The effect of citrate anions on the kinetics of cupric ion–catalysed oxidation of ascorbic acid

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The kinetics of uncatalysed and Cu(II) ion-catalysed oxidation of ascorbic acid by molecular oxygen in the presence of citric acid and its anions were studied at 25°C. In the pH range investigated (2·5–6·0), the rate of cupric ion-catalysed oxidation was found to be first order with respect to ascorbic acid. The observed effects of pH and cupric ion concentration on the reaction rate suggests a mechanism which may involve the formation of a transition complex between monoascorbate ion and Cu(II)–citrate chelate. The catalytic ability is in the order $Cu(II) > H_3CiCu^+ > HCiCu^- > CiCu^{-2}$. Hydrogen peroxide was also detected during the reaction.

INTRODUCTION

Because of its significance in food and pharmaceutical industries, the Cu(II)-catalysed oxidation of ascorbic acid (H_2A) by molecular oxygen has attracted our attention in recent years. This reaction has been studied in various media by different workers (Khan & Martell, 1967a; Khan & Martell, 1967b; Ogata *et al.*, 1968; Davidson & Grieger-Block, 1977). The complexation of Cu(II) by certain ligands such as EDTA, CDTA, NTA and poly-L-histidine modifies its catalytic activity (Pecht *et al.*, 1967; Khan & Martell, 1967b; Schwertnerova *et al.*, 1976; Mikova *et al.*, 1981), but no information on the catalytic effects of citrate–Cu(II) chelates in H_2A oxidation is in the literature. Citric acid, on the other hand, merits special interest due to its presence in numerous natural products and its allowance in food for various technical purposes.

Although the citric acid and its anions which interacted to different extents with Cu(II) to form complexes was a complicating factor, the present study was undertaken to elucidate the catalytic activity of Cu(II)–citrate chelates in H_2A oxidation in the pH range 2·5–6·0 and provide new information on the mechanism of this important reaction.

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MATERIALS AND METHODS

Reagents

All the reagents used in this investigation were of analytical reagent grade and supplied by E. Merck, Darmstadt, FRG. Thrice distilled water was used for the preparation of the samples and at all other stages of analysis.

Buffer solution (pH 2·5–6·0)
The pH of 0·5 M citric acid was adjusted to the desired value by dropwise addition of 0·5 M KOH, using a Knick model pH-meter. Buffer solutions are stable for two weeks.

Sample preparation

Sample solution containing $2·27 \times 10^{-3}$ M ascorbic acid and $1·58 \times 10^{-5}$, $7·87 \times 10^{-5}$ or $15·8 \times 10^{-5}$ M Cu(II) was prepared at the beginning of each run. At the same time, 2·5 ml of citrate buffer was added into the polarographic cell and the temperature was adjusted to 25°C. During this time, the cell was saturated with oxygen by passing air through it for 15 min. Then 2·5 ml of the sample solution prepared as mentioned above was added to the citrate buffer in the cell. In the final solution the ascorbic acid concentration was $1·14 \times 10^{-3}$ M

and that of Cu(II) was 7.9×10^{-6}, 3.9×10^{-5} or 7.9×10^{-5} M.

Kinetic measurements

The experimental set-up mainly consisted of a polarographic cell with thermostat and its auxiliary equipment. The cell was obtained from Princeton Applied Research and fitted with the air-supply system. A stream of air (flow rate = 40 ml min^{-1}) was passed through the cell during reaction, except when the anodic current was recorded. The flow-rate of air was adjusted by a flow-meter (Gilmont No. 11) from Cole-Parmer Company (Great Neck, New York, USA). The air was presaturated with water vapour by streaming through a wash-bottle. As the rate of reaction is low compared to the rate of dissolution of oxygen, the reacting solution was considered to be saturated with oxygen at all times. All the experiments were carried out at 25°C and the temperature was controlled by means of a thermostat system (±0.5°C) which contained an immersion circulator (Model 1266-00) from Cole-Parmer Company (Chicago, Illinois, USA).

