A TIME-RESOLVED ELECTRON SPIN RESONANCE STUDY OF THE OXIDATION OF ASCORBIC ACID BY HYDROXYL RADICAL

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ABSTRACT Time-resolved electron spin resonance (ESR) spectroscopy for the study of radicals produced by pulse radiolysis is illustrated by a study of the oxidation of ascorbic acid by OH radical in aqueous solution. In basic solution, the direct oxidation product, the ascorbate mono-anion radical, is formed within less than 2 μs of the radiolysis pulse. In acid solutions (pH 3–4.5, N_2O:saturated) three radicals are initially formed, the ascorbate mono-anion radical, an OH adduct seen also in steady-state ESR experiments, and an OH adduct at C2 with the main spin density at C3 of the ring. The first OH adduct decays with an initial half-life of about 100 μs, probably by bimolecular reaction. The second OH adduct, which shows one hyperfine splitting with $a^H = 24.4 \pm 0.3$ G and $g = 2.0031 \pm 0.0002$, decays with a half-life of about 10 μs. On this same time scale the concentration of the ascorbate radical approximately doubles. It is concluded that the adduct at C2, but not the other adduct, loses water rapidly to form the ascorbate radical.

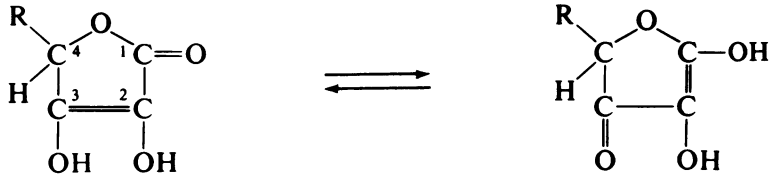
INTRODUCTION

Electron spin resonance (ESR) experiments with microsecond time resolution to study radicals produced by pulse radiolysis have been carried out in two laboratories (1–5). Much of the initial thrust has been directed toward understanding chemically induced dynamic electron polarization (CIDEP) a process by which chemical reactions either producing or destroying radicals can be spin-dependent and thereby modify the population of the various spin states and the intensities of the ESR transitions. In addition, however, several kinetic and mechanistic applications have been reported (6–8). To illustrate the potential of such experiments, this paper will present data from time-resolved ESR experiments which help clarify the complex chemistry involved in the oxidation of ascorbic acid by hydroxyl radical. A brief preliminary report of some observations on this system was included in an earlier paper reviewing *in situ* radiolysis-ESR experiments (9).

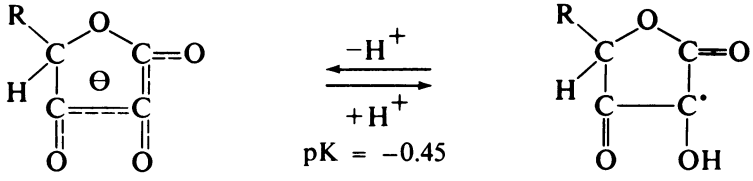
The oxidation of ascorbic acid by OH has been studied by both steady-state radiolysis-ESR experiments and pulse radiolysis with optical spectrophotometric and conductometric detection. The ESR experiments (10) showed that the ascorbate mono-anion radical formed by the net loss of an electron from ascorbate was present over

the pH range 0–13 and a second radical also was formed in acid solution (pH < 5). The most detailed optical study is that by Schöneshöfer (11), who studied changes in the optical spectra as a function of pH for acid solutions. He proposed a very complex reaction scheme. Although some effects are seen by both spectrophotometric and ESR methods, the present data show that some of the details of that proposed mechanism are incorrect. The earlier steady-state ESR work (10) showed clearly that the ascorbate anion radical has a pK of −0.45, in contrast to the value of 3 concluded from the optical study (11).

The oxidation of ascorbic acid by OH is potentially complex because of the possibility of addition to either end of the double bond of either of the tautomeric forms.



The radical found in neutral and basic solutions can result either from loss of water



from an OH adduct or by direct electron transfer oxidation. The various intermediates either have similar absorption spectra with peaks at 360 nm or have little absorption in the accessible region (>300 nm). The ESR method can be used to advantage in such a situation because of the better resolution of different spectra.

