The Detection of a Complex Intermediate in the Oxidation of Ascorbic Acid by Ferric Ion

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The oxidation of ascorbic acid by Fe^{3+} ion has been studied using the stopped-flow technique in an attempt to detect and study the formation of an intermediate. It has been shown that a transient species, believed to be a ferric ascorbate complex, and exhibiting a broad absorption band with $\lambda_{max.}$ = 560 nm is formed by a two-step mechanism. Values of 11 ± 6 l mol^{-1} cm^{-1} and 0·55 ± 0·4 for the extinction coefficient and formation constant respectively at 25 °C have been obtained for the overall complex formation. At 0 °C, the first step in the reaction is governed by the law, Rate = $k[Fe^{3+}][H_2A]$, where k has the value of 30 ± 10 l mol^{-1} s^{-1}. The probable mechanism of both the complex formation and overall redox reaction are discussed.

For many years it has been known that the rate of oxidation of ascorbic acid solutions by molecular oxygen is increased by reducible metals ions, and that the reaction often goes to completion. Thus many analyses of both ascorbic acid and metal ions have been devised using this fact.[1-3]

Different studies on the kinetics of the metal ion catalysis have given rise to conflicting opinions of the mechanism. For example, in the Cu^{2+} system, Weissberger and Luvalle [4] have suggested that the rate-determining step is expressed by

$$HA^- + Cu^{2+} \longrightarrow HA^{\bullet} + Cu^+$$

(H_2A = ascorbic acid, HA^- = monodissociated acid, $HA^{\bullet}$ = semiquinone) but Nord [5] points out that this does not account for the observed dependence of the rate on the concentration of dissolved oxygen. Grinstead,[6] in studying a model peroxidase system showed that the rate of oxidation of ascorbic acid by the Fe^{III}–EDTA complex was determined by a one-electron oxidation to a radical intermediate. It was suggested that electron transfer might occur *via* a ferric EDTA–ascorbic acid complex, but its existence could not be proved.

Equilibrium evidence for the existence of the complex $CuHA^+$ has been obtained by Taqui Khan and Martell [7a] and they have also suggested [7b] that the corresponding ferric complex may exist as a transient intermediate. In the study of a series of reactions of Fe^{III} chelates of aminopolycarboxylic acids,[7c] they proposed a mechanism involving a loose combination of the ascorbate anion and the oxidising agent. More recently they have reported the existence of corresponding vanadyl [7d] and uranyl [7e] complexes.

The kinetics of the reduction of hexacyanoferrate(III) by ascorbic acid has also received attention, and it is believed [8] that the rate-determining step is the interaction between a univalent ascorbate ion and the $Fe(CN)_6^{3-}$ ion.

It was felt that the slight differences in proposed mechanisms may have been due to the variety of metal species in solution, particularly where buffer solutions were used to maintain high pH values, and to the possibility of there being both a direct redox reaction between M^{z+} and reductant, and a metal ion-catalysed oxidation of ascorbic acid by molecular oxygen. The present study attempts to clarify the situation for the Fe^{3+} system by using degassed solutions to lower the oxygen content, low pH values to avoid the interference of the various hydroxy-species, and with particular attention to the detection of any transient intermediates.

EXPERIMENTAL

All solutions were prepared from demineralised water which was distilled successively from alkaline potassium permanganate and acidified $K_2Cr_2O_7$ in an all-glass apparatus. Ferric solutions were prepared from $Fe(ClO_4)_3$ (G. Frederick Smith) which was purified by three recrystallisations from $HClO_4$ (AnalaR). Ferric concentrations were determined by stannous chloride reduction and titration with $KMnO_4$, and the acid was analysed by titration to pH = 9 with freshly prepared NaOH. Stock $HClO_4$ solutions were determined volumetrically using sodium tetraborate. Sodium perchlorate solutions which were used to adjust the ionic strength were prepared from $NaClO_4$ (B.D.H., low in chloride).

The ascorbic acid was B.D.H. biochemical grade L-ascorbic acid which was stored at 0 °C. Solutions of known concentrations were made by accurately weighing out the required amount and dissolving this quickly in perchlorate solutions. Fresh solutions were prepared prior to each experiment.

