

Is there an Equilibrium between Ascorbic and Dehydroascorbic Acids?

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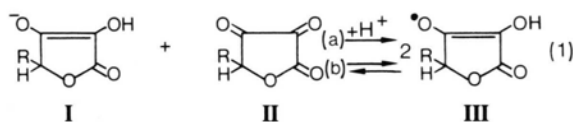
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The previously reported interaction between ascorbate monoanion and dehydroascorbic acid yielding semidehydroascorbic acid has been shown to be thermodynamically unfeasible and non-existent. No evidence for radical formation in the reinvestigation of the reaction using ESR spectroscopy under careful exclusion of air could be found. Our results show that the detection of the free radical in earlier studies resulted from the reaction of ascorbate with traces of oxygen and/or metals.

The possibility of a spontaneous reaction between the monoanion of ascorbic acid (**I**), Scheme 1, and its first oxidation product, dehydroascorbic acid (**II**) yielding the semidehydroascorbate free radical (**III**) has been suggested on many occasions. Weissberger *et al.* [1] postulated process a (Scheme 1) as the rate determining step in the CN^-/CNS^- inhibited oxidation of **I** in the presence of oxygen. The reaction was proposed to account for induction periods observed during low pH experiments.



R = $CHOHCH_2OH$

Scheme 1.

Twenty years later Yamazaki *et al.* [2–4] also observed **III** by ESR. These authors postulated a mechanism in which enzymic oxidation of ascorbic acid occurs essentially by simultaneous two-electron (or hydrogen) transfer, and that the free radicals are produced by a secondary reaction between **I** and **II** in the equilibrium (b). A more complete ESR spectroscopic study was undertaken by von Forester

et al. [5]. These authors claimed to have determined the equilibrium constant of (b) and its pH dependence (pH 4.0; $pK_e = 11.25$ and pH 6.4; $pK_e = 8.29$). In a biochemical context Lewin [6] considers the ascorbic acid-dehydroascorbic acid equilibrium as crucial to the redox balance of the mammalian body. Bearing in mind the ease of trace metal catalysed formation of **III**, especially by Cu^{2+} ions [7, 8] and noting that no report mentions any precautions taken to avoid such complications we doubted the validity of both possibilities. For these reasons we have reinvestigated the reaction using ESR spectroscopy to detect **III**: i) under careful exclusion of air, ii) in the presence of a large excess of EDTA to suppress the effects of trace metals and iii) with small amounts of typical trace metal impurities added.

Materials and Methods

All materials were of the highest purity commercially available and were used without further purification. Atomic Absorption Spectroscopy showed typical concentrations in the nM range (Cu, Ag, Fe) in the experimental aqueous solutions.

Apparatus – The ESR apparatus has been described elsewhere [7]. It is capable of routinely measuring radical concentrations in the 0.1–0.01 μM range. All preparations were made in a carefully cleaned and dried modified double-Schlenk-tube [9]. For purposes of comparison we conducted our investigations exclusively under those conditions which yielded the highest concentration of **III** (ca. 7 μM) in the work of von Foerster [5], that is AH_2 0.3 M, A 0.03 M and using both phosphate buffer and sodium hydroxide to adjust the pH value to 6.8.

The weighed amounts of dried ascorbic acid (AH_2) and dehydroascorbic acid (A) (+ additive when appropriate) were placed in one arm of the Schlenk-tube and the buffer or sodium hydroxide in the other arm. Sufficient solution (2 ml) was available for several measurements. Subsequently solution and solid material were subjected to three freeze – thaw cycles at a pressure of 2×10^{-5} torr. After completion of the evacuation the materials were mixed, shaken vigorously and a portion of the solution removed to the ESR cell for analysis. When applicable aeration of the remaining solution in the

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arm was performed by simply stirring in air for ca. 5 min. Such samples were not reevacuated prior to ESR measurement.

Results and Discussion

Feasibility of the reaction – To decide whether or not such an equilibrium or reaction is feasible we have examined the standard electrode potential of the process. Reaction (1) and its thermodynamics can be easily derived by subtraction of the half-reactions of Eqs. (2) and (3).



Table I Radical formation in the ascorbic acid–dehydroascorbic acid system^a: (AH₂)¹ = 0.3 M, (A) = 0.03 M; pH value 6.8 (phosphate buffer)^b.

Experi- ment No.	Reaction mixture and conditions	Signal amplitude ^c
1.1	AH ₂ evacuated	–
1.2	AH ₂ aerated	++
2.1	AH ₂ -Cu ²⁺ evacuated ^d	+
2.2	AH ₂ -Cu ²⁺ aerated ^d	++
3.1	A evacuated	–
3.2	A aerated	–
4.1	A-Cu ²⁺ evacuated	–
4.2	A-Cu ²⁺ aerated	–
5.1	AH ₂ -A evacuated	–
5.2	AH ₂ -A aerated	+
6.1	AH ₂ -A-Cu ²⁺ evacuated ^d	+
6.2	AH ₂ -A-Cu ²⁺ aerated ^d	++
7.1	AH ₂ -A-Cu ²⁺ -EDTA evacuated ^e	+
7.2	AH ₂ -A-Cu ²⁺ -EDTA aerated ^e	+
8.1	AH ₂ -A-Fe ³⁺ evacuated	+
8.2	AH ₂ -A-Fe ³⁺ aerated ^d	++
9.1	AH ₂ -A-Ag ⁺ evacuated	+
9.2	AH ₂ -A-Ag ⁺ aerated ^d	++
10.1	AH ₂ -A-Ce ³⁺ evacuated	+
10.2	AH ₂ -A-Ce ³⁺ aerated	++
11.1	AH ₂ -A-Au ³⁺ evacuated	+
11.2	AH ₂ -A-Au ³⁺ aerated	++