Polarographic measurements

Ascorbic acid
A normal polarographic technique was used to follow the concentration of ascorbic acid during each run. Ascorbic acid has a well-defined anodic current in citrate buffers and the half-wave potential ranged from +170 to +20 mV as the pH changed from 2.5 to 6.0 (Şahbaz & Somer, 1992). At the beginning of each experiment, the anodic current of ascorbic acid corresponding to the initial concentration was measured and then anodic waves were recorded in 30-min intervals during the reaction. Thus, ascorbic acid content was
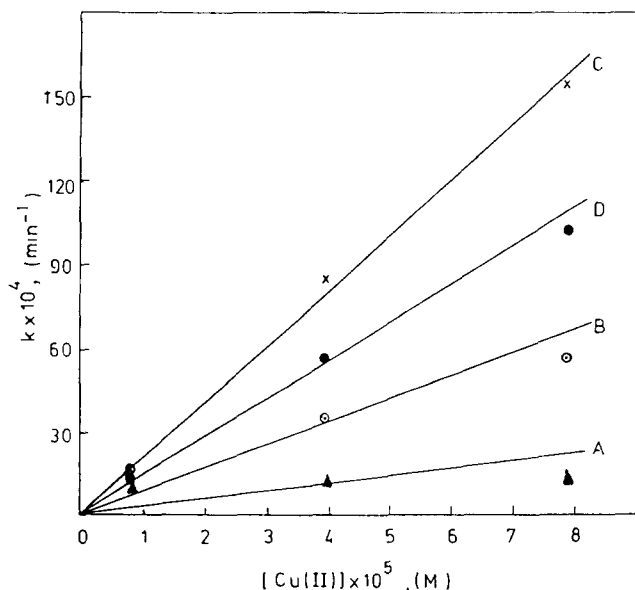

Fig. 1. Catalytic effect for the oxidation of H_2A in the presence of Cu(II)-citrate complexes at 25°C at pH values of A, 2.5; B, 3.5; C, 4.5; D, 6.0. k = Difference between the first-order rate constants in the presence and in the absence of copper.

determined by the comparison of wave heights measured at the beginning and during the run.

Oxygen
By measuring the cathodic waves of oxygen, which occurred at about 0 and -0.8 V in citrate buffers (pH 2.5–6.0), the concentration of oxygen was controlled and determined to be constant during the reaction.

Hydrogen peroxide
The formation of hydrogen peroxide as a reaction product was also followed qualitatively (Somer & Green, 1973; Somer & Temizer, 1984) by recording the current of the hydrogen peroxide wave with half-wave potential of about -0.8 V. This wave was the secondary wave of oxygen as mentioned in Oxygen Measurement. For this reason, oxygen present in the solution had to be removed by passing through nitrogen to observe the hydrogen peroxide wave formed during the reaction.

RESULTS

Spontaneous and copper(II) ion-catalysed oxidation

In order to determine the spontaneous and Cu(II) ion-catalysed oxidation of H_2A (1.14×10^{-3} M), ascorbic acid solutions with copper levels of 0, 7.9×10^{-6}, 3.9×10^{-5} and 7.9×10^{-5} M were studied at 25°C. The reaction was followed for 330 min over the pH range 2.5–6.0. Except for all Cu(II) concentrations at a pH value of 2.5 and for the spontaneous oxidation in the pH range 2.5–4.5, the reaction was followed for 2 or more half lives.

Most authors have reported that the oxidation reaction studied in various model systems followed a first-order rate equation (Khan & Martell, 1967a,b; Ogata et al., 1968; Ogata & Kosugi, 1969). In our experiments, the plot of the logarithm of ascorbic acid concentration versus time yielded a straight line in each case, which indicated a first-order reaction. Each experiment under specified conditions, was carried out at least three times. The first-order rate constants were calculated by linear regression analyses. In all cases at a certain pH, the rate increased with copper concentration.

