reported[32] for the tetrahedral systems $NiCOL_3$ and NiL_4 and suggests that strong P–P coupling may occur without the phosphorus atoms being in *trans* positions. The increase in the P–H coupling constant for $PRhC_5H_5$ in going from compounds containing phosphine ligands to those with phosphite ligands (Table VI) also suggests a difference of bonding with rhodium between these two types of ligands.

Acknowledgment. This research is supported by National Science Foundation Grant GP-2505 to Northwestern University and the Technischen Hochschule, München. We wish to thank Professor E. O. Fischer for helpful suggestions and interest in this research.

---

[32] J. G. Verkade, R. E. McCarley, D. G. Henricker, and R. W. King, *Inorg. Chem.*, **4**, 228 (1965).

The Iron(III)-Catalyzed Oxidation of Cysteine by Molecular Oxygen in the Aqueous Phase. An Example of a Two-Thirds-Order Reaction

Jay E. Taylor, Johnson F. Yan, and Jin-liang Wang

Contribution from the Department of Chemistry, Kent State University, Kent, Ohio.

Received November 8, 1965

Abstract: A careful investigation of the iron(III)-catalyzed oxidation of cysteine has demonstrated that the reaction is zero order with respect to both cysteine and oxygen but two-thirds order with ferric ion. There is a marked pH effect with a pronounced maximum in rate at pH 8.1, and the existence of an iron–cysteine complex has been well documented. A quantitative interpretation of these observations leads to the following proposal.

$$Fe^{3+} + 3CySH \rightleftarrows Fe(CyS)_3 + 3H^+$$

The $Fe(CyS)_3$ may undergo ionization due to the carboxyl and amino groups on the ligand, cysteine. One of the ionic forms is assumed to be specially reactive and is so designated as $Fe(CyS)_3{}^*$. The equilibria with the ionic forms of lesser activity on each side of the pH maximum explain the pH dependency of the reaction.

$$Fe(CyS)_3{}^* \rightleftarrows Fe(CyS) + 2CyS^*$$

$$2CyS^* \longrightarrow \text{cystine}$$

$$Fe(CyS) + 2CySH + 0.5O_2 \longrightarrow Fe(CyS)_3 + H_2O$$

The second and last steps can be altered in different ways giving modifications which yield the same basic mathematical rate expression. The CyS* represents an intermediate which combines with itself to form cystine. There is not sufficient information available to attempt to define the nature of the CyS* intermediate. It may be a radical, an ionic species, a combination of activated units, or a molecular species of unusual reactivity. Since both cysteine and oxygen are in excess to the ferric ion concentration, the reason for the zero order in each case is obvious from the first and last steps. The two-thirds order in Fe^{3+} arises from the second and third steps. This mechanism gives a mathematical expression which very adequately correlates all of the presently known rate data. Obviously this proposal is not conclusive; however, if the actual mechanism is not as described above, it must give the same mathematical model.

The determination of the rate of the oxidation of cysteine by the method of measuring oxygen uptake has been the subject of various investigations. These were generally done at an early date and were of a low order of accuracy. A consistent evaluation of the rate data in terms of a valid mechanism does not appear in the literature. Among the investigations which have been made, Gerwe[1] found that pure cysteine, free from iron, is oxidized at a very slow rate; Mathews and Walker[2] reported that cysteine is oxidized in alkaline but not in acidic solutions. They also found that at 20° the maximum rate is reached at pH 8 and concluded that only the neutral nonionized molecule of cysteine oxidizes rapidly. The more rapid oxidation of cysteine in the presence of cystine was noted by Abderhalden and Wertheimer.[3] The influence of various catalysts was systematized. The most recent study on this oxidation is probably that by Robert,[4] who reported the influence of copper on the oxidation of cysteine in alkaline solutions. A brief review has been made by Tarbell.[5]

In most of the works mentioned above, experiments were conducted in vessels shaken vigorously and connected to an air or oxygen source, and the whole system was kept airtight. The decrease in a mercury barometer was recorded as a measure of the amount of

---

[1] E. G. Gerwe, *J. Biol. Chem.*, **92**, 399 (1931).

[2] A. P. Mathews and S. Walker, *ibid.*, **6**, 21, 289 (1909).

[3] E. Abderhalden and E. Wertheimer, *Arch. Ges. Physiol.* (Pflüger's), **197**, 131 (1922); **199**, 336 (1923).

