DOI: 10.1007/s10967-007-0919-1 *Journal of Radioanalytical and Nuclear Chemistry, Vol. 273, No.3 (2007) 609–614*

ESR study of ascorbic acid irradiated with gamma-rays

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(Received June 27, 2006)

The interest in application of high-energy ionizing radiation for sterilization of pharmaceutical products and foodstuffs has led a number of workers to investigate the radiation sensitivity of vitamins. Aside from its use as a vitamin, ascorbic acid (AA) or some derivatives are employed as antioxidants in foodstuffs. The effects of ionizing radiation on AA in simple solutions and in mixture of naturally occurring compounds have been extensively reported in the literature. However, the effects of ionizing radiation on solid AA were reported in few works which described rather dosimetric features of AA. No reports, except one, are available describing the characteristic features of the radiolytic intermediates produced after irradiation of polycrystalline AA. Irradiation studies performed on single crystal of AA has led us to reinvestigate our previous work on the radiolytic intermediates produced in irradiated polycrystalline AA. Three radical species, rather than two, having different characteristics were decided contributing to the formation of experimental electron spin resonance (ESR) spectrum of γ-irradiated polycrystalline AA. Spectral parameters of these species were calculated after exhaustive spectrum simulation calculations based on data derived from experimental microwave saturation and dose-response studies.

Introduction

Ascorbic acid (AA) is a water-soluble vitamin and most important free oxygen scavenger. AA is stable when dry, but in solution it is readily oxidized to dehydroascorbic acid (DHAA),[1] especially in the presence of trace amounts of copper, iron and alkali. Its molecular structure is given in Fig. 1. AA or some derivatives are employed as antioxidants and radiation protector in foodstuffs, to prevent rancidity, browning of cut apples and other fruits and in meat curing.[2] AA is noted for its complex multi-functional effects.

Depending on medium conditions, AA can act as an antioxidant, pro-oxidant, metal cheaters, a reducing agent or an oxygen scavenger. Radiosensitivity and stability of AA in different in vitro systems and in different matrices were the subject of different research works using ESR spectroscopy and other methods.[3–9] Radiation damage produced in irradiated solid AA were also reported in the literature using ESR spectroscopy, but those investigations which considered only the variations of the intensities of the resonance line of doublet appearance, were rather oriented toward the determination of AA dosimetric properties.[10,11] Spectroscopic parameters of the radicalic species produced upon irradiation were not reported in none of these works except the ESR work carried out in our laboratory. Spectrum simulation calculations based on the presence of two different radical species and on signal intensity data derived from a room temperature spectrum were performed in this work. However, our later studies on irradiated single crystals of AA revealed that ESR spectra of crystalline samples consist, in general, more than nine resonance lines of different intensities except some specific orientations. Hence, it was concluded that such a spectrum cannot be explain along with radical species with unpaired electrons localized on carbon and oxygen atoms of five member rings as advised in Reference 10. Therefore, it was decided that species produced after the damage of the $-CHOHCH_2OH$ molecular part of AA should also take part in the formation of experimental ESR spectra of irradiated solid AA. Consequently, in the present work, simulation calculations performed in our previous work were repeated adopting a model consisting of the presence of three different radical species using, as input, the data derived from experimental microwave and dose-response studies carried out for irradiated polycrystalline AA.

Experimental

AA fine powder was supplied by Vankim Ltd. (Istanbul, Turkey). The AA samples, in polycarbonate vials, were irradiated in the dose range 0.5–25 kGy. All irradiations were performed at room temperature using a ^{60}Co ionizing radiation source at the Sarayköy Establishment of the Turkish Atomic Energy Agency in Ankara. The dose rate (1.6 kGy/h) was previously calibrated using Fricke dosimetry.

The ESR spectra were recorded at room temperature using a Bruker EMX 131 X-band spectrometer equipped with a cylindrical cavity. Numbers were assigned to the observed resonance peaks and intensity measurements were performed with respect to the spectrum base line. An unirradiated sample was kept as a reference.

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Results and discussion

While unirradiated polycrystalline AA presents no ESR signal, irradiated AA was found to present a nonsymmetrical spectrum with many resonance lines as shown in Fig. 2. The resonance lines are not distinguishable at low doses but they are well developed at high doses.