The spontaneous oxidation rate of H_2A in the absence of added Cu(II) was relatively high at pH 6.0. In each case the difference between the first-order rate constants in the presence and in the absence of added Cu(II) ion was taken as the rate characteristic. Figure 1 shows the variation of rate constant (k) with total Cu(II) concentration at each pH, where $k = K[\text{Cu(II)}]$. At 7.9×10^{-6} M Cu(II) concentration, there was no effect of pH on the rate constant. The specific rate constants (K) given in Table 1 were calculated from the gradients of the straight lines in Fig. 1. The results showed that K changed with pH, reaching a maximum at pH 4.5.

Table 1. Specific rate constants (K) of Cu(II) ion–catalysed oxidation of ascorbic acid

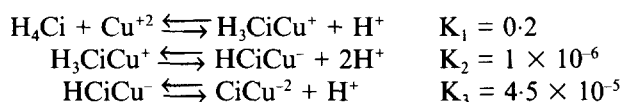| pH	K $(M^{-1} min^{-1})$
2·5	4·1
3·5	57·9
4·5	193·1
6·0	123·3

Undissociated and monionic forms of ascorbic acid

The K_{a1} and K_{a2} values reported for ascorbic acid are $7·9 \times 10^{-5}$ at 24°C and $1·62 \times 10^{-12}$ at 16°C (Weast, 1982). The undissociated (H_2A) and monionic (HA^{-1}) forms of ascorbic acid were the main species present in solution over the pH range 2·5–6·0 and their relative concentrations as a function of pH can be calculated by using K_{a1}.

Citrate complexes of copper

Some authors reported that complexes were formed between Cu(II) ions and citric acid or its dissociated forms (Meites, 1950; Parry & Du Bois, 1952; Warner & Weber, 1953).

Warner and Weber (1953) reported that three different complexes (H_3CiCu^+, $HCiCu^-$ and $CiCu^{-2}$) were formed as a function of pH. The following reactions and the equilibrium constants for the formation of complexes reported by the same authors were as follows:

$$H_4Ci + Cu^{+2} \rightleftharpoons H_3CiCu^+ + H^+ \qquad K_1 = 0·2$$
$$H_3CiCu^+ \rightleftharpoons HCiCu^- + 2H^+ \qquad K_2 = 1 \times 10^{-6}$$
$$HCiCu^- \rightleftharpoons CiCu^{-2} + H^+ \qquad K_3 = 4·5 \times 10^{-5}$$

Citric acid is tribasic and the first, second and third dissociation constants are equal to $7·5 \times 10^{-4}$, $1·7 \times 10^{-5}$ and $4·0 \times 10^{-7}$, respectively (Skoog & West, 1976). In the reactions, citric acid was represented as H_4Ci because four protons were displaced from acid to form $CiCu^{-2}$. The first stable chelate formed was $HCiCu^-$ [stability constant $\sim10^7$, calculated by using K_1 and K_2 and first, second and third acid dissociation constants of citric acid] and was present between pH 3·0 and 5·0. $CiCu^{-2}$ was the most stable chelate [stability constant $\sim10^{18}$, reported by Warner & Weber (1953)] and practically all cupric ion is bound to this complex at pH 6·0.

In this connection, we have studied the polarography of solutions containing cupric ion (1×10^{-3} M) and the citrate ions over the pH range 2·5–6·0. The potential for the oxidation or reduction of a metallic ion is greatly affected by the presence of species that form complexes with that ion. It is not surprising, therefore, that similar effects are observed with polarographic half-wave potentials ($E_{1/2}$). The $E_{1/2}$ for the reduction of a metal complex is generally more negative than that for reduction of the corresponding simple metal ion. Moreover, the magnitude of this shift in half-wave potential is related to the stability of the complex. The
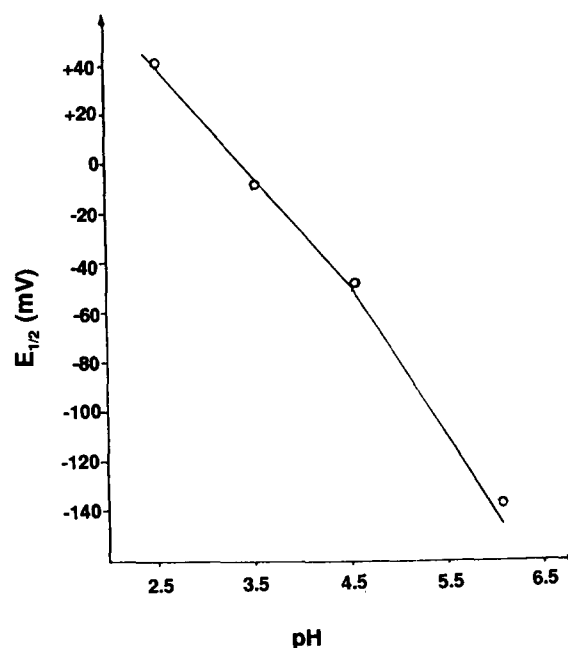
Fig. 2. Variation of $E_{1/2}$ with pH.