THE TIME-RESOLVED ESR METHOD

ESR experiments with a time resolution of better than 1 μs have been demonstrated (3, 5). Radicals are produced by pulse radiolysis with 3 MeV electrons with pulse periods of 0.1–1 μs. Direct detection of ESR absorption is preferred because quantitative analysis of the resulting curves is much easier (3). In contrast to optical techniques, fast ESR spectroscopy has a number of potential complications, which must be kept in mind in analyzing curves of absorption vs. time after the radiolysis pulse. The strength of an ESR absorption does not necessarily reflect the concentration of radicals producing it. In dynamic experiments the chemical reactions forming or destroying radicals can be spin-selective, perturbing the relative spin populations upon which the absorption intensities depend. This latter effect, as mentioned above, is called CIDEP. In the usual form the low-field lines of a spectrum appear weak or even in emission and

high-field lines are in enhanced absorption. The strength of the effect is proportional to the rate of the radical-radical encounters and so the amplitude of a high-field line will depend more than linearly on the radical concentration. When observing radical disappearance with such a line, the apparent half-life will be somewhat shorter than the correct chemical value. A related effect involves the fact that in any radical transformation, such as addition to a double bond, the product radical will initially possess the spin populations of the reactant. This effect is particularly pronounced in radicals formed from H atoms, because the latter can develop strongly perturbed populations. Another important consideration is that the ESR signal does not appear immediately after formation of the radicals. The rise time of the ESR signal is related to the spin relaxation time of the radical, the initial population difference for the particular transition, and the microwave power level. A final effect occurs at high microwave power levels, where overshoot and damped oscillations on the rise of the ESR signal are possible. This transient ESR response disappears with the spin relaxation time. A quantitative treatment of all of these effects has been given in a previous paper (3).

In spite of all these potential problems, the observation of ESR signals in such pulse experiments provides very useful information, which often can be analyzed quantitatively to determine rate constants. The rates of reaction of H atoms with several alcohols (7) and of phenyl radicals with isopropyl alcohol (8) have been determined in this way. An important conclusion is that for times long with respect to the spin relaxation time the signals reflect mainly the chemical kinetics. In the work to be reported here, the relaxation times are fairly short (1–2 μs) so that the transient effects and the effects of the initial spin populations are minor. Only one of the radicals appears to be affected by CIDEP.

Implicit in the above discussion is the idea that the spectrometer is set on a given ESR line and a time profile of the absorption recorded. To provide sufficient signal-to-noise ratio, a number of curves must be recorded for signal averaging. A similar number of curves are taken at a nearby position to provide a base line with no ESR adsorption. The difference of the two accumulations is the true time profile. Because the ESR lines usually are narrow with respect to the total extent of a spectrum (0.1 vs. 100 G), it is difficult to search for lines of new radicals. A continuous scan using a boxcar integrator is possible, but this mode has not proved as sensitive as recording time dependences. The time-resolved experiments are clearly less sensitive than the steady-state ones.

EXPERIMENTAL DETAILS

The ESR spectrometer was as described previously (3). Absorption was detected directly. Radiolysis was with 2.8 meV electrons. Each pulse produced about 3×10^{-5} M total radicals. To provide for signal averaging, the pulses were repeated at 100 s^{-1} repetition rate and formation-decay curves for about 5,000 pulses were digitized with a Biomation 8100 transient recorder (Biomation, Cupertino, Calif.) and processed in a PDP8 minicomputer (Digital Equipment Corp., Marlboro, Mass.). The computer also provided a field-frequency lock to maintain a given position in the spectrum throughout an accumulation. The microwave

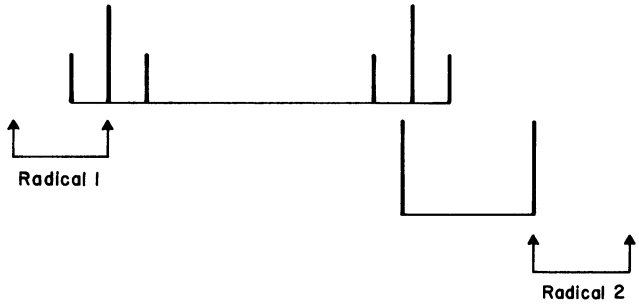


FIGURE 1 A schematic representation of the ESR spectra of the first and second radicals observed in acid, N_2O-saturated ascorbic acid solutions at about pH 4 (10). The arrows mark the positions of signal and base-line accumulations for the principal radical (low-field side) and the second radical (high-field side). The doublet splitting for radical 1 is 1.76 G. The lines for radical 3 are centered at fields 15.7 G above and 8.7 G below the center of this spectrum.

power level was about 1 mW and the spectrometer time constant 1 μs. Sample solution flowed through the flat aqueous cell at about 0.5 cm^3/s so that each volume element (20 μl) received only a few irradiation pulses. Increases in the flow rate had no significant effect on the data obtained.