[4] C. Robert, *Bull. Soc. Chim. Biol.*, **36**, 253 (1954).

[5] D. S. Tarbell, "Organic Sulfur Compounds," Vol. I, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, p 97.

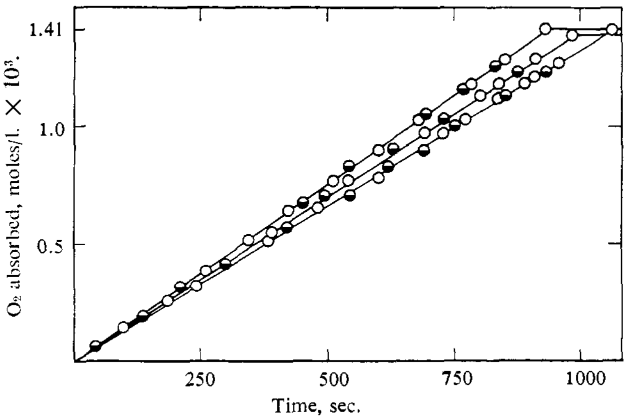


Figure 1. Typical runs showing the extreme zero-order character for both oxygen and cysteine at 28.7° with 5.628×10^{-3} M cysteine and 1.00×10^{-5} M $FeNH_4(SO_4)_2$. At each pH (top curve, pH 8.10; middle, pH 7.88; bottom, pH 7.78) the oxygen (open circles) and the air (half-circles) follow precisely the same curve indicating zero order for oxygen. Note that the reaction is complete upon absorption of 1.410×10^{-3} mole/l. of oxygen, which represents one-fourth of the molarity of the cysteine.

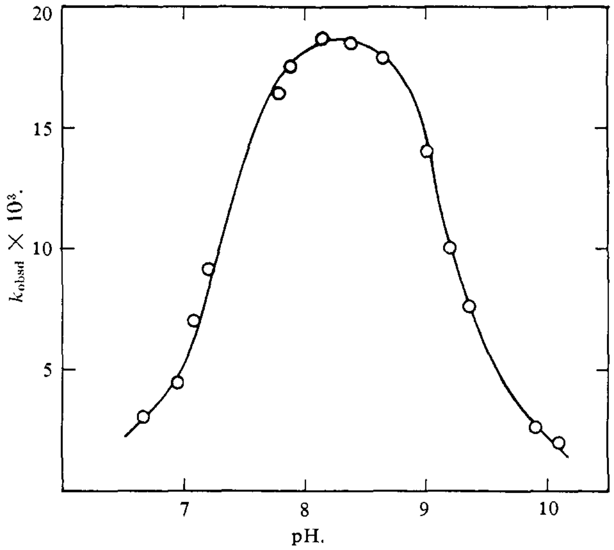


Figure 2. A typical bell-shaped curve obtained upon plotting k_{obsd} *vs.* pH (cysteine = 5.628×10^{-3} M, $[Fe^{3+}] = 1.00 \times 10^{-5}$ M).

oxygen absorbed. Mathews and Walker[2] did not even control the temperature of the reaction vessel, while another investigator[6] reported that the rate of oxygen absorption is independent of shaking.

In this paper a very careful experimental investigation of the oxidation of cysteine in the presence of ferric ion is described. A precise mathematical interpretation of the effects of variation of pH and ferric ion is also presented.

Experimental Section

Materials. L-Cysteine hydrochloride monohydrate, from Nutritional Biochemicals Corp., was noted to be of sufficiently high purity that there is no measurable absorption of oxygen at the temperatures used in these experiments. All other chemicals were of analytical grade or equivalent and were used without further purification.

Apparatus and Procedure. The general type of apparatus, incorporating a highly sensitive, constant pressure gas buret of about 10-ml volume and a reaction flask with an aeration stirrer, is described in another publication.[7] The reaction flask holds 475 ml of solution which was the volume of the reacting solution in all experiments. The total volume of the flask was 500 ml, allowing space for oxygen circulation. Since the rate of oxygen absorption was zero order with respect to both oxygen and cysteine, it was treated as a simple linear plot with time. The apparatus as described[7] was adapted for this particular research by the addition of a small cup plunger fitted into the flask through a 24/40 joint. This cup was designed to hold either 1–2 ml of ferric ammonium sulfate solution or the solid cysteine. After the aqueous solution was saturated with oxygen and allowed to come to temperature, the reaction was started by plunging the ferric ammonium sulfate solution (or solid cysteine) into the reactor and adjusting the volume of oxygen to the desired pressure.