All kinetic experiments were performed on a spectrophotometric stopped-flow apparatus similar to the design of Sturtevant.[9] The dead time of the instrument was approximately 5 ms, and the optical path-length of the observation

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cell was 2 mm. The whole apparatus could be thermostatted to within 0·05 °C. Absolute optical densities were calculated by comparison with some point on the trace (usually at $t = 0$ or $t = \infty$) for which the O.D. could be measured statically.

Prior to use, all solutions were degassed by melting the frozen solution *in vacuo*, refreezing, evacuating, and melting *in vacuo*. The concentration of dissolved gases was thus reduced by *ca.* 98% for each cycle. As well as preventing cavitation in the mixing chamber, this reduced the concentration of dissolved oxygen to something less than 10^{-6}M.

RESULTS

The Overall Reaction.—The stoicheiometry has previously been reported [6,7a] as the reduction of two moles of Fe^{3+} for every one mole of ascorbic acid oxidised.

i.e. $$2Fe^{3+} + H_2A \longrightarrow 2Fe^{2+} + 2H^+ + A \qquad (1)$$

(H_2A = ascorbic acid; A = dehydroascorbic acid)

This was confirmed in the present study by adding a known concentration of ascorbic acid to a solution of Fe^{3+} ion,

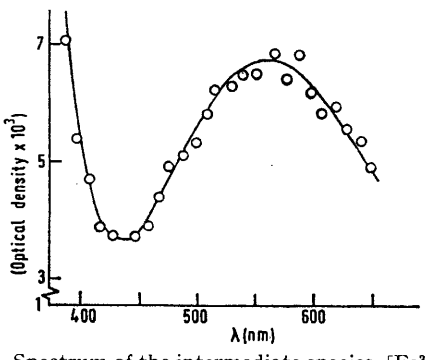

FIGURE 1 Spectrum of the intermediate species, $[Fe^{3+}] = 2·5 \times 10^{-2}$M; $[H_2A] = 5 \times 10^{-2}$M; $[HClO_4] = 0·2$M. Temp. = 25·0 °C; Time after mixing = 0·4 s (corresponds to maximum complex formation)

and measuring the change in Fe^{3+} concentration colorimetrically using the ferric thiocyanate method.

Since neither the reactants nor products showed any appreciable light absorption at wavelengths greater than 400 nm, the kinetics of the overall reaction (1) could only be studied at lower wavelengths. Because of the stray light transmitted by the optics of the instrument in the u.v. region (10% at $\lambda = 320$ nm), it was not possible to calculate optical densities very reliably in this wavelength region, but some kinetic runs were monitored at $\lambda = 340$ nm, and these showed that at 25 °C, using 0·2M-H^+, $2·5 \times 10^{-2}$M-Fe^{3+} and ascorbic acid concentrations in the range 2×10^{-2}M to 5×10^{-2}M, the first half-life was of the order of 500 ms, with a dependence on ascorbate concentration.

Detection of the Coloured Intermediate.—On monitoring the reaction with light in the wavelength range 450 to 650 nm, there was found to be an initial rapid increase followed by a slower decrease in optical density. The maximum optical density reached was wavelength dependent for a given set of reactant concentrations, and by observing the change in absorbance as a function of wavelength, the spectrum at any time could be compiled with the result shown in Figure 1.

In many cases there was considerable colour formed during the dead time of the instrument, but accurate optical densities were measured by reference to the infinite time value for complete reaction which was shown independently to be zero optical density. Blank runs excluding one or the other of the reactants showed that the above effect was due to reaction between ferric ion and ascorbic acid.

Variation of Fe^{3+} *and* H_2A *at* 25 °C.—The effect of changing the H_2A concentration on the formation of the intermediate is shown in Table 1. As well as the variation

TABLE 1

Variation of H_2A in intermediate formation. $t = 25$ °C; $\lambda = 560$ nm; $H^+ = 0·2$M