$E_{1/2}$ of Cu(II) in the absence of complexing agent was reported as +20 mV (Skoog & West, 1976). Compared to this value, the data in Fig. 2 ($E_{1/2}$ *versus* pH) just reveal the formation of complexes between Cu(II) and different citrate anions for all pH values considered. Moreover, $E_{1/2}$ shifts to more negative values as the pH increases. The decrease of $E_{1/2}$ with increase in pH is smaller in the pH range 2·5–4·5 than in the range 4·5–6·0, showing that the complexes formed in the range 4·5–6·0 are more stable, compared to those in the range 2·5–4·5. Thus we have similar results to the data reported in the literature (Meites, 1950; Parry & Du Bois, 1952; Warner & Weber, 1953).

DISCUSSION

The mechanism proposed for Cu(II) chelate–catalysed oxidation of H_2A must explain the following experimental results:

(1) the increase of rate with the concentration of Cu(II),

(2) the increase of rate with pH to about 4·5 and the decrease at pH 6·0,

(3) the formation of H_2O_2 at all pH values.

In the presence of Cu(II) ions, the initiation of the oxidation reaction is through the transfer of an electron from HA^- to Cu(II) (Khan & Martell, 1967a,b). As a consequence of this, the reaction rate was expected to increase with increasing Cu(II) and pH. This was true for Cu(II) but seemed to be a contradiction when the increase in HA^- concentration was considered. At this point, the Cu(II)–citrate chelates formed as a function of pH and their relative stabilities should be taken into account. For Cu(II) chelate–catalysed oxidation of H_2A, two mechanisms may be considered:

Table 2. Rate constants for the oxidation of ascorbic acid as catalysed by free metal ion

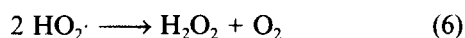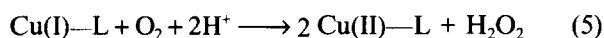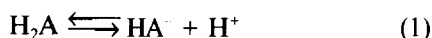| Total [Cu^{+2}] concentration (M)	k (obsd) (min^{-1})	k_f^{a} (min^{-1})
7.9×10^{-6}	14.9×10^{-4}	4.17×10^{9}
3.9×10^{-5}	56.7×10^{-4}	4.95×10^{9}
7.9×10^{-5}	102.9×10^{-4}	5.40×10^{9}

$^{a}k_f$ = Rate constant attributed to free copper ion.

(1) the catalytic effect may be due to the free Cu(II) ion in equilibrium with the chelate compound or (2) Cu(II)–citrate chelates may directly participate in the electron transfer. Other works have shown that very stable Cu(II) chelates like Cu–EDTA ($K = 10^{18}$) have participated in the catalytic oxidation (Khan & Martell, 1967b; Schwertnerova et al., 1976).

The main Cu(II)–citrate chelates are H_3CiCu^+ and $CuCi^{-2}$ at pH 2·5 and 6·0, respectively (Warner & Weber, 1953). As a test of the first mechanism, the concentration of free cupric ion in equilibrium with H_3CiCu^+ (at pH 2·5) and with $CuCi^{-2}$ (at pH 6·0) were calculated, taking into account the complex formation reactions and constants, and the acid dissociation constants of citric acid. For the total Cu(II) ion concentration range studied, the corresponding concentrations of free Cu(II) ion were found to be between $2·9 \times 10^{-7}$ and $2·9 \times 10^{-6}$ M at pH 2·5 and between $3·1 \times 10^{-14}$ and $3·1 \times 10^{-13}$ M at pH 6·0, respectively. The concentrations of HA$^-$ at the same pHs were $2·8 \times 10^{-5}$ M and $1·1 \times 10^{-3}$ M.