The gas buret was placed in an auxiliary constant-temperature bath which was held at 30.50° while the reactor was kept in a bath maintained at 28.7°. Both baths were constant to ±0.01°.

The kinetic runs were made by two procedures which gave, as will be discussed, differing results.

Procedure 1. An amount of cysteine, 0.475 g for all experiments, was weighed into a 500-ml volumetric flask. Sodium hydroxide solution was then added to adjust the pH of the medium to the desired value, and the resulting solution was diluted with triply distilled water to 500 ml. The amount of sodium hydroxide to be added was previously determined by titrating the given concentrations of cysteine and catalyst in an auxiliary flask. The major portion, 475 ml, of this mixture was transferred to the reactor which was then placed in the water bath. An appropriate amount of ferric ammonium sulfate solution (between 1 and 2 ml) was metered into the plunger. The reaction was then started as previously described.

Procedure 2. The ferric ammonium sulfate solution was placed in the reaction flask and the cysteine in the plunger. Otherwise the second procedure was the same as the first. The difference was that in this procedure the ferric hydroxide aged for about 30 min before the start of the reaction.

Commercial oxygen was used as an oxidant for most experiments. However, upon substituting air the same results were obtained within experimental error.

Results and Calculations

The results of this study may be summarized as follows.

(1) In keeping with the findings of earlier observers, the oxidation of cysteine in the presence of metal ions was found to be zero order with respect to both oxygen and cysteine as shown in Figure 1. The reaction was extremely slow in the absence of metal ions.

(2) At low concentrations of ferric ion, oxygen was consumed in the ratio 4:1 cysteine to oxygen, indicating formation of cystine (see Figure 1).

(3) A rather detailed study of the catalytic effect of iron(III) ion upon this reaction has been made. There was a maximum in rate at pH 8.10 with a marked decrease in the rate of reaction as the pH was either increased or decreased. These data are correlated in Figure 2.

(4) A determination of the order with respect to the metal ion indicated that the reaction is two-thirds order with respect to iron(III) ion. The experimental support is presented in Figure 3, where log k_{obsd} is plotted with log $[Fe^{3+}]$.

It has been emphasized that cysteine forms a pronounced colored complex with a number of ions.[8–11] With iron the color is reddish blue to blue.

---

(6) D. C. Harrison, *Biochem. J.*, **18**, 1009 (1924).

(7) J. E. Taylor, *J. Chem. Educ.*, **42**, 618 (1965).

(8) J. P. Greenstein and M. Nimitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1961, p 487.

(9) I. M. Kolthoff and W. Stricks, *J. Am. Chem. Soc.*, **77**, 1996 (1955).

(10) F. M. Page, *Trans. Faraday Soc.*, **51**, 919 (1955).

(11) E. Sakakibara, *Seikagaku*, **28**, 246 (1956); *cf. Chem. Abstr.*, **54**, 22131*a* (1960).

The combination of observations as described above makes this reaction an extremely interesting one from a mechanistic aspect. Obviously the reaction is controlled by the concentration of ferric ion which, of course, is never in excess. The two-thirds order is explained by a proposal that the ferric ion forms a complex with three cysteine molecules. The results may be equally well explained by an iron–dicysteine complex, but the tris complex is chosen because of the more reasonable octahedral structure. This complex is essentially quantitative in its formation but is subject to decomposition to the iron–monocysteine complex and two cysteinyl species (as yet undefined) which are in equilibrium with the iron–tricysteine complex. Two of these cysteinyl species may combine together to form cystine. The final reaction is the re-formation of the iron–tricysteine complex. The role of oxygen is not clear. It may serve to displace two cysteinyl groups or it may act in a final step to oxidize the Fe(I) complex, formed by the loss of two cysteines, back to the Fe(III)–tricysteine complex.

The pH effect is attributed to the Fe(III)–cysteine complex (by virtue of the several ionic species of cysteine) and/or the amphoteric character of the cysteinyl intermediate.

