this phenomenon.

Recently, the kinetics of the reactions of ferricyanide with 3-mercaptopropionic acid (*6*) and with 1-octanethiol (*7*) have been investigated. In the former case, the possibility of catalysis by traces of metal ions was not specifically considered, while in the latter case the inhibitory effect of cyanide was explained by a mechanism that did not involve metal-ion catalysis. In the present work, the behavior of the above-named mercaptans was reinvestigated to determine whether such catalysis was in fact involved.

METHODS

Materials. Cysteine hydrochloride monohydrate was obtained from the California Corp. for Biochemical Research, Los Angeles (sample I, B grade; sample II, A grade); 3-mercaptopropionic acid from Evans Chemetics, Watertown, New York (label purity, 99.2%); 1-octanethiol from Matheson, Coleman and Bell, Cincinnati. Potassium ferricyanide was of ACS reagent grade; sample I was was obtained from Fisher Scientific Company, St. Louis, and sample II from Baker and Adamson, New York. Other chemicals were also ACS reagent grade, except acetone, which was of CP grade.

The water used in the experiments was obtained by condensation of steam and passed through a bed of ion-exchange resin, Rexyn IRG-501, reagent grade (Fisher Scientific Co.). The water was then boiled, cooled slowly with a stream of nitrogen bubbling through, and stored under nitro-

gen. The nitrogen was of commercial grade. It was passed through a vanadium(II) solution to remove oxygen (*8*).

Acetate buffer, pH 4.05, was prepared by mixing 9.18 ml of glacial acetic acid and 3.282 g of sodium acetate in deaerated water to give 1 liter; acetate buffer, pH 5.57, was prepared from pH-4 buffer by adding 5.600 g of sodium hydroxide per liter. Carbonate buffer, pH 9.80, was prepared by dissolving 2.103 g of sodium bicarbonate, 2.650 g of sodium carbonate, and 0.5845 g of sodium chloride in deaerated water to give 1 liter. These buffers were stored under nitrogen.

Unless otherwise specified, the solutions of cysteine and of ferricyanide were used within 6 hrs from the time of preparation.

Apparatus. Spectrophotometric measurements were made with a Beckman DU spectrophotometer using 1-cm silica cells. Measurements of pH were made with a Beckman Model G meter, standardized with commercial buffers.

Experimental procedure. Weighed amounts of cysteine hydrochloride or of 3-mercaptopropionic acid were dissolved in acetate buffer; 1-octanethiol was dissolved in 30 ml of carbonate buffer and this diluted with acetone to 100 ml. Solutions of EDTA and ferricyanide were prepared in the same buffer as the mercaptan and the reagents were mixed to give the following concentrations at zero time: mercaptan, 0.0038–0.004 M; ferricyanide, 4.0×10^{-4} M; EDTA, 0 to 3×10^{-5} M, as designated.

To measure the rate of reactions which would be complete in a few minutes, the reagents were first brought to 25° ± 0.1° and then mixed in a spectrophotometer cell; the absorbance was measured at appropriate intervals of time, without attempting to control the temperature of the cell. In experiments extending for longer periods of time, larger amounts of reagents were mixed and kept in the thermostat and absorbance measurements were done on aliquot portions that were withdrawn and placed in the spectrophotometer cell at the appropriate time.

Ferricyanide has a strong absorption maximum at 418 mμ (*9, 6*) while the other reagents do not absorb at this wavelength. The progress of the reaction could therefore be conveniently followed by spectrophotometric measurements. The measurements were actually made at 410 mμ, and the molar absorbancy index of ferricyanide was taken as 990.