The solutions were prepared in water which had been distilled and passed as vapor with oxygen through a silica oven. Oxygen was removed by bubbling with N_2O. The ascorbic acid was obtained from Calbiochem (San Diego, Calif.) and was the same as that used for the steady-state experiments (10). The pH was changed by addition of Baker analyzed KOH or $HClO_4$ (J. T. Baker Chemical Co., Phillipsburg, N.J.).

RESULTS AND DISCUSSION

The ESR spectrum of N_2O-saturated solutions of ascorbic acid at pH 4, as observed in continuous radiolysis experiments (10), is shown schematically in Fig. 1. The first (or principal) radical shows a doublet of triplets and is present with unchanged line positions from pH 1 to 13. (This radical starts to protonate at pH < 1 and the lines then shift.) The second radical shows a doublet and its lines start to shift below about pH 3.

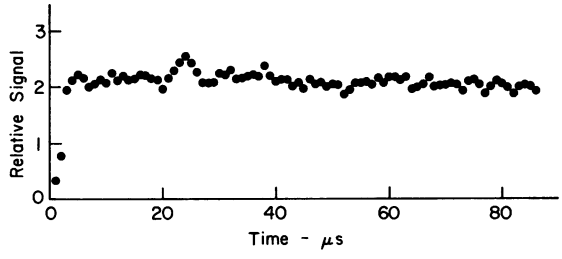


FIGURE 2 Time dependence of the ESR absorption signal of the first radical in basic (pH 11), N_2O-saturated solutions of ascorbic acid (5 mM). The time scale is 1 μs/point. The time origin is the end of the 1-μs irradiation pulse. The 2-μs delay in response after the pulse is the result of switching the signal amplifier off during the irradiation period to prevent interference. The data represents the average of about 5,000 pulses.

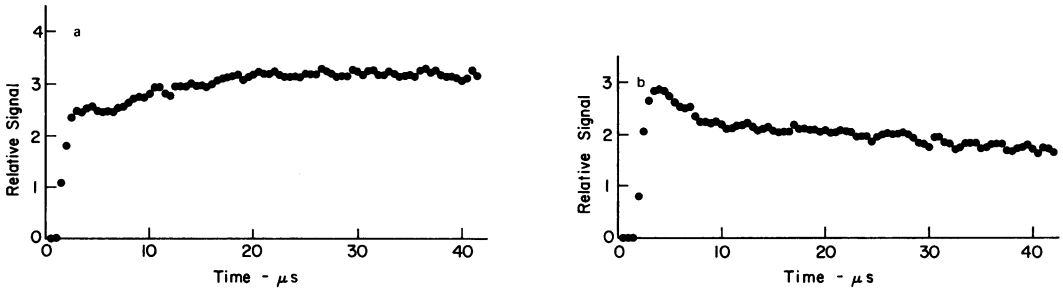


FIGURE 3 Time dependence of the first (*a*) and second (*b*) radicals in an N_2O-saturated solution of 5 mM ascorbic acid at pH 3.9. The time scale is 0.5 μs/point.

The pulse experiments described here show the time profiles taken at the positions indicated.

A time profile for the first radical at pH 11 is given in Fig. 2. This curve shows that the transient effects associated with the rise of the ESR signal are not a problem because of a relatively short relaxation time for the radical. Based on the observed rise time, the relaxation time cannot be longer than about 2 μs. The lack of decay is as expected from the known disappearance rate constant (11). Because of the slow reaction, no effects of CIDEP should be present. A curve (not shown) for the central line of the upper triplet of this radical (the only one present at pH 11) is essentially identical to that shown, confirming this expectation.

Data taken at pH 3.9 and 3.5 are shown in Figs. 3 and 4 for the lines of the first and second radicals. In each case the signal of the second radical rises within 3–4 μs to a level which then decays with a half-life of about 100 μs. Such a decay is typical of radical-radical reaction at the concentration levels used. (The slight overshoot on the curve at pH 3.9 is a result of transient ESR response as described above. At a lower power level this overshoot is absent.) The signal from the first radical shows a growth on two time scales. In each case there is a fast growth, as observed at pH 11, followed by a slower growth. At pH 3.9 (see Fig. 3) 65% of the signal (extrapolated to the end of the pulse) appears rapidly, with the remaining 35% growing with a half-life of about

