Oxidation of ascorbic acid by nitrous acid: conditions where autoxidation of nitric oxide is rate determining

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In air-saturated aqueous acid (0.01–0.1 mol dm^{-3}) with equimolar (1 × 10^{-4} mol dm^{-3}) ascorbic acid and nitrous acid, the second-order reaction has a first-order kinetic form due to rapid regeneration of nitrous acid by autoxidation of the nitric oxide produced, and the rate constant takes the form $k = a[H^+] + b[H^+][Cl^-]$, typical of a rate-determining nitrosation, whereas in 0.5–1 mol dm^{-3} HCl with a deficiency of nitrous acid autoxidation of nitric oxide is for the most part rate-determining.

The reaction under anaerobic conditions of ascorbic acid (H_2A) with nitrite or nitrous acid to form dehydroascorbic acid (A) has been thoroughly investigated.[1] As the acidity increases, the reaction in which formation of N_2O_3 is rate-determining gives way to the rate determining reaction of N_2O_3 with HA^- and then H_2A. Below about pH 2 rate determining acid catalysed reaction of nitrous acid with H_2A takes over, and in this region chloride ion catalysis has been noted, though no details are given.[1]

The presence of oxygen has been shown to affect the reaction stoichiometry[2] and this affects the ability of ascorbic acid to act as an *in vivo* nitrosation inhibitor. It has been suggested that oxygen could exert its influence by reaction with nitric oxide reforming nitrous acid, or by direct oxidation of the radical intermediate $HA^{\bullet}$ (or its conjugate base[3]). We now provide evidence that both roles are indeed important.

As in our previous studies of nitrosation/oxidation of hydroquinone and related compounds,[4] we have used a numerical integration package in order to take into account the accompanying inorganic reactions under acidic and aerobic (air-saturated) conditions.

All reactions were studied by UV spectroscopy and with EDTA (1 × 10^{-3} mol dm^{-3}) present to remove any possibility of catalysis by traces of, for instance, copper ion.[5]

Between pH 1 and 2 and with *equimolar* (1 × 10^{-4} mol dm^{-3}) ascorbic acid and nitrous acid (added last) the reaction appears accurately first-order kinetically, and the first order rate constant shows a clear dependence on the concentrations of hydrogen ion and chloride ion (Table 1). In 0.5–1 mol dm^{-3} HCl with 1–2 × 10^{-4} mol dm^{-3} H_2A and a deficiency (1–3 × 10^{-5} mol dm^{-3}) of nitrous acid, the reaction proceeds to completion but a completely different kinetic form is observed. After an initial burst corresponding to the consumption of one mole of ascorbic acid per mole of nitrous acid, there is an initially almost linear (zeroth order) decrease in the concentration of H_2A (Fig. 1).

The following mechanism accounts quantitatively for these observations.

$$2HNO_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} NO_2 + NO\,(+H_2O)$$

$$2NO + O_2 \xrightarrow{k_3} 2NO_2$$

$$H_2A + HNO_2 \xrightarrow{k_4} NO + HA^{\bullet}\,(+H_2O)$$

$$HA^{\bullet} + O_2 \xrightarrow{k_5} A + HO_2^{\bullet}$$

$$HA^{\bullet} + HO_2^{\bullet} \xrightarrow{k_6} A + H_2O_2$$

Table 1 Observed and calculated[a] first-order rate constants in the reaction of ascorbic acid with nitrous acid (both initially 1 × 10^{-4} mol dm^{-3} unless otherwise stated) in air saturated aqueous solution at 25 °C and I = 1 mol dm^{-3}

$[H^+]$/mol dm^{-3}	$[Cl^-]$/mol dm^{-3}	$k_{obs}/10^{-3}$ s^{-1}	$k_{calc}/10^{-3}$ s^{-1}
0.02	0	2.4	2.3
0.02	0.2	5.6	5.1
0.02	0.4	8.1	7.7
0.02	0.6	12	10
0.02	0.8	14	13
0.04	0	4.3	4.5
0.04[b]	0[b]	3.2[b]	4.0[b]
0.04[c]	0[c]	8.6[c]	9.4[c]
0.04	0.8	21	23
0.04[b]	0.8[b]	16[b]	18[b]
0.04[c]	0.8[c]	48[c]	52[c]
0.06	0	6.4	6.5
0.06	0.8	28	32
0.08	0	8.1	8.4
0.08	0.8	34	41
0.10	0	10	10
0.10	0.2	22	22
0.10	0.4	28	31
0.10	0.6	38	41
0.10	0.8	50	50

[a] Concentration–time data were calculated by numerical integration using the mechanism and rate constants given in the text, and first-order rate constants were then computed from these. [b] Initial concentration of ascorbic acid 2 × 10^{-4} mol dm^{-3}. [c] Initial concentration of nitrous acid 2 × 10^{-4} mol dm^{-3}.

The inorganic part (steps 1–3) is as previously,[4] but does not include the reversible reaction of $2NO_2$ with water to form nitrous acid and nitrate because the inclusion of these steps made no difference to the concentrations of any of the species as calculated by numerical integration. Clearly under the conditions investigated here the sink for NO_2 is step 2.[6]

The calculations correctly predict the first-order form under equimolar conditions (Table 1); the reason is that the nitrous acid is reformed relatively rapidly through steps 3 and 2 and its concentration, though not constant (the numerical integrations indicate that it decreases rapidly by up to 40% of its initial value and then increases slowly), is constant enough during the bulk of the reaction to make departures from a first-order form undetectable.