Results

In acetate buffer of pH 4.0 with 3.8×10^{-3} M cysteine and 4×10^{-4} M ferricyanide, the reaction was essentially complete in less than 1 min and no measurements of rate were made. When EDTA was added to the reaction mixture, the rate was reduced, as exemplified by the curves in Fig. 1. In the range of EDTA concentrations

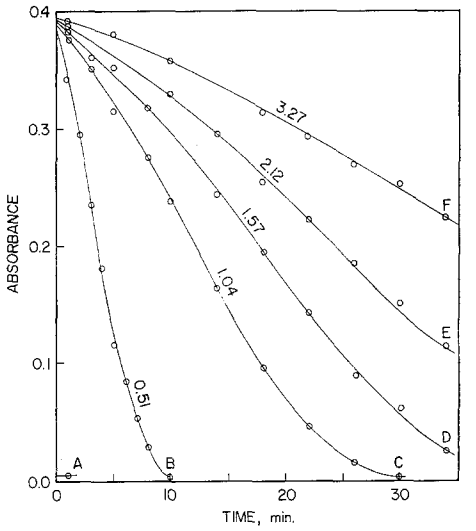

Fig. 1. Rate of reaction of ferricyanide (I), initially 4×10^{-4} M, with cysteine (I), initially 3.8×10^{-3} M, in acetate buffer (II) of pH 4.0. Curve A: no EDTA; curves B-F: EDTA concentration 0.51 to 3.27×10^{-5} M.

that gave conveniently measurable rates, reproducible results could be obtained with freshly prepared solutions made up from the same samples of solid reagents and the same batch of distilled water, but quite different rates might be observed with different samples (for purposes of identification, these will be denoted by Roman numerals, sequentially assigned). With a particular sample of cysteine (I) and ferricyanide (I) made up in acetate buffer

(I) in the presence of $9 \times 10^{-6} M$ EDTA, for example, the average half-reaction time was found to be 3.5 min with an average deviation of ±0.3 min; with the same reagents made up in acetate buffer (II), the half-time was 13.6 min; with everything the same as in the first instance except a different sample of ferricyanide (II), the half-time was 7.7 min. The age of the ferricyanide solutions was an additional variable, the same solution giving a faster rate after standing 1–2 days; in general, freshly prepared solutions were used, so this effect did not come into play. Cysteine samples (I) and (II) reacted at nearly the same rate, but this may not be the case generally.

Figure 2 shows the results obtained in a

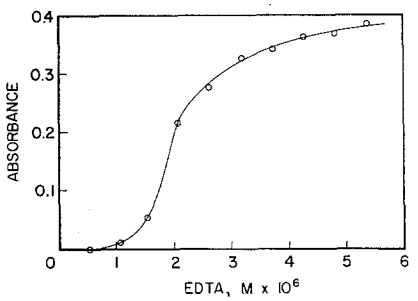

FIG. 2. Absorbance at 410 mμ 45 sec after mixing ferricyanide (I), initially $4 \times 10^{-4} M$, with cysteine (I), initially $3.8 \times 10^{-3} M$, in acetate buffer (III), pH 4.0, in the presence of varying concentration of EDTA.

different type of experiment, in which the ferricyanide remaining after a short reaction period, 45 sec, was measured in the presence of increasing EDTA concentration (cysteine, I; ferricyanide, I; acetate buffer, III). It can be seen that a plot of the data has an inflection point at about $1.9 \times 10^{-6} M$ EDTA. With other samples of ferricyanide and buffer, including some of pH 5.6, similar results were obtained but the inflection point occurred at different EDTA concentrations.

Another set of experiments was done with cysteine (I), ferricyanide (I), and acetate buffer (III), the reagents used in obtaining the data represented in Fig. 2. With no EDTA added, the ferricyanide absorbance remaining after 45 sec was negligible; with $2 \times 10^{-6} M$ EDTA, a little more than corresponds to the inflection point, the absorbance after 45 sec was 0.261, corresponding to 34% reaction; with $4 \times 10^{-6} M$ EDTA, the absorbance was 0.375. Now, several aliquot portions of the cysteine solution containing $4 \times 10^{-6} M$ EDTA were taken, and to each was added a metal salt in the amount needed to give $2 \times 10^{-6} M$ concentration; then ferricyanide was added, and the absorbance after 45 sec was measured. A representative set of results is given in Table 1.