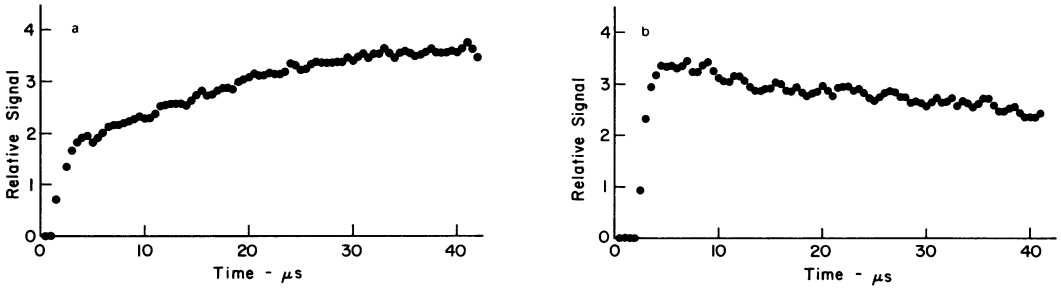


FIGURE 4 Time dependence of the first (*a*) and second (*b*) radicals in ascorbic acid at pH 3.5. Other conditions were as in Fig. 3.

8 μs. At pH 3.5 (see Fig. 4) the faster portion represents 40% and the rest grows with about 13-μs half-life. The division between the two portions does not continue to change with lower pH, as curves (not shown) for pH 3.2 give 50% for the fast portion. (The difference between 40 and 50% represents the experimental error of the determination.) The smaller slow portion at pH 3.9 is probably the result of a change in mechanism, as a considerable amount of ascorbate anion (pK = 4.1) is present. At pH 4.5 the slow portion is not readily discernable. The estimate of the half-period of the slower component is rather crude, because the level shown at 40 μs is not a true plateau, as some decay occurs at longer times. A curve taken at twice the time scale showed 20% decay from 30 to 80 μs. Corrections for this effect give 15 μs for the lifetime at pH 3.5.

The longer period of decay of the second radical ($\sim$100 μs) as compared with that for the growth for the first radical, as discussed above, shows that the decay of the second radical probably does not lead to formation of the first radical. Thus a third radical must be present. The most likely species is one formed by addition of OH at position C2 of the ring with the main spin density at C3. This radical would have one large hyperfine splitting of >20 G from the single hydrogen atom on C4. A search for such a species was carried out by running accumulations at successive magnetic fields in the appropriate regions of the spectrum. In fact, lines were found as shown by data for pH 2.9 in Fig. 5. Although it is not certain that this spectrum does not contain other lines, the structure matches the expected radical. In contrast to the other radicals, this radical has broad lines—about 2 G full width at half-amplitude. The parameters are a^H = 24.4 $\pm$ 0.0002 G and g = 2.0031 $\pm$ 0.0002. The accuracy of these values is limited because of the weakness of the spectrum and the width of the lines. This radical is also present at pH 3.2 and 3.9 but is barely detectable at pH 4.5. It is not present when 0.1 M KBr is added so that oxidation of the ascorbic acid is by Br_2^- and only the first radical is formed as previously described (11). These latter results also show that the third radical is not formed from the direct yield of H atoms in water. The emission observed for the low-field line is a result of CIDEP, which would be expected for a radi-

