Interaction of L-ascorbate with substituted nitrosobenzenes. Role of the ascorbate 2-OH group in antioxidant reactions

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NJC Letter

L-Ascorbate reduces substituted nitrosobenzenes in aqueous solution, in a process that includes a cyclic transition state composed of ascorbate and nitrosobenzene and in which concerted electron and proton transfer leads to the products. The results suggest that the mechanism by which ascorbate interacts with the α-tocopheroxyl radical in the exceptionally important α-tocopherol–ascorbate redox cycle also includes the corresponding cyclic transition state.

L-Ascorbic acid is a 'simple yet mysterious molecule'.[1] Ascorbate is of exceptional importance in the antioxidant protection of the human and other aerobic organisms. It is considered the most important antioxidant in the human plasma,[2] where ascorbate decreases, among others, the oxidative damage of DNA in lymphocytes[3] and prevents the metal-ion-dependent peroxydation of low-density lipoprotein.[4] Ascorbate also acts synergistically with other physiological antioxidants[5] and is a biological reductant of the human carcinogen Cr^{VI}.[6] Support of the antioxidant activity of α-tocopherol by ascorbate in biological membranes, lipoproteins, cells and plasma has been reported.[7] In addition, nitric oxide (NO) interacts with the redox cycle involving α-tocopherol and ascorbate, where ascorbate regenerates α-tocopherol from the α-tocopheroxyl radical.[8]

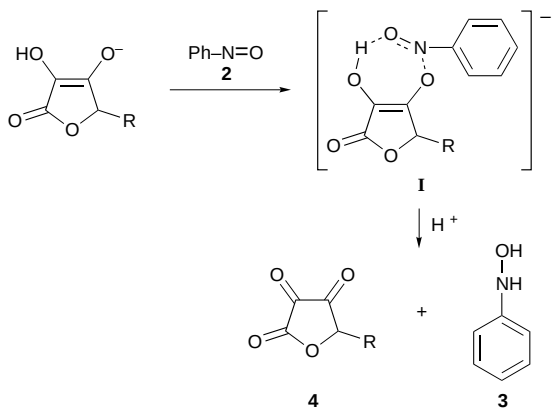
The inactivation of peroxy and similar radical species *via* the interaction with ascorbate is, in many cases, a fundamental part of the antioxidant action of ascorbate. Ordinarily, an electron and a proton are transferred to the radical, giving the corresponding reduced product and the ascorbyl radical. On the other hand, interaction of ascorbate with the α-tocopheroxyl radical is of key importance for sustaining the antioxidant capacity of α-tocopherol.[7]

Recently, it has been observed[9a] that ascorbate **1** reduces nitrosobenzene **2**, giving the corresponding phenylhydroxylamine **3** and dehydroascorbic acid **4** (see Scheme 1). The reaction is of particular interest because of the cytotoxic and antiretroviral properties of the nitrosobenzenes.[9] Nitrosobenzenes also can be present in the human blood as products of the reduction of aromatic nitro compounds, introduced into the organism as a toxin.[10] We report here the results of our study on the mechanism of the biochemically important interaction between ascorbate and the aromatic nitroso group[11] of substituted nitrosobenzenes. Our observations are as follows:

(*i*) At constant hydronium ion concentration, the observed reaction kinetics are second-order overall, and first-order with respect to both ascorbate and nitrosobenzene.

(*ii*) The dependence of the observed rate constants on the hydronium ion concentration indicates (Fig. 1) that the ascorbate anion as well as ascorbic acid interact with nitrosobenzene.[13]

(*iii*) The Hammet plot of log k_{obs} *vs.* σ^+ ($\rho^+ = 1.31$, $r = 0.990$) indicates that the order of reactivity of the substituted nitrosobenzenes is related to the electron-withdrawing



Scheme 1 The interaction of ascorbate and nitrosobenzene giving phenylhydroxylamine and dehydroascorbic acid