Variations of the resonance line intensities with microwave power

To investigate different features of the observed resonance peaks, a number is assigned to each peak and variations of the intensities of these peaks with applied microwave power were investigated first.

As seen from Fig. 3, microwave saturation characteristics of the observed resonance peaks are fairly different. Hence, variations of the intensities of these peaks with applied microwave power were investigated in the power range of 0.0016–20 mW to get insight into the characteristic behaviors of these peaks and possibly classify them into subgroups. To achieve this goal the signal intensities measured with respect to the spectrum base line of the observed resonance peaks were plotted as a function of the square root of applied microwave power. Intensities of all resonance peaks were observed to increase linearly with microwave power at low power. However, after certain critical power values, they all begun to decrease implying the behaviors of homogeneously broadened resonance lines except peaks 1 and 11 which exhibited the behavior of nonhomogeneously broadened resonance lines. Furthermore, power values corresponding to turning points from linearly increase to homogeneously broadening regions were found to be different for different peaks suggesting the presence of radical species presenting different saturation characteristics in irradiated AA. The results are summarized in Fig. 4.

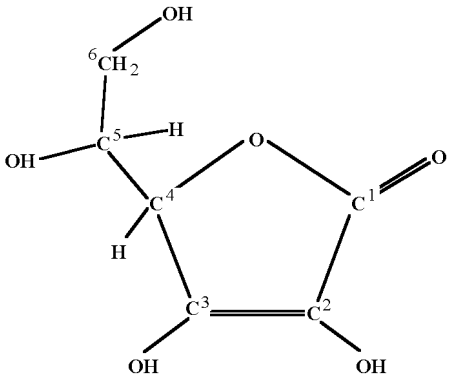

Fig. 1. Molecular structure of ascorbic acid

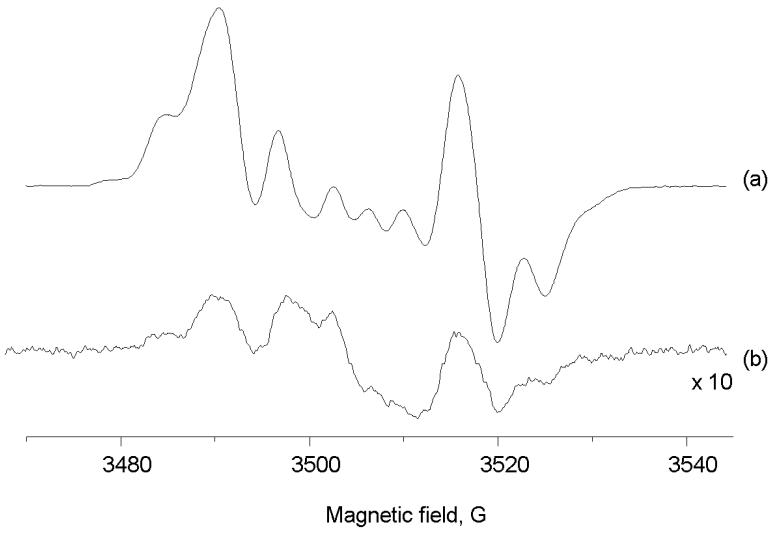

Fig. 2. ESR spectra of irradiated AA at two different radiation doses, (a) 25 kGy; (b) 0.5 kGy

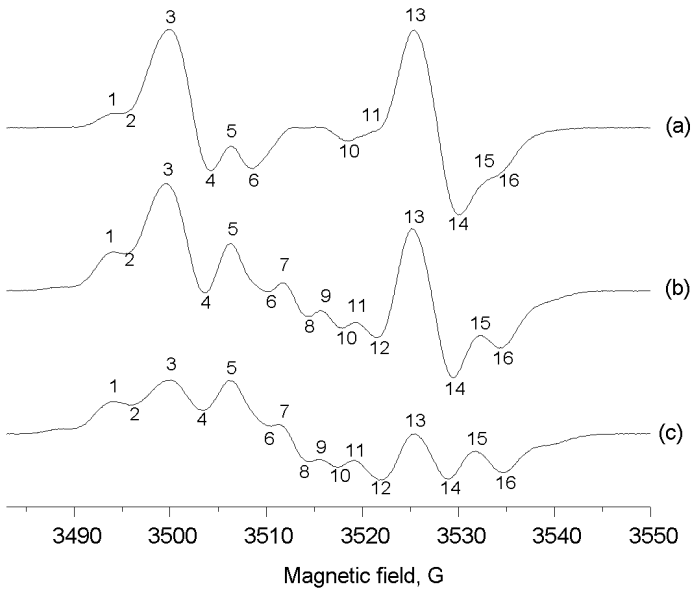

Fig. 3. ESR spectra of AA irradiated at a radiation dose of 10 kGy for three different microwave powers; (a) 0.02 mW, (b) 1 mW and (c) 5 mW