The first-order rate constants from the calculated concentrations fit well with the observed ones (Table 1) with k_4 given by eqn. (1) and a = 1300 dm^6 mol^{-2} s^{-1}, b = 8400 dm^9 mol^{-3} s^{-1}.

$$k_4 = a[H^+] + b[H^+][Cl^-] \qquad (1)$$

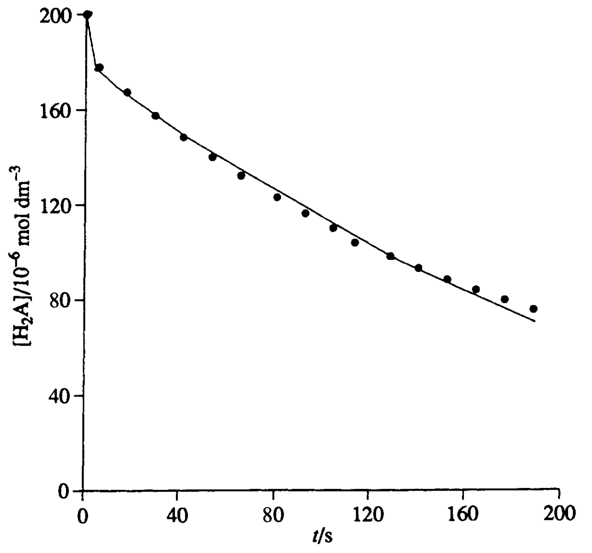

Fig. 1 Concentration of ascorbic acid with time for a reaction in 0.5 mol dm^{-3} HCl at 25 °C containing initially 2 × 10^{-4} mol dm^{-3} ascorbic acid and 2 × 10^{-5} mol dm^{-3} nitrous acid. The large circles are observed concentrations and the line is calculated by numerical integration using the mechanism and rate constants given in the text.

This is completely in accord with nitrosation being rate determining, and occurring concurrently through the nitrosonium ion or nitrous acidium ion and through an equilibrium concentration of NOCl.[7] The nitroso intermediate in step 4 is not kinetically significant and is not shown. It is to be contrasted with the reaction of hydroquinone where there is no chloride ion catalysis and the later step of homolysis of the nitroso intermediate to form nitric oxide and the aryloxy radical is rate determining.[4]

The numerical integrations indicated that if step 4 is made fast enough (high $[H^+]$ and $[Cl^-]$) and with a deficiency of nitrous acid the reformation of HNO_2 from NO (step 3, followed rapidly by step 2) could become rate determining. This proved to be the case. After the initial rapid burst in which we suggest that the nitrous acid is almost quantitatively converted to nitric oxide, the rate of consumption of the remaining ascorbic acid is almost constant, though decreasing slowly as the reaction proceeds (Fig. 1). This is in accord with eqn. (2). The

$$-\mathrm{d}[H_2A]/\mathrm{d}t = 4k_3[NO]^2[O_2] \qquad (2)$$

concentration of nitric oxide remains constant and equal to the initial concentration of nitrous acid and the curvature of the concentration–time graph relates only to the changing concentration of oxygen. The factor of 4 arises because each oxygen molecule regenerates four nitrous acid molecules through steps 3 and 2, and because in the mechanism proposed above it takes only one nitrous acid molecule to remove one ascorbic acid molecule, the second part of the oxidation being effected by oxygen or the hydroperoxy radical (steps 5 and 6).

A value of k_3 was estimated from the initial slope in this region (obtained by least squares quadratic fit to the concentration–time curve). Mean values of 2.9 × 10^6 dm^6 mol^{-2} s^{-1} in 1 mol dm^{-3} HCl and 2.5 × 10^6 dm^6 mol^{-2} s^{-1} in 0.5 mol dm^{-3} HCl–0.5 mol dm^{-3} NaCl were obtained. These should be compared with the most recently reported values[8,9] of 2.1 × 10^6 dm^6 mol^{-2} s^{-1} (25 °C) and 1.6 × 10^6 dm^6 mol^{-2} s^{-1} (23 °C). It is encouraging that the value is of the same order of magnitude as the literature values, though the reason why it is somewhat larger is not clear. It could be regarded as further evidence for an acid catalysed component for this reaction.[9] This point will be studied further. For the purposes of the numerical integrations the value of k_3 was taken to be given by eqn. (3). The inclusion of the acid catalysed term in previous work[4,6] would have made no significant changes.

$$k_3 = 2.1 \times 10^6\ \mathrm{dm^6\ mol^{-2}\ s^{-1}} + 0.8 \times 10^6\ \mathrm{dm^9\ mol^{-3}\ s^{-1}}\ [H^+] \qquad (3)$$

Quantitative agreement with the observed degree of curvature for various initial concentrations of ascorbic acid could only be obtained if steps 5 and 6 were included, in which $HA^{\bullet}$ is further oxidised by O_2 and $HO_2^{\bullet}$. This was a surprise; we started with the view that the further oxidation would be effected by a nitrogen species, either nitrous acid or nitrogen dioxide, but this gave the wrong stoichiometry for the initial burst, double the measured value of k_3 (taking it further from literature values) because the factor of 4 must be replaced by a factor of 2 in eqn. (2), and gave insufficient curvature of the absorbance–time curve which followed the initial burst. Using the sequence shown however, all the features of the reaction could be quantitatively reproduced. Oxidation of $HA^{\bullet}$ by a nitrogen species must also occur (the reaction proceeds under anaerobic conditions[1]), but its contribution under our conditions must be small.

The rate constants used were as follows: k_1 = 15 dm^3 mol^{-1} s^{-1} as previously;[4] k_2 = 9 × 10^7 dm^3 mol^{-1} s^{-1} as previously;[6] k_3 as given by eqn. (3); k_4 as given by eqn. (1); $k_5/k_6 \approx 2$. The values of k_5 and k_6 were not determined by the procedure but needed to be large.

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