Journal of Photochemistry, *17* (1981) 517–520

LASER FLASH PHOTOLYSIS AND TIME-RESOLVED ELECTRON SPIN RESONANCE STUDIES: THE PHOTOREDOX REACTIONS OF VITAMINS C, E AND K_1*

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Interest in the biological significance of vitamins C and E continues to be stimulated by reports of their involvement in many biochemical processes including cancer and iron nutrition. It has been established that vitamin C is readily oxidized in aqueous solution to a relatively stable free radical whose controversial structure has been determined by electron spin resonance (ESR) spectroscopy. In the non-enzymatic systems, formation of the ascorbate (vitamin C) radical may involve either an electron transfer process and/or a molecular elimination process. It is of fundamental importance that the detailed dynamics and mechanisms of these free-radical processes be understood.

In the past 5 years we have extensively used the photoreduction of quinones by phenols as model systems for chemically induced magnetic polarization studies. In addition to providing some insights into the mechanistic aspects of free-radical reactions, the time-resolved ESR technique can also be employed to probe the dynamic and/or structural properties of the transient radicals, *e.g.* their spin–lattice relaxation times in solution. The interesting ascorbate radical has a semiquinone-like structure and various probable sites for hydrogen-bonding interactions with the water solvent. It is our hope that the application of laser flash photolysis combined with time-resolved ESR spectroscopy to the photoredox reactions of vitamins in aqueous solution may yield further valuable information on the mechanism and the dynamic properties of the reaction intermediates in these biochemical processes.

In this paper we have carefully chosen four model systems: (1) the oxidation of vitamin C by excited triplet pyruvic acid; (2) the oxidation of vitamin C by excited triplet duroquinone; (3) the oxidation of vitamin C by excited vitamin K_1; (4) the redox reaction between vitamins C and E. The choice of the first two systems is most significant in that the excited triplet states of pyruvic acid and of duroquinone have distinctly different spin dynamic properties which lead to an initial polarization of the vitamin C radical anion being totally enhanced absorptive in system (1) and totally emissive in system (2). Spin–lattice relaxation times T_1 estimated from both the emissive and the absorptive ascorbate radical anions are found to be the same and this provides a critical test for the phototriplet mechanism as well as the reliability of the chemically induced dynamic electron polarization (CIDEP) method in estimating T_1 for transient free radicals. Furthermore, by using sodium pyruvate as a control, the oxidation of vitamin C by pyruvate affords an interesting opportunity to study a primary photo-

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* Extended abstract of a paper presented at the Xth International Conference on Photochemistry, Iraklion, Crete, Greece, September 6 - 12, 1981.

0047-2670/81/0000-0000/$02.50

chemical reaction between two anions. In the photoredox reaction between vitamins C and K_1 it is expected that triplet vitamin K_1 is involved, and the time-resolved ESR study reveals that the spin dynamic properties of the excited triplet vitamin K_1 are similar to those of quinones. For system (4) it has been suggested that vitamins C and E are both biochemically important antioxidants and that they may act synergistically, with the initially formed vitamin E radical being regenerated by the reaction with vitamin C. For CIDEP study it is necessary to introduce some quinones into the system to enhance the photolytic efficiency and therefore to enhance the spin polarization of the vitamin E radical.

A brief summary of the photoexcited triplet mechanism in CIDEP and the method of estimating T_1 for polarized transient radicals is given. In order to understand qualitatively the T_1 results in terms of dynamic properties of some of these vitamin radicals such as vitamin E, we developed (in collaboration with Professor D.A. Hutchinson) a "rotating rod" model by treating the long-chain radicals as a collection of rotating rods. There is free rotation about the rod axis but the motion is frozen about the transverse axes. The angular displacement and angular velocity variables for the rotation about the rod axis are random variables. They contribute to T_1 through **G** tensor modulation and spin rotation interactions. In this simple model we neglect many more complex motions such as the "crankshaft" motion and bending vibrations. None the less it provides satisfactory qualitative explanations of the experimental results.

In the photooxidation system involving vitamin C and pyruvic acid or sodium pyruvate, the adjustment of the pH of the aqueous solution afforded an opportunity to select the photochemical reaction between either two anions or one anion and a neutral molecule. The experimental results clearly showed that the CIDEP intensity and the mode of polarization were unchanged as the pH of the solution was altered. Similar results were obtained for the sodium pyruvate–vitamin C system. We thus speculate that the photoredox reaction between the triplet pyruvic acid–pyruvate anion and vitamin C involves mainly a hydrogen abstraction mechanism. The approach of the triplet pyruvate anion towards another anionic reactant, the vitamin C anion, apparently does not slow down the reaction as the triplet anion possesses sufficient excitation energy to overcome any small energy barrier for two approaching anions. However, an electron transfer mechanism is still possible but it would be expected that electron transfer between two anions would have modified the transient ESR observations compared with electron transfer between two neutral molecules. In this respect we studied the photooxidation of 2,3-dimethyl ascorbate by duroquinone. Here neither radicals nor CIDEP were observed. Although negative ESR results cannot be used to exclude the possibility of chemical reactions, it is fairly safe to say that under identical experimental conditions the free-radical processes involved in the photooxidation of 2,3-dimethyl ascorbate by quinone are insignificant. This can be attributed to the fact that the "efficient" hydrogen abstraction reaction by the triplet quinone from the OH group on the vitamin C is no longer operative when the molecule has methyl group substituents. However, the substituted ascorbate is probably un-ionized in solution, but an electron transfer mechanism between the triplet quinone and the neutral dimethyl ascorbate is still possible if the photooxidation does involve such a mechanism.