Run No.	$[Fe^{3+}]$(M)	$[H_2A]$(M)	Ionic strength	O.D.$_0$ [a]	O.D.$_{max.}$ [b]
(1)	$2·5 \times 10^{-2}$	10×10^{-2}	0·5	0·0054	0·0112
(2)	$2·5 \times 10^{-2}$	5×10^{-2}	0·5	0·0024	0·0063
(3)	$2·5 \times 10^{-2}$	$2·5 \times 10^{-2}$	0·5	0·0017	0·0033
(4)	$2·5 \times 10^{-2}$	$1·25 \times 10^{-2}$	0·5	0·0011	0·0017
(5)	5×10^{-2}	50×10^{-2}	1·0	0·0478	0·0749
(6)	5×10^{-2}	40×10^{-2}	1·0	0·0419	0·0660
(7)	5×10^{-2}	20×10^{-2}	1·0	0·0207	0·0395
(8)	5×10^{-2}	8×10^{-2}	1·0	0·0116	0·0204
(9)	5×10^{-2}	6×10^{-2}	1·0	0·0070	0·0125
(10)	5×10^{-2}	4×10^{-2}	1·0	0·0050	0·0090
(11)	5×10^{-2}	2×10^{-2}	1·0	0·0028	0·0041

[a] Optical density reached in instrument dead time. [b] Maximum optical density attained prior to slow fading reaction.

in O.D. with concentration, note should be made of the relatively large extent of reaction which occurred within the instrument dead time. When compared with the time taken to reach maximum O.D. (order of hundreds of ms), it is apparent that the formation of the colour may well occur *via* two steps. Similarly, Table 2 shows that there is

TABLE 2

Variation of Fe^{3+} in intermediate formation. $t = 25$ °C; $\lambda = 560$ nm; ionic strength = 1·0M, $H^+ = 0·2$M

Run No.	$[Fe^{3+}]$(M)	$[H_2A]$(M)	O.D.$_0$	O.D.$_{max.}$
(12)	10×10^{-2}	20×10^{-2}	0·0439	0·0781
(13)	8×10^{-2}	20×10^{-2}	0·0332	0·0629
(14)	6×10^{-2}	20×10^{-2}	0·0200	0·0439
(15)	4×10^{-2}	20×10^{-2}	0·0158	0·0303
(16)	2×10^{-2}	20×10^{-2}	0·0070	0·0120

TABLE 3

Study of the first step of intermediate formation at 0 °C. $\lambda = 560$ nm; ionic strength = 1·0M; $H^+ = 0·2$M

Run No.	$[Fe^{3+}]$(M)	H_2A(M)	Initial rate (O.D. per s)	Estimated [a] O.D.$_{max.}$
(17)	2×10^{-2}	50×10^{-2}	0·75	0·0075
(18)	5×10^{-2}	50×10^{-2}	2·20	0·0200
(19)	10×10^{-2}	50×10^{-2}	4·24	0·0400
(20)	10×10^{-2}	5×10^{-2}	1·25	0·0140
(21)	10×10^{-2}	3×10^{-2}	0·14	0·0024
(22)	10×10^{-2}	1×10^{-2}	0·10	0·0017

[a] See text.

linear dependence of the optical density on ferric concentrations although it is not possible to make meaningful kinetic measurements because of the very fast initial step in the complex formation.

Complex Formation at 0 °C.—Reduction of the temperature to 0 °C reduced the overall reaction rate by a factor of *ca.* 25, but the first step in complex formation was still only

marginally observable. Using the most sensitive scales of the instrument and a limited range of concentrations, it was possible to observe the initial part of reaction, and to estimate approximately the maximum optical density which would have been reached, had only the first step been involved.

The dependence of this part of the reaction on the reactant concentrations at 0 °C is shown in Table 3.

DISCUSSION

The detection of an absorption band other than those due to reactants or products shows that some other species is present during the course of the overall reaction. Figure 1 shows the species to have a broad absorption with $\lambda_{max.} \simeq 560$ nm, and the data in Tables 1 and 2 suggest that it may not be formed by a simple one-step process. Even though it was not possible to study the rate of formation of the intermediate at 25°, the dependence of the extent of its formation under differing concentration conditions is of considerable interest.

The following discussion only applies strictly to a system in which complex formation occurs to some well-defined equilibrium state at infinite time. In the present study this is not possible because of the subsequent decay of the intermediate. For the purpose of discussion, however, the assumption is made that the measured maximum optical density does correspond closely to what the equilibrium value would be, in the absence of the decay. From a consideration of the rate profiles, we consider that in most cases this assumption is valid within the limits of experimental uncertainty, and even in the extreme, is good to within 10—20%.