These generalizations are defined below.

$$\mathrm{Fe^{3+} + 3HA^-\ (or\ other\ ionic\ forms) \rightleftharpoons Fe(A^-)_3 + 3H^+} \qquad (1)$$

$$\mathrm{Fe(HA)_3 \leftrightharpoons Fe(HA)_2(A^-) \leftrightharpoons Fe(HA)(A^-)_2 \leftrightharpoons} \\ \mathrm{Fe(A^-)_3 \leftrightharpoons Fe(A^-)_2OH \leftrightharpoons Fe(A^-)(OH)_2} \qquad (2)$$

If it is assumed that only $\mathrm{Fe(A^-)_3}$ is the reactive intermediate, the pH effect is readily explained.[12]

$$\mathrm{Fe(A^-)_3 \rightleftharpoons Fe^{I}A^- + 2A^-} \qquad (3)$$

$$\mathrm{2A^- \longrightarrow cystine} \qquad (4)$$

$$\mathrm{Fe^{I}A^- + 2HA^- + 0.5O_2 \longrightarrow Fe(A^-)_3 + H_2O} \qquad (5)$$

where $\mathrm{A^{2-}}$, $\mathrm{HA^-}$, $\mathrm{H_2A}$, and $\mathrm{H_3A^+}$ represent the possible general ionic forms for cysteine; that is, $\mathrm{A^{2-}}$ is $\mathrm{^-SCH_2CHNH_2CO_2^-}$, etc. The ligands are represented by $\mathrm{A^-}$ which is $\mathrm{\cdot SCH_2CHNH_2CO_2^-}$, HA which is $\mathrm{\cdot SCH_2CHNH_2CO_2H}$, and $\mathrm{H_2A^+}$ which is $\mathrm{\cdot SCH_2CH(NH_3^+)CO_2H}$. It is further assumed that the $\mathrm{Fe{-}A^{2-}}$ bond is a very weak one since the S must be ionized and no Fe–S bond is possible. The contribution of $\mathrm{H_3A^+}$ or $\mathrm{Fe(H_2A^+)_3}$ is negligible since it would not be formed in significant concentrations at the pH range under consideration.

An alternate explanation assumes that the pH effect is due to the amphoteric character of the cysteinyl intermediate $\mathrm{A^-}$ from (3). This may be expressed as

$$\mathrm{H^+ + A^- \rightleftharpoons HA} \qquad (5a)$$

Assuming $\mathrm{Fe(H_2A^+)_3}$ is unimportant and $\mathrm{Fe(A^{2-})_3{}^{3+}}$ may not be readily formed, a second ionization effect may yet be incorporated. In highly basic solutions the ionization of the mercaptide ion

$$\mathrm{OH^- + HA^- \rightleftharpoons A^{2-} + H_2O} \qquad (5b)$$

should markedly decrease the tendency to form the complex in (1). This could, therefore, serve as a second equilibrium effecting a decrease in complex formation at high pH.

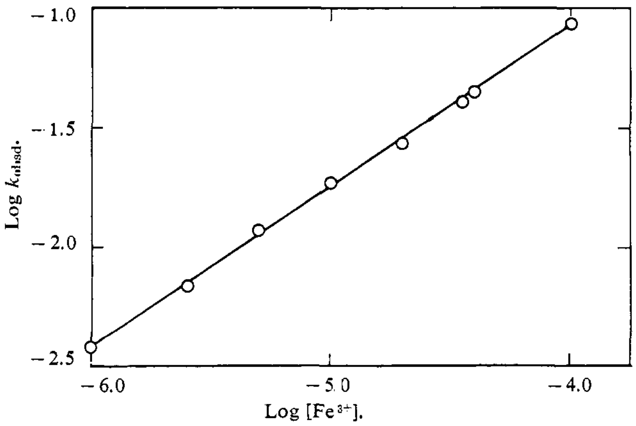


Figure 3. A plot of log of the rate constant *vs.* log ferric ion. The slope of $^2/_3$ indicates a two-thirds order with respect to ferric ion (data taken at 28.70° and 5.628×10^{-3} *M* cysteine).