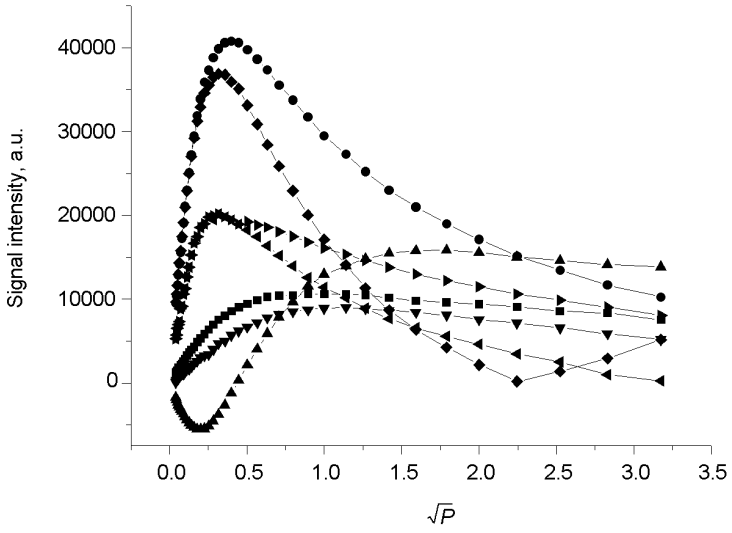

Fig. 4. Variations of the observed resonance peaks intensities with the square root of microwave power;
■ 1; ● 3; ▲ 5; ▼ 11; ♦ 13; ◄ 15; ► 16

Dose-response curves

Beside qualitative detection, the ESR spectroscopy can also be used for dose estimation. However, critical point in this respect is the choice of mathematical functions used to describe the dose-response curves. Samples of AA irradiated to doses of 0.5, 2.0, 5.0, 7.0, 10.0, 15.0, 20.0 and 25.0 kGy were used to construct the dose-response curves associated with assigned resonance peaks. Variations of the intensities of observed peaks with the absorbed radiation dose are given in Fig. 5. Different mathematical functions were tried to describe the variations of the resonance peak intensities with absorbed radiation dose due to its advantage of simplicity and giving better results than alternative methods such as double integration. A linear function of the applied dose of the type, was found to describe best experimental dose response data:

$$I = a\,D + b$$

where I and D stand for experimental peak intensity and applied dose (in kGy), respectively, and a and b are two constants. Parameter values and correlation coefficients derived for the observed principal peaks given be used to estimate applied radiation dose in the range of 0.5–25 kGy (Table 1).

Table 1. Parameter values calculated from fitting experimental dose-response data to linear functions for principal resonance peaks

Resonance lines	Parameter	Correlation coefficient
	a = 3931.00	
1	b = -2224.29	0.92150
	a = 9925.35	
3	b = 3529.53	0.98061
	a = 6460.99	
13	b = 770.41	0.99622
	a = 3912.24	
15	b = 8963.41	0.96597
	a = 6018.92	
16	b = 2222.30	0.97914