From Tables 1 and 2 it can be seen that the amount of intermediate formed is dependent on the concentrations of both reactants, suggesting that the species involved is a product of reaction between Fe^{3+} and H_2A. Since $\lambda_{max.}$ corresponds closely to that of the *d–d* absorption of the aquated Fe^{3+} ion, it is not unreasonable to postulate that the intermediate is a complex of Fe^{3+} ion with some form (associated molecule or dissociated anion) of ascorbic acid. On this basis it is possible to calculate both the formation constant and extinction coefficient of the 1 : 1 complex. Although a higher order complex is possible, the available data indicates that the 1 : 1 complex predominates under the experimental conditions used.

For reasons mentioned above, most experiments were carried out at relatively high acid concentrations, but a few runs at lower acidity showed a marked increase in the extent of complex formation, which is interpreted as indicating that the ligand present in the complex is probably HA^-. Previous investigators[4,7b] have also suggested that the intermediate is $FeHA^{2+}$, and not FeH_2A^{3-}.

$$i.e. \quad Fe^{3+} + H_2A \overset{K_1}{\rightleftharpoons} FeHA^{2+} + H^+ \qquad (2)$$

$$H^+ + HA^- \overset{K_2}{\rightleftharpoons} H_2A \qquad (3)$$

$$or \quad Fe^{3+} + HA^- \rightleftharpoons FeHA^{2+} \qquad (4)$$

If the initial concentration of Fe^{3+}, H_2A and H^+ are given by a, b, and c respectively, and the equilibrium concentration of $FeHA^{2+}$ is x, it is possible to derive equation (5), making the assumption that $c + x \simeq c$, and that $FeHA^{2+}$ is the only species significantly absorbing light at 560 nm.

$$\frac{a \,.\, b}{\text{O.D.}} = \frac{c}{E \,.\, l \,.\, K_1} + \frac{1}{E \,.\, l}(a + b) \qquad (5)$$

(E = extinction coefft., l = path length of observation cell).

Figure 2 is a plot of $\frac{a \,.\, b}{\text{O.D.}}$ *versus* $(a + b)$ for runs 1 to 4, and from this, values of E and K_1 are found to be 11·0 l mol^{-1} cm^{-1} and 0·55 respectively. Using these

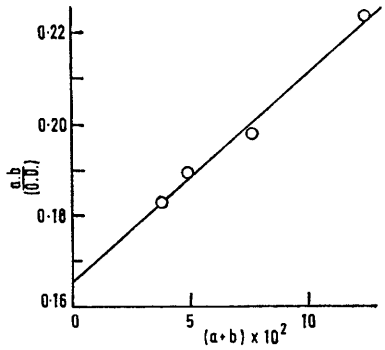

FIGURE 2 Determination of E and K_1; plot of equation (5)

values, the complex concentration in Run 1 is calculated to be only 2½% of the total $[H^+]$, showing the approximation $c + x \simeq c$ to be valid.

It must be noted that the measured optical densities are only 0·01 or less and are not measured relative to a reference cell as in normal spectrophotometry, so that the values are subject to 5—10% error. In addition, the assumption that $\text{O.D.}_{max.}$ corresponds to the full extent of complex formation must lead to some inaccuracy in the value of K_1.

A consideration of these possible errors shows that the values of E and K_1 can only be quoted as 11 ± 6 l mol^{-1} cm^{-1} and 0·55 ± 0·4 respectively.

Table 4 shows the values of K_1 which are obtained for the other runs when using $E = 11$ and taking into

TABLE 4

Calculated values for K_1, using $E = 11$

Run No.	K_1	Run No.	K_1
(5)	1·07	(11)	0·44
(6)	0·93	(12)	0·80
(7)	0·65	(13)	0·53
(8)	0·67	(14)	0·61
(9)	0·49	(15)	0·61
(10)	0·47	(16)	0·40

Av. 0·64 ± 0·20

account the term $c + x \simeq c$ where necessary. Despite the variation, all values are within the limits quoted above.

At 0 °C, reaction was sufficiently slow to enable us to

make rate measurements on the initial step of the complex formation. The results in Table 3 indicate that both the initial rate and the extent of colour formation for the first step are proportional to the first power of the Fe^{3+} concentration. The changes in runs 19—22 are so small as to be subject to very large errors, but the results suggest that the rate is dependent on H_2A concentration to the first power only.