Disregarding pH effects, the following expressions may be developed. A is ionically unidentified and represents the total complexed cysteine.

$$K_{\mathrm{F}} = \frac{[\mathrm{Fe^{I}A}][2\mathrm{A}]^2}{[\mathrm{Fe^{III}A_3}]} \qquad (6)$$

Since $[\mathrm{Fe^{I}A}] = [\mathrm{A}]$

$$K_{\mathrm{F}}[\mathrm{Fe^{III}A_3}] = [\mathrm{Fe^{I}A}][2\mathrm{A}]^2 = 4[\mathrm{A}]^3 \qquad (7)$$

Under the condition that cysteine is in excess and that equilibrium 1 is far to the right, it may be assumed that $[\mathrm{Fe^{III}A_3}]$ equals the initial concentration of $\mathrm{Fe^{+3}}$.

$$[\mathrm{A}] = ([\mathrm{Fe^{III}A_3}]K_{\mathrm{F}}/4)^{1/3} = ([\mathrm{Fe^{3+}}]K_{\mathrm{F}}/4)^{1/3} \qquad (8)$$

Only two equilibria are necessary to explain the primary effect of pH as shown in Figure 2. Two alternatives for the equilibria are given under (2) and (5a) and (5b). Under (2) it is, of course, necessary only to consider the equilibria immediately pertaining to $\mathrm{Fe(A^-)_3}$ (or whichever intermediate species is being assumed). Since it is not yet possible to identify these equilibria, another symbol X will represent the unknown ionic forms.

The equilibria are written as

$$\begin{aligned} K_1 &= [\mathrm{X^\pm}][\mathrm{H^+}]/[\mathrm{X^+}] \\ K_2 &= [\mathrm{X^-}][\mathrm{H^+}]/[\mathrm{X^\pm}] \end{aligned} \qquad (9)$$

Letting $[\mathrm{A}] = [\mathrm{X^\pm}] + [\mathrm{X^-}] + [\mathrm{X^+}]$ and upon assuming that the most reactive form is $\mathrm{X^\pm}$, it follows that

$$[\mathrm{X^\pm}] = \frac{[\mathrm{A}]}{1 + \frac{[\mathrm{H^+}]}{K_1} + \frac{K_2}{[\mathrm{H^+}]}} \qquad (10)$$

Since the reaction is zero order with respect to cysteine, the rate of cysteine consumption equals

$$[\mathrm{AH}]_{\mathrm{con}}/t = [\mathrm{Cy}]/2t = [\mathrm{O_2}]_{\mathrm{con}}/4t = \\ k_{\mathrm{obsd}}/4 = k'[\mathrm{X^\pm}]^2 \qquad (11)$$

---

(12) It should be noted that owing to uncertainty in the effect of the complex upon the ionization of the cysteine molecule, this form or another or a combination of several forms may be the responsible intermediate. For example, the three ions with $\mathrm{FeX(A^-)_2}$ could be a collective intermediate.

The $\mathrm{Fe(A^-)(OH)_2}$ and $\mathrm{Fe(A^-)_2OH}$ forms are, at this moment, simply reasonable guesses as to the nature of these complexes in high pH. The only supporting evidence is that as the pH of the reacting solution increases the color of the complex changes from blue to red. At about pH 12, yellow ferric hydroxide appears to precipitate rather than forming the complex.

The rate of oxygen consumption, $[O_2]_{con}/4t$, contains the factor of 4 since only 0.25 mole of oxygen is utilized by 1 mole of cysteine. Thus upon incorporating eq 8

$$[O_2]_{con}/t = k_{obsd} = \frac{k[Fe^{3+}]^{2/3}}{\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right]^2} \tag{12}$$

where $k = k'(2K_F)^{2/3}$. The success of this equation is demonstrated in Table I, where the values of k_{obsd} are listed along with the calculated values of k. It is noted that the deviation of k is insignificant except at the extremes of the pH range. This drift may well be due to either of two factors: (a) the reactivity of ions other than $Fe(A^-)_3$ or (b) additional equilibria. In view of the complexity of this particular reaction, the good correlation of the experimental and the calculated values of the rate constants appears to be excellent proof of the mathematical acceptability of eq 12.

Table I[a]

$[Fe^{3+}]$, *M*	pH	k_{obsd}, ml. of O_2/sec	k
10^{-4}	6.30	0.783×10^{-2}	112
	6.45	1.175	97
	6.97	2.960	54
	7.35	5.280	49
	7.75	7.930	51
	7.88	8.650	52
	8.10	8.740	50
	8.16	8.650	49
	8.50	8.460	49
	8.80	7.930	53
	9.00	6.200	49
	9.20	4.530	46
	9.60	2.067	46
	10.77	0.216	347
10^{-5}	6.67	3.02×10^{-3}	56
	6.95	4.40	39
	7.08	6.96	47
	7.20	9.12	49
	7.78	16.40	48
	7.88	17.50	49
	8.14	18.70	49
	8.38	18.50	49
	8.64	17.90	51
	9.00	14.00	52
	9.20	10.00	47
	9.35	7.54	46
	9.90	2.79	69
	10.10	1.98	98
10^{-6}	6.50	5.34×10^{-4}	80
	6.90	9.36	43
	7.40	25.00	47
	7.90	35.10	45
	8.10	37.60	46
	8.28	37.30	46
	8.45	36.30	45
	8.90	32.60	51
	9.15	20.90	43
	10.10	4.61	105
	10.70	0.73	179