TABLE 1
EFFECT OF METAL SALTS ON REACTION

Added substance	Absorbance
$CuSO_4$	0.005
$FeSO_4$	0.005
$Fe_2(SO_4)_3$	0.014
$CoCl_2$	0.005
$SnCl_2$	0.005
$MnSO_4$	0.313
$Ni(NO_3)_2$	0.220
$CrCl_3$	0.245

Figure 3 represents data obtained with $4 \times 10^{-3} M$ 3-mercaptopropionic acid and $4 \times 10^{-4} M$ ferricyanide in acetate buffer

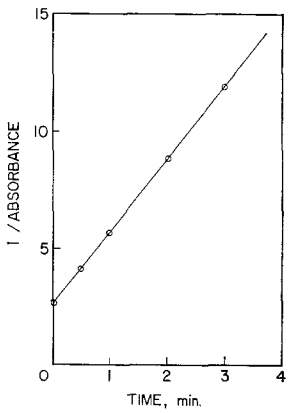

FIG. 3. Rate of reaction of ferricyanide (II), initially $4 \times 10^{-4} M$, with 3-mercaptopropionic acid, initially $4.0 \times 10^{-3} M$, in acetate buffer, pH 4.0.

of pH 4.0, plotted so as to give a straight line if the rate of consumption of ferricya-

nide were of second order (*6*). Figure 4 shows the data obtained with $4 \times 10^{-3} M$ 1-octanethiol in acetone-water-carbonate

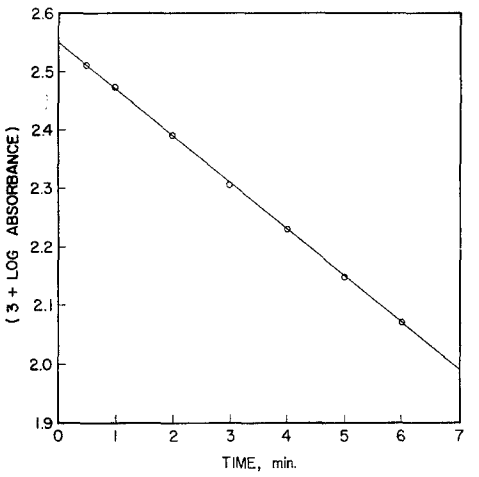

FIG. 4. Rate of reaction of ferricyanide (II) initially $4 \times 10^{-4} M$, with 1-octanethiol, initially $4.0 \times 10^{-3} M$, in acetone-water-carbonate buffer.

buffer, plotted so as to give a straight line if the rate of consumption of ferricyanide were of first order; a second order rate constant can be calculated from the experimental first order constant by dividing into it the mercaptan concentration.

Figure 5 represents the dependence of half-reaction times on EDTA concentration for cysteine (I) and for 3-mercaptopropionic acid. Figure 6 represents the

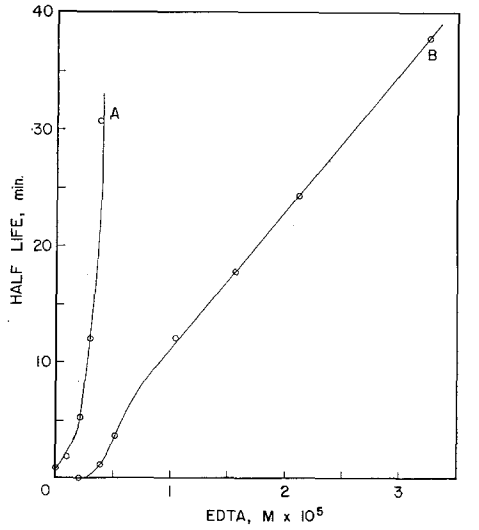

FIG. 5. Dependence of half-reaction times on EDTA concentration. Curve A: 3-mercaptopropionic acid; curve B: cysteine (I), ferricyanide (II), acetate buffer (I).