Assuming a rate law of the form Rate $= k[Fe^{3+}][H_2A]$ a value of 30 ± 10 l mol^{-1} s^{-1} can be calculated for k at 0 °C. It is clear that two stages are involved in the formation of the intermediate, and since the shape of the spectrum does not change appreciably, it is felt that the second step involves some rearrangement of the complex formed initially. The possibility of a diascorbate complex cannot be ignored, but the dependences on H_2A tend to indicate that its formation is negligible. In all cases the optical density at the end of the first stage is *ca.* 0·5—0·7 times the $O.D._{max.}$, regardless of the reactant concentrations. If the second complex was involved, it would be expected that at high ascorbate concentration, the ratio [2nd complex] : [1st complex] would be much larger than at low ascorbate concentrations.

Carlyle and Espenson [10] have discussed a similar two-step complex formation in the case of the monobromoiron(III) ion. Some of their evidence was obtained in the same way as in the present study, and they have been able to show by independent experiments that the steps involved are the rapid formation of an ion pair, followed by the slower formation of an inner-sphere complex. They point out that it is not possible to say whether the ion pair is the precursor of $FeBr^{2+}$.

It may be that this type of process occurs in the Fe^{3+}–H_2A system, but there is yet another possibility to be considered. The ascorbate molecule can be thought of as a bidentate ligand, and the two steps could be the formation of the monoassociated complex followed by closure of the chelate ring. It is difficult to estimate the

$$Fe^{3+} + H_2A \rightleftharpoons H^+ + Fe^*HA^{2+} \quad (6)$$
monoassociated complex

$$Fe^*HA^{2+} \rightleftharpoons FeHA^{2+} \quad (7)$$
chelated complex

relative rates of the two steps in the process because of their mutual interaction, and the interaction of the final steps in which the colour fades, hence the use of initial rates in attempting to measure the rate constant for the first step only.

The values of K_1 and E refer to the overall formation of the blue colour. If one accepts the chelating mechanism, and assumes that at the point of maximum optical density virtually all the complex exists in this form, then K_1 and E refer directly to the chelated complex. If however, the equilibrium constant for reaction (7) is small, then the optical density is due to both species, and it would not be possible to separate the two terms.

A similar argument holds for the ion pair to inner-sphere reaction. Carlyle and Espenson [10] showed that for the Fe^{3+}/Br^- system, the formation constant of the ion pair is considerably larger than for the inner-sphere complex, and in that case it was only possible to separate the two extinction coefficients and dissociation constants by independent determinations of one or the other.

The Overall Reaction.—It is unfortunate that the properties of the intermediate complex are such as to make a complete analysis very difficult. It is clear however, that the reaction does proceed *via* an intermediate, and the values obtained for the formation constant, extinction coefficient, and rate of formation are all of the expected order of magnitude for the suggested complex.

Following the slower rearrangement of the complex, it is proposed that there is an intramolecular electron-transfer from ascorbate to ferric ion. The reduced metal complex then dissociates to produce Fe^{2+} and an ascorbate radical which undergoes further rapid reaction with another Fe^{3+} ion to produce another Fe^{2+} ion and the dehydroascorbic acid: to yield overall two moles of Fe^{2+}

$$FeHA^{2+} \rightleftharpoons (Fe^{2+} \cdots HA^\bullet) \quad (8)$$

$$(Fe^{2+} \cdots HA^\bullet) \rightleftharpoons Fe^{2+} + H^+ + A^{\bullet -} \quad (9)$$

$$A^{\bullet -} + Fe^{3+} \longrightarrow Fe^{2+} + A \quad (10)$$

for every mole of ascorbic acid oxidised. Preliminary experiments indicate that it may be possible to 'stabilise' the blue complex to some extent by freezing the solution rapidly on mixing, or by adding a large excess of Fe^{2+} ion which presumably exerts its effect by the reverse of equation (9).

For the proposed process to occur, it is unnecessary for a transition state containing more than one Fe^{3+} ion and one ascorbate molecule to be formed and yet the 2 : 1 stoicheiometry is readily explained. If the mechanism is correct, a further study of the rate of the overall reaction should indicate a first-order dependence on both Fe^{3+} and H_2A concentrations.

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