Taking the observed rate constant at pH 2·5 as the basis, and assuming that the rate changed linearly with the free Cu(II) and HA$^-$ anion concentrations, a new rate constant was calculated for each Cu(II) concentration at pH 6·0. If the mechanism was operative, the rates calculated (Table 2) should be equal to the rates observed. Thus the results in Table 2 seemed to be incompatible with the dissociation mechanism. The second alternative is the direct participation of the Cu(II)-chelate species in the oxidation of H_2A and the following mechanism is proposed.

$$H_2A \rightleftharpoons HA^- + H^+ \quad (1)$$

$$HA^- + Cu(II){-}L \rightleftharpoons HA{-}Cu(II){-}L \quad (2)$$

$$HA{-}Cu(II){-}L \xrightarrow{slow} \cdot AH + Cu(I){-}L \quad (3)$$

$$\cdot AH + O_2 \longrightarrow A + HO_2\cdot \quad (4)$$

$$Cu(I){-}L + O_2 + 2H^+ \longrightarrow 2\ Cu(II){-}L + H_2O_2 \quad (5)$$

$$2\ HO_2\cdot \longrightarrow H_2O_2 + O_2 \quad (6)$$

where L stands for citrate anions as a ligand.

In this mechanism, a transition complex between HA$^-$ and Cu(II) chelate is formed. This is followed by a rate-determining electron transfer within the transition complex, from ascorbate to metal ion. In the rate-determining step, the transition complex dissociates to cuprous chelate and monodehydroascorbate radical

($\cdot$AH), the presence of which was detected by some workers (Yamazaki et al., 1960; Bielski et al., 1971; McAlpine et al., 1972). Oxidation of this semiquinone and the lower valence metal chelate by molecular oxygen takes place in subsequent steps, and H_2O_2 and dehydroascorbic acid (A) are formed as the reaction products. The formation of H_2O_2 was detected in our experiments as mentioned in the experimental section. Reactions (2) and (3) involve the oxidation of H_2A by Cu(II) chelates in the absence of oxygen. In this connection, during this work we observed this reaction polarographically with equimolar H_2A and Cu(II) in citrate buffers.

From the proposed mechanism, it is obvious that the rate should depend on HA$^-$ and Cu(II)–L concentrations as well as the catalytic activities of Cu(II)–L formed in the pH range considered. In the citrate buffers (pH 2·5–6·0), different Cu(II)-citrate chelates are present at each pH, which have different catalytic effects. The main species are Cu(II) and H_3CiCu^+ at pH 2·5; $HCiCu^-$ at pH 3·5; $HCiCu^-$ and $CiCu^{-2}$ at pH 4·5 and $CiCu^{-2}$ at pH 6·0 (Warner & Weber, 1953). As the pH increases, more stable complexes are formed and the binding between Cu(II) and citrate anions becomes stronger and consequently the tendency to form HA–Cu(II)–L decreases. Secondly, the affinity for monoascorbate anion (HA$^-$) to form HA–Cu(II)–L decreases as the negative charge on Cu(II)–L increases. Thus it is obvious from the charge and stability constants that the catalytic activity of Cu(II)-chelates should be in the order Cu(II) > H_3CiCu^+ > $HCiCu^-$ > $CiCu^{-2}$. According to this, catalytic activity must decrease with increasing pH. In spite of this, the reaction rate increased with increase in HA$^-$ to about 4·5 and decreased at pH 6·0. At this pH, as stated before, $CiCu^{-2}$ is the main specie, which has the highest stability with the greatest negative charge. Thus its low tendency to form a transition complex lowers its catalytic activity, resulting in the decrease in reaction rate.

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