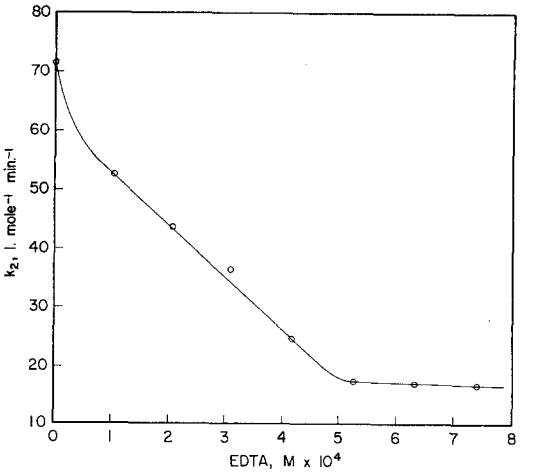

FIG. 6. Dependence of second order rate constant on EDTA concentration for reaction of ferricyanide (II), initially $4 \times 10^{-4} M$, with 1-octanethiol, initially $3.8 \times 10^{3} M$, in acetone-water-carbonate buffer.

dependence of calculated second order rate constants on EDTA concentration for octanethiol. It must be stressed that different samples of chemicals might be expected to give quantitatively different results though the shape of the curves might be similar.

DISCUSSION

The variation in rates of reaction observed with different samples of ferricyanide, cysteine, and buffer salts, as well as the inhibitory effect of EDTA on the reaction, can be reasonably explained by the hypothesis that the reaction is catalyzed by traces of metal ions which are present as impurities.

If EDTA combines with the catalyst to form a slightly dissociated complex, the concentration of free catalyst should change very rapidly near the equivalence point and cause a corresponding sharp inflection

in the rate of reaction. Just such a situation is represented in Fig. 2. The inflection point, at about 1.9×10^{-6} M, is presumably in stoichiometric correspondence to the concentration of catalyst present in this case. Since the concentration of solid reagents, mostly buffer salts, was more than 0.2 M, this amount of impurity corresponds to only 0.001 mole %, not an unreasonable value. The fact that similar behavior was found with other samples of chemicals but that the inflection occurred at different EDTA concentrations is consistent with the proposed interpretation.

What is the catalyst present in these samples? It is natural to suspect the ions of iron, because they are known to act as catalysts in other oxidation processes, for instance in the reaction of cysteine with oxygen, and also because they are of sufficiently widespread occurrence that they may well be present in the reagents used to the extent indicated (*10*). When sufficient EDTA was added to inhibit the reaction almost completely, adding ferrous or ferric ions restored the reaction, which is consistent with the suspicion expressed above. However, the demonstration is not unambiguous, because it could be that the iron added displaced the original catalyst from its EDTA complex. Also, other ions, namely copper(II), tin(II), and cobalt(II) had an effect equal to iron (insofar as this could be tested by the experiment). It is not likely that the latter two ions would have been present in the original reagents, but copper(II) might have been. It is clear that the question cannot be settled at this time, and that the results of the experiments show only that iron and/or copper *could be* the catalyst(s) involved.

Presumably, the metal ion mediates the transfer of electrons from mercaptan to ferricyanide. For the reaction of 3-mercaptopropionic acid with ferricyanide, Bohning and Weiss proposed a mechanism which involves three steps, namely oxidation to ($RS\cdot$), then to (RS^+), and reaction of (RS^+) with RSH to give RSSR (*6*). This mechanism is based in large part on evidence not considered in the present work, and therefore it will not be discussed critically at this time. So far as the metal-ion catalysis is concerned, it can be introduced into the mechanism by postulating that ferricyanide reacts not with the mercaptide ion but with a metal-mercaptan complex, e.g., for a divalent ion.

$$RSM^+ + [Fe(CN)_6]^{3-} \rightarrow RS\cdot + [Fe(CN)_6]^{4-} + M^{2+}$$

In the interpretation of the kinetics of oxidation of 1-octanethiol, Kolthoff *et al.* (*7*) found that cyanide inhibits the reaction and postulated that the effect comes about because cyanide repressed the equilibrium

$$[Fe(CN)_6]^{3-} + RS^- \rightleftharpoons [Fe(CN)_5RS]^{3-} + CN^-$$

However, there is a striking similarity between the inhibition by cyanide (see Fig. 6, ref. 7) and the results obtained in this work with EDTA (Fig. 6). Since cyanide is a good complexing agent for iron and copper ions, as is EDTA, it seems logical to suggest that the inhibition is due in both cases to complexation of catalytic impurities present in the reaction mixtures.

Acknowledgments

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