[a] The calculated values of k are based on eq 12; determination at 28.7° with 5.628×10^{-3} *M* cysteine and 10^{-5} *M* ferric ammonium sulfate catalyst. $K_1 = 1.1 \times 10^{-7}$, $K_2 = 3.0 \times 10^{-10}$. O_2 was measured at 706 mm (dry) and 30.5°.

Although the reaction mechanics are explained as indicated above by the formation of two cysteinyl species from one molecule of complex, there are certain complications which necessitate a further elaboration of this idea. One defect is that the equilibrium as proposed should occur in steps.

$$Fe^{III}A_3 \rightleftarrows Fe^{II}A_2 + A \rightleftarrows Fe^{I}A + 2A \tag{13}$$

With such an equilibrium why does not the reaction stop at $Fe^{II}A_2$ giving first-order kinetics with respect to Fe^{3+}? The continued decomposition of $Fe^{II}A_2$ can be explained if there is assumed a selective reaction of $Fe^{I}A$ with elemental oxygen. Since the decomposition of the complex takes place by a stepwise process (13) and since $Fe^{II}A_2$ is presumed to be slow to react with oxygen whereas $Fe^{I}A$ reacts rapidly, the $Fe^{III}A_3$ must necessarily decompose to $Fe^{I}A$ before oxidation, and re-formation of $Fe^{III}A_3$ may occur as in eq 5. The assumption of the discrete formation of a $Fe^{I}A$ complex suggests both an unusual valence state and a higher energy bond dissociation, $Fe^{II}A_2 \rightarrow Fe^{I}A$, as compared to $Fe^{III}A_3 \rightarrow Fe^{II}A_2$. In the latter case the bond energy is very low because of the instability of the Fe^{III}–S bond. In view of this an alternative is presented.

$$Fe^{III}A_3 \overset{K_{II}}{\rightleftarrows} Fe^{II}A_2 + A \tag{14}$$

$$Fe^{II}A_2 + O_2 \overset{K_{III}}{\rightleftarrows} FeA \cdot O_2 + A \tag{15}$$

or

$$Fe^{III}A_3 + O_2 \overset{K_{III}}{\rightleftarrows} FeA \cdot O_2 + 2A \tag{16}$$

$$2AH + FeA \cdot O_2 \longrightarrow Fe^{III}A_3 + H_2O + 0.5O_2 \tag{17}$$

From (15) we may write

$$K_{II} = \frac{[Fe^{II}A_2][A]}{[Fe^{III}A_3]} \tag{18}$$

$$K_{III} = \frac{[FeA \cdot O_2][A]}{[Fe^{II}A_2][O_2]} \tag{19}$$

Combining, $K_{II}K_{III} = K_I$, or using (16) and letting $[Fe^{3+}] = [FeA \cdot O_2] + [FeA_3]$

$$[FeA \cdot O_2] = \frac{[Fe^{3+}][O_2]}{[A]^2/K_I + [O_2]} \tag{20}$$

It is obvious that at higher oxygen concentrations the value of $[O_2]$ may become much larger than $[A]^2/K_I$ causing the $[O_2]$ values in (20) to cancel. Then $[FeA \cdot O_2]$ becomes equal to $[Fe^{3+}]$. Accordingly, no additional increase in rate due to a continued increase in the partial pressure of oxygen is to be noted.

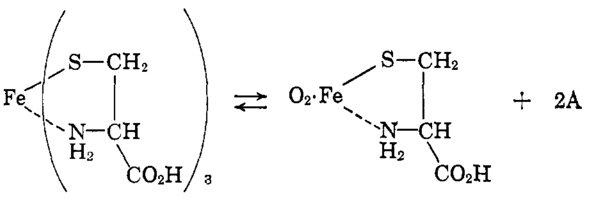
The over-all rate equation at higher oxygen partial pressures is the same as (12), but at low oxygen pressures the order with respect to oxygen should change. A careful investigation is planned.