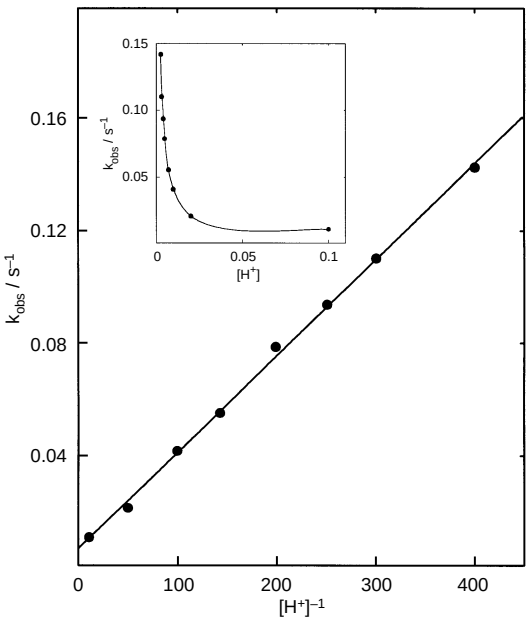


Fig. 1 The dependence on the hydronium ion concentration (mol dm^{-3}) of the pseudo-first-order rate constants for the reaction of ascorbate/ascorbic acid in water at 25 °C. Rate constants were determined spectrophotometrically by measuring the disappearance of the absorbance of nitrosobenzene at 308 nm. Initial concentrations of total ascorbate/ascorbic acid and nitrosobenzene were 0.0024 and 0.00024 mol dm^{-3}, respectively. The corresponding values of k_{HA^-} and k_{H_2A}, the second-order rate constants for the reaction of nitrosobenzene with ascorbate and undissociated ascorbic acid, are 2830(83) mol^{-1} dm^3 s^{-1} and 2.90(0.49) mol^{-1} dm^3 s^{-1}, respectively

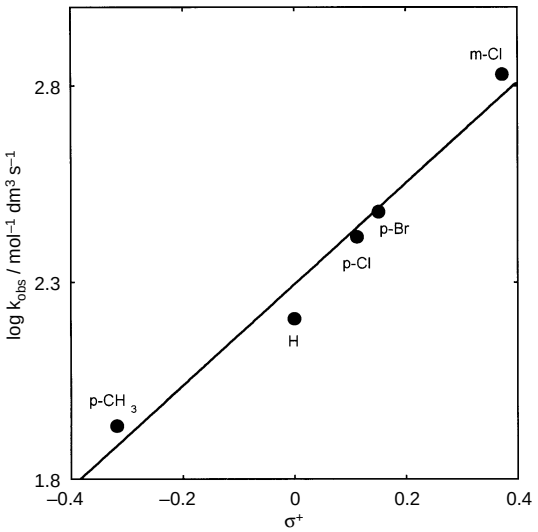


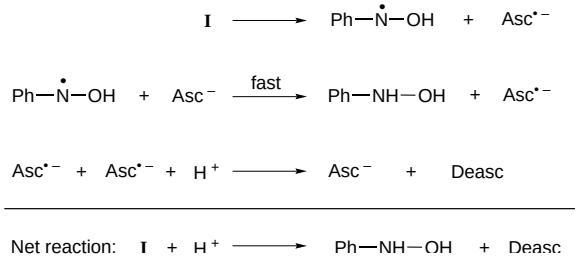
Fig. 2 Plot of log k_{obs} *vs.* Hammett σ^+. $T = 25\,^{\circ}C$. Rate constants were determined spectrophotometrically (see Fig. 1), using the second-order kinetics with $[\varphi NO] = [ascorbate]_{total} = 0.00024$ mol dm^{-3} and $[H^+] = 0.0008$ mol dm^{-3}

properties of the ring substituents, R (Fig. 2). This suggests that the electron transfer could participate in the rate control of the process. For example, the correlation of the rate constants for the reaction of various tocopherols with variously substituted phenoxyl radicals *vs.* Hammett σ parameters was observed, and the proposal was made that charge transfer should play an important role in this reaction.[17b] On the other hand the enthalpy of activation $\Delta H^{\neq} = 20.3$ kJ mol^{-1} is relatively low, similar to the values observed in the reactions of ascorbate with α-tocopheroxyl radicals, for which a concerted electron and proton transfer was proposed.[7]

(*iv*) The value of the rate constant ratio between H_2O and D_2O, at pH 3.15, is 8.81, which leads[14] to a value of 3.1 for the kinetic primary deuterium isotope effect k_H/k_D. Although lower than the maximum isotope effect expected for the rate-controlling O—H bond breaking,[15] this number is still consistent with a rate-controlling proton transfer in the transition state.[15,16] Moreover, similar isotope effects were observed in the reaction of ascorbate with the α-tocopheroxyl radical[7] and in the reactions of the α-tocopheroxyl radical with alkyl hydroperoxides,[17] and have been interpreted as a rate-controlling proton transfer concerted with an electron transfer in the process.

(*v*) A large negative entropy of activation $\Delta S^{\neq} = -111$ J mol^{-1} K^{-1} was observed for this reaction (see Table 1). This is consistent with a quite ordered transition state and is strongly in support of a cyclic transition state like **I**, as proposed for the reaction.

In our view, the results are consistent with the mechanism described by Scheme 1 and Scheme 2 (which is added separately for clarity). We believe that the reaction proceeds *via* a

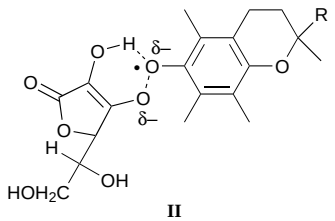


Scheme 2 Proposed mechanism for the conversion of **I** to the final products. (Asc = ascorbate, Deasc = dehydroascorbic acid)

cyclic transition state similar to **I**, where transfer of the 2-OH proton of ascorbate to the nitroso oxygen is concerted with the transfer of an electron from the anionic oxygen of ascorbate to the nitroso nitrogen.[18]

The significance of the observations could be to help in the further elucidation of the mechanism by which ascorbate interacts with the α-tocopheroxyl radical in the exceptionally important α-tocopherol–ascorbate redox cycle, and perhaps on the interactions of ascorbate with peroxy and similar radical species.