^a In experiments where applicable added metal = 0.1 mM and EDTA = 0.01 M.

^b Identical results were obtained on adjusting the pH value with NaOH.

^c Signal amplitude of the radical observed: –, absent; +, present; ++, very high. In experiments with high initial radical concentration the ESR signal was observable for several hours.

^d Solutions turbidified and precipitated slowly.

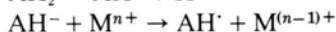
^e Solutions remained colourless.

¹ The abbreviations used are: AH₂, ascorbic acid; A, dehydroascorbic acid; AH[–], ascorbate monoanion; AH[·], semidehydroascorbic acid.

Taking $E_2^0 = -0.33$ V versus NHE [10] and $E_3^0 = 0.08$ V versus NHE [11] we estimate that E_1^0 will be -0.58 V indicating that the direct reaction between **I** and **II** to yield **III** will not be feasible. This prediction was confirmed in all cases by experimental work.

Testing the radical formation – The results shown in Table I show unambiguously that processes a) and b)* do not take place between monoanionic ascorbic acid and dehydroascorbic acid (exp. 5.1). Much more likely is that incomplete removal of traces of oxygen and/or reducible metals led previous workers to assume that their species **III** arose via a) or b).

In fact experiments 6–11 show that several commonly occurring impurity metals are capable of inducing radical formation under both sets of conditions. Indeed our experiments using EDTA as complexing agent show that radical formation cannot be completely suppressed even in the presence of vanishingly small concentrations of free copper (**II**) ions; $\text{pK}_{\text{Cu-EDTA}} = 18.8$. The origin of the ESR signal in these cases is probably via direct interaction with the chelate itself since this, as was the radical concentration also, is independent of the oxygen tension [12]. The visually striking reactions (colour change, precipitation, cloudiness) in the presence of metals in deaerated solutions probably occur as follows:



The kinetics and mechanism of such reactions have been amply discussed elsewhere [10, 13].

In analogy to the above problem we have further examined the affinity of various metals to radical formation and destruction in ascorbic acid systems. Such reactions (Table II) have been frequently postulated in recent kinetic schemes involving metal catalysed ascorbic acid oxidation [14–18]. The results show that for undissociated AH₂ one electron oxidation is unlikely to be a primary step for most metals in their normal uncomplexed valency states. Thus in experiments involving anaerobic ascorbic acid solutions buffered with acetate (pH 4.6) we

* The equilibrium expression (p. 167) in the work of von Foerster *et al.* [5] has not been balanced in protons and hence the “equilibrium constants” of Table I are probably several orders of magnitude in error.

Table II. Redox potentials for the interaction of ascorbic acid species with some metal ions^a.

Process ^b	$E^{\circ}_{\text{process}}$									
	Ag ⁺	Co ³⁺	Cu ²⁺	Cu ⁺	Fe ³⁺	Fe(CN) ₆ ³⁻	Mn ³⁺	V ⁵⁺ ^c	Ce ⁴⁺	
$M^{n+} + AH_2 = AH_2^+ + M^{(n-1)+}$	(1) -0.6	+0.44	-1.24	-0.88	-0.63	-0.71	+0.11	-0.4	+0.06	
$M^{n+} + AH^{\cdot-} = AH^{\cdot-} + M^{(n-1)+}$	(2) +0.5	+1.51	-0.17	-0.19	+0.40	+0.36	+1.2	+0.7	+1.13	
$M^{n+} + AH^{\cdot-} = A^{\cdot-} + M^{(n-1)+} + H^+$	(3) +1.4	+2.1	+0.41	+0.77	+1.02	+0.94	+1.7	+1.3	+1.71	

^a The values of $E^{\circ}(M^{n+}/M^{(n-1)+})$ were taken from (1980–1981) "Handbook of Chemistry and Physics".

^b The redox potential ($E^{\circ}_{AH^{\cdot-}/A} = 0.25$ V) is calculated from the couples: $A + H^+ + 2e^- = AH^{\cdot-}$, $AH^{\cdot-} + e^- = A^{\cdot-}$; $E^{\circ} = 0.08$ V, $E^{\circ} = 0.33$ V.

^c Reduction $VO_2^+ - VO^{2+}$ considered.

were unable to observe any signal from the radical **III** for any of the metals of Table I. In contrast, in most cases processes (2) and (3) in Table II will be strongly favoured.

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