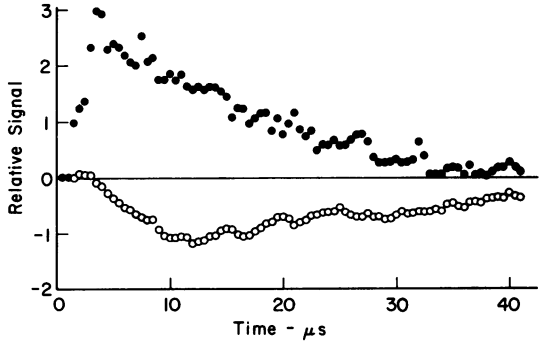
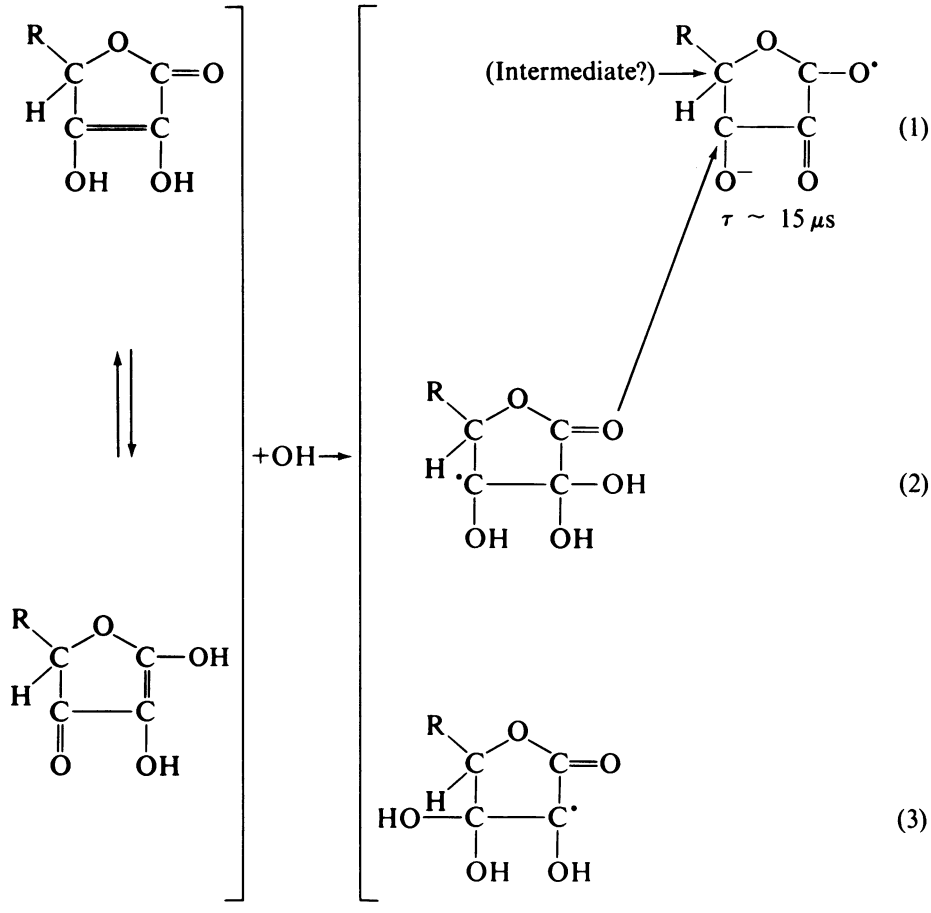


FIGURE 5 Time dependences found for the high- and low-field lines of the third radical in N_2O-saturated 5 mM solutions of ascorbic acid at pH 2.95. The low-field line (negative going) shows emission as a result of CIDEP produced by fast radical-radical reaction.

cal with a sizable hyperfine splitting. The high-field line must represent enhanced absorption and so the apparent half-life (~ 10 μs) will be shorter than the actual chemical value. The approximate values does, however, correspond to that for the slower growth of the first radical. It is very likely that the third radical converts to the first radical with such a period.

On the basis of these results, the mechanism for reaction of OH with ascorbic acid in acid solutions can be written as follows:



where R represents $HOCH_2CHOH$-. The curve of Fig. 4*a* shows that about 50% of the radical 1 is formed in a few microseconds and that the other 50% is formed with a half-period of about 15 μs. Conversion of radical 3 to 1 is the most likely path of this reaction. Radical 3 is clearly identified as the result of addition of OH at C2. This radical can be produced from either of the two forms of ascorbic acid because of an expected fast equilibration between radicals protonated at the oxygen atoms on C1 and C3. Radical 2 represents one of the two remaining adducts. Although it is not certain

that this form is responsible for the spectrum observed in steady-state experiments, the adduct at C1 should be the least stable as a result of three oxygen substituents on that carbon atom. It is likely that this form is produced and loses water rapidly to form 1.

This mechanism differs in a number of ways from the very detailed one given by Schöneshöfer (11). His mechanism involved only OH adducts at C1 and C3. Changes seen on the 25-μs time scale were attributed to loss of water from an OH adduct at C3. On the basis of the present results, this reaction is clearly loss of water from 3. We cannot comment on many of the other features of his mechanism except to note that the steady-state ESR experiments clearly show pK for 1 and 2 to be -0.45 and 2.0, respectively.

These results on ascorbic acid are relatively qualitative but illustrate very well the power of the ESR method in resolving the separate radicals in a system where the optical spectra do not do so. It is to be emphasized that the qualitative nature of these experiments results from the difficulty in carrying them out because of the relatively long accumulation times necessary. The detection of radical 3 was the most difficult experiment carried out to date and all portions of the equipment had to be functioning at peak consistency. With the present data in hand it is possible that more quantitative experiments could be contemplated.

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