There are open questions about the structure of the transition state for the reaction of the α-tocopheroxyl radical with ascorbate.[7] Now, the comparison of the activation parameters and kinetic isotope effects for this reaction[7] and the values obtained in this study (see Table 1) suggest the close similarity between the transition states of the two reactions. Therefore, it seems reasonable to conclude that the reaction of the α-tocopheroxyl radical with ascorbate proceeds also *via* a cyclic transition state, similar to structure **II** (see Scheme 3). In addition, it is normally expected that the anionic oxygen of ascorbate interacts with the α-tocopheroxyl radical centre. Many reactions of ascorbate with oxygen radicals are known,[19] and the ascorbate 2-OH group enables the proton transfer concerted with the electron transfer to the radical centre in the transition state. Probably, this conclusion is corroborated by the observation that a proton can be transferred from the solvent molecule to the transition state for the reduction of peroxide radicals with organic reductants.[20] In both of the reactions, that of ascorbate with the α-tocopheroxyl radical and that of ascorbate with nitrosobenzene, formation of a cyclic transition state that includes the 2-OH proton makes the reaction thermodynamically more



II

Scheme 3 Proposed transition state for the reaction of the α-tocopheroxyl radical with ascorbate

Table 1 Comparison of the activation parameters and kinetic isotope effects for the reactions of ascorbate with nitrosobenzene and ascorbate with α-tocopheroxyl radicals

Oxidant	$\Delta H^{\neq}$/kJ mol^{-1}	$\Delta S^{\neq}$/J mol^{-1} K^{-1}	k_H/k_D	k_2[e]/dm^3 mol^{-1} s^{-1}
Nitrosobenzene[a]	20.3(1.8)[c]	−110.9(7.5)[c]	3.06[d]	2.83×10^3
α-Tocopheroxyl radical[b,f]	18.9(0.5)	−103.3(1.7)	7.97	4.97×10^4
α-Tocopheroxyl radical[b,g]	28.8(1.3)	−57.9(4.1)	3.11	3.05×10^5[g]
Trolox C radical[b,h]	2.2(0.8)	−109.0(2.8)	7.00	1.44×10^7

[a] This work. [b] Ref. 7. Trolox C is the α-tocopherol analogue 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid. [c] From measurements at five temperatures, in the range of 15.35–44.45 °C. At least three runs at each temperature were made. In order to obtain values of k_2, the second-order rate constants for the reaction of ascorbate with nitrosobenzene, the temperature dependence of the pK_as was determined at the same temperatures. [d] The ratio of rate constants in H_2O/D_2O, corrected for the solvent isotope effect on the dissociation of ascorbic acid (see above). Rate constants are an average of three paired measurements. [e] At 25 °C, except otherwise noted. [f] In sodium dodecyl sulfate micelles. [g] At 35 °C, in dimirystoylphosphatidylcholine bilayers. [h] In water.

favourable. Moreover, the enthalpy of activation in the reaction of ascorbate with the α-tocopheroxyl radical (Table 1) is small or near zero, and the reaction is entropy-governed.

We also have investigated (in part) the reaction of 2-nitroso-2-methylpropane with ascorbate. The reaction is about three orders of magnitude slower than the one with nitrosobenzene. Another difference is that the pH rate profile shows a minimum at about pH 2.4, while the observed rate constants increase above and below this point. We are continuing the investigation of this reaction, as well as the reaction of ascorbic acid with nitrosobenzenes.

Acknowledgements

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11 This study presents a part of our ongoing investigations on the mechanisms of interactions of *C*-nitroso compounds with biochemically important molecules.[12] To our knowledge, no report on the kinetics or mechanism of the reaction of nitrosobenzene with ascorbate has been reported.
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13 Under the conditions employed, the reaction of undissociated ascorbic acid with nitrosobenzene is a minor part of the overall process (see also Fig. 1) and the obtained results can be, to a good approximation, referred to the reaction of nitrosobenzene with ascorbate.
14 Taking into account the value of 2.88 for the antilog ΔpK_a between D_2O and H_2O for ascorbic acid. This ΔpK_a is 0.46, whereas we have determined the pK_a for ascorbate to be 4.30 in 'pure water', without any added salt. The concentration of the total ascorbic acid/ascorbate was 1.2×10^{-3} mol dm^{-3} throughout. We have determined spectrophotometrically the solvent deuterium isotope effect on the dissociation of ascorbic acid (to the best of our knowledge, this isotope effect was not determined previously).
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