Rationale

In the previous paragraphs a mechanism which explains the observed data on the oxidation of cysteine has been presented briefly. It is intended in this section to develop these ideas and certain related ideas in more detail.

Step 3, or correspondingly eq 14–16, is the critical step upon which the explanation of the two-thirds order with respect to the Fe^{III} ion depends. Obviously the significant rate step is (4), but it is the equilibrium formation of the cysteinyl intermediate, eq 3 or 14–16, that effectively controls the rate of cystine formation. The dependence of this intermediate upon the concentration of complex is responsible for the zero-order kinetics for cysteine and oxygen. Numerous other proposals were considered but were found to be inadequate. Certainly this general proposal must not be unique, but a good alternate has not yet appeared.

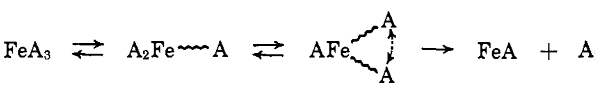
The cysteine–iron(III) complex is most probably an octahedral structure with the cysteine acting as a bidentate ligand. Presumably, the sulfur and either carboxyl or amino groups are the points of coordination. The changes, occurring in the complex as the oxidative process takes place, may be represented as


(21)

The significantly sharp maximum in the pH curve (Figure 2) requires a complex hydrogen ion function and is definitely indicative of the second-order character of the essential reaction, *i.e.*, step 4.

The nature of $X^{\pm}$ is not clearly defined and especially since it has not been possible to relate the calculated values of K_1 and K_2 (Table I) to previously determined ionization constants for cysteine. These have been recently reevaluated[13] with pK = 8.33 and 10.78 and may be compared to our values of pK_1 = 6.96 and pK_2 = 9.52. The obvious discrepancy indicates either that the values of this paper do not represent a unified form or that a different molecule is being dealt with; the latter is, of course, the conclusion from the other evidences.

Whatever the nature of A it is obviously different from cysteine. It may be a radical intermediate or a dual ionic ($-S^+$ and $-S^-$) species. Another possibility is to look upon A as an activated segment of the complex FeA_3. Such a proposal might be represented as



$$FeA_3 \rightleftarrows A_2Fe{\sim}A \rightleftarrows AFe\overset{A}{\underset{A}{\updownarrow}} \longrightarrow FeA + A_2 \qquad (22)$$

The equilibrium as employed in eq 6 may be applied if $\sim$A is treated as a unique species.[14]

The role of ferrous ion was considered. Certain preliminary experiments in which ferrous ammonium sulfate was substituted for the ferric compound were inconclusive because of the ease of oxidation of the former to the latter, particularly under the slightly basic conditions of the reaction. It was noted, however, that ferrous ammonium sulfate in higher concentrations did not form a colored complex with cysteine.

The possibility of hydrogen peroxide being a product of the reaction was eliminated after some consideration. It was observed that hydrogen peroxide was not detectable in the reaction mixture by the simple test of adding $KMnO_4$ to determine if there is evolution of oxygen. Conversely, added hydrogen peroxide did not decompose readily to form oxygen under the reaction conditions. Finally, the stoichiometry does not permit the inclusion of hydrogen peroxide as a stable product of the reaction.

When the order of addition of reagents was changed so that the cysteine was added to the reaction mixture last (see Experimental Section), the rate decreased to about one-fifth of the k_{obsd} values of Table I. The preliminary mixing of the base with the ferric salt caused it to be converted to ferric hydroxide which upon standing for about 0.5 hr lost four-fifths of its reactivity with respect to complex formation. However, the same order of reaction and the same type of pH–rate curves, etc., were obtained. The 20% activity seemed to be quite consistent over a range of conditions.

New and interesting concepts concerning the nature of a given intermediate and the role of a specific metal ion in catalysis have been presented. The investigation of these phenomena is being continued.

Acknowledgment. The authors are greatly indebted to Dr. Waldo Semon (Semon Oxidation Fellowship) and the National Institutes of Health for financial support of this project. Without this support this work could not have been done.

---

(13) E. Elson and E. Edsall, *Biochemistry*, **1**, 1 (1962); and D. P. Wrathall, R. M. Izatt, and J. J. Christensen, *J. Am. Chem. Soc.*, **86**, 4779 (1964), with acknowledgment to referee II.

(14) The intermediate $\sim$A and likewise A or FeA_3, etc., should not be classified as an "activated complex." The term, activated intermediate, might be applicable, however.