When the photoredox reaction between vitamins C and K_1 was carried out in a mixed water–ethanol solvent, the salient conclusion that was drawn from the experimental facts was that the triplet vitamin K_1 was photoreduced much more efficiently by vitamin C than by ethanol. Here the electron transfer mechanism from vitamin C to triplet vitamin K_1 is particularly attractive as it can account for both the direct formation of the semiquinone K_1 radical anion observed and the fast reaction rate required by the CIDEP phototriplet mechanism. Unequivocal evidence of an electron transfer mechanism was observed in the photooxidation of vitamin C by Cu^{2+} ions in aqueous solution at 77 K. Our results show that vitamin C and Cu^{2+} form a paramagnetic complex with well-defined ESR parameters. Photoexcitation of this complex at the ligand to metal charge transfer (LMCT) band leads to a one-electron transfer from the vitamin C ligand to Cu^{2+}. The subsequent decomposition of the ascorbate radical–Cu^+ complex yields an unexpected hydrogen atom together with copper and dehydroascorbic acid. Confirmation of hydrogen atom production was obtained by performing the experiment in a deuterated pyridine solvent at 77 K, which established that the hydrogen atom observed must have been derived from the vitamin C ligand and not from the solvent. Apart from the metal–vitamin C systems, it is obvious that any definitive conclusion for the detailed mechanism must await further elucidation. Our current instrumental capability (a nanosecond pulse laser and a 200 ns ESR time resolution) is not sufficient to monitor the rapid processes involving

TABLE 1

Estimated T_1 values from CIDEP measurements of transient radicals in solutions

Radicals in the primary pair	*Mode of initial polarization*	*Temperature (°C)*	*Solvent*	T_1 (μs)
L-ascorbate radical anion	Absorptive	6	Water	2.1 ± 0.4
Pyruvic ketyl radical	Absorptive	6	Water	3.4
L-ascorbate radical anion	Absorptive	29	Water	1.0
Pyruvic ketyl radical	Absorptive	29	Water	2.3
L-ascorbic radical anion	Absorptive	27	Water	1.4
Pyruvic ketyl radical anion	Absorptive	27	(pH 10.2)	2.5
L-ascorbate radical anion	Emissive	19	Ethanol	2.0
Vitamin K_1 semiquinone	Emissive	19	Ethanol	4.0
L-ascorbate radical anion	Emissive	−20	Ethanol	2.4
Durosemiquinone	Emissive	−20	Ethanol	3.0
D-arabascorbate radical anion	Absorptive	11	Water	2.0
$CH_3CH_2\dot{C}(OH)COO^-$[a]	Absorptive	11	Water	3.6
Vitamin E semiquinone anion	Emissive	0	Ethanol	5.4

[a] T_1 in the $CH_3CH_2\dot{C}(OH)COO^-$ radical is slightly dependent on the hyperfine components with T_1 for the centre component 0 being slightly larger than those associated with the +1 and −1 components.

electron transfers. It is our hope that the results illustrated here will stimulate further development of the instrumental techniques perhaps to the subnanosecond range.

In the relaxation studies the data (Table 1) show that in general T_1 for the L-ascorbate is the same as that for the D-arabascorbate. The apparently shorter T_1 for the ascorbate radical anion than for its counter radicals such as pyruvic ketyl radicals under the same experimental conditions is rather surprising. It had been expected that in aqueous solution the ascorbate might interact with water via substantial hydrogen bonding and this effect would have increased T_1. If we take into account that similar but less extensive hydrogen-bonding interactions are also probable for the counter pyruvic ketyl radical, it is reasonable to argue that the difference in T_1 values between the ascorbate and the pyruvic ketyl radicals is not mainly due to hydrogen bonding. Another interesting observation is that T_1 for the large vitamin K_1 semiquinone radical appears to be of the same order of magnitude as that for the corresponding unsubstituted naphthosemiquinone radical in solution. This, however, is not too surprising as our recent theoretical treatments on T_1 values for semiquinone radicals in solution have shown that a combined argument based on a modest size effect and the extent of the π system provides a better understanding of the T_1 observations than either consideration by itself.

While our current efforts in T_1 studies have been concerned mainly with radicals in solution, we are exploring the application of this combined laser flash photolysis and time-resolved ESR technique to examine the dynamics of radicals in multiphase systems.

Acknowledgments

This research was supported by the Natural Sciences and Engineering Research Council of Canada. The author is most grateful to the members of his group, particularly Drs. M. Catharine Depew, B.B. Adeleke, S. Emori and J.W.M. deBoer, who did much of the experimental work presented in this paper.

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