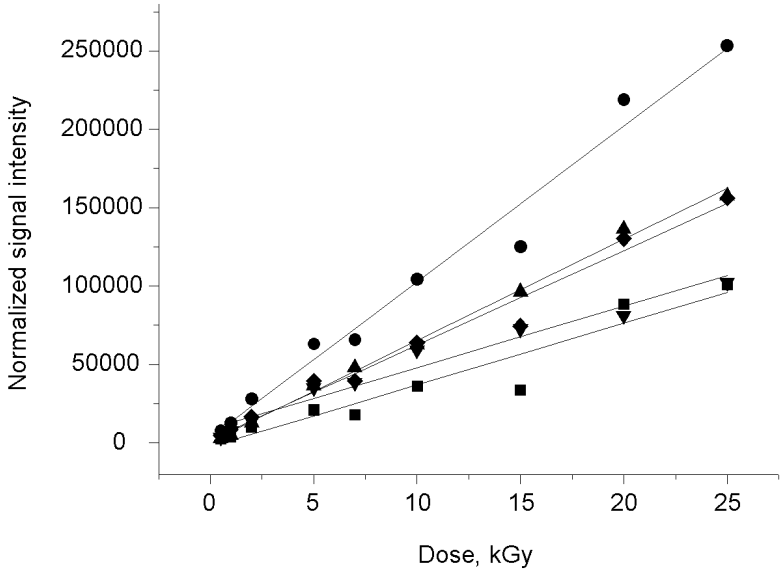

Fig. 5. Variations of the intensities of observed resonance peaks with applied radiation dose; ■ 1; ● 3; ▲ 13; ▼ 15; ♦ 16

Table 2. Spectroscopic parameters calculated for proposed radical species

Radical	Relative weight	Linewidth ΔH_{pp} (G)	g tensor				A tensor		
			g_{xx}	g_{yy}	g_{zz}		A_{xx} (G)	A_{yy} (G)	A_{zz} (G)
						H^1	25.62	19.53	21.11
I	0.414	1.48	2.0028	1.9973	2.0058	H^2	7.26	7.26	8.43
						H^3	7.26	7.26	8.43
						H^1	3.24	1.04	6.72
II	0.500	1.23	1.992	2.0075	2.00079	H^2	1.06	1.06	2.76
III	0.086	10.07	2.0054	2.0087	2.0082				

Spectrum simulation calculations

The results of long and exhaustive spectrum simulation calculations based on the presence of three different radical species are given in Table 2. As seen from this table, two species having orthorhombic g factors and orthorhombic hyperfine splitting and another species with orthorhombic g factor but not exhibiting hyperfine splitting contribute to the formation of experimental room temperature spectra of irradiated AA. While unpaired electron of radical I interact with three protons two of which are equivalent, which of the radical II exhibits hyperfine interactions with two nonequivalent protons. Theoretical spectrum calculated using spectroscopic and hyperfine splitting parameters given in Table 2 is presented in Fig. 6 with its experimental counterpart for comparison. As seen, except some minor differences, the model based on the presence of three different radical species describes quite well the experimental spectrum at room

temperature. Radicals produced through the mechanisms are seen in Fig. 7, believed to be the species responsible from observed experimental spectra of irradiated polycrystalline AA.

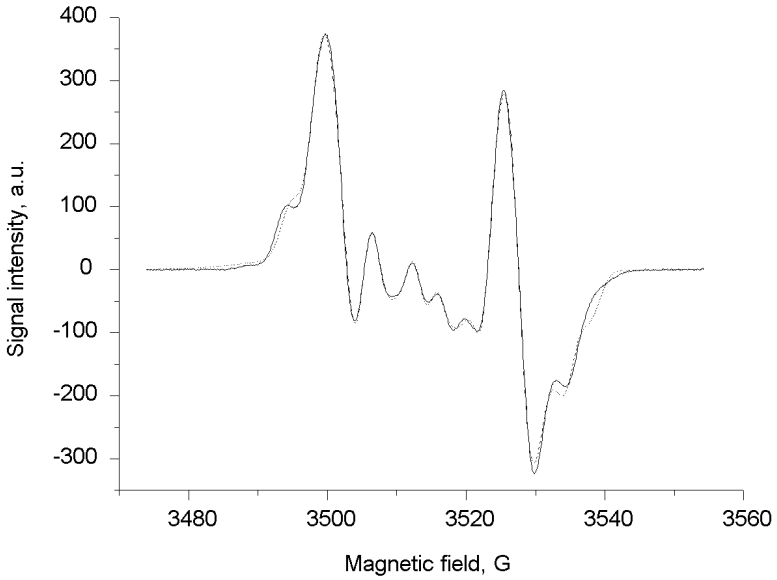

Fig. 6. Experimental (solid line) and theoretical (dashed line) ESR spectra calculated using parameter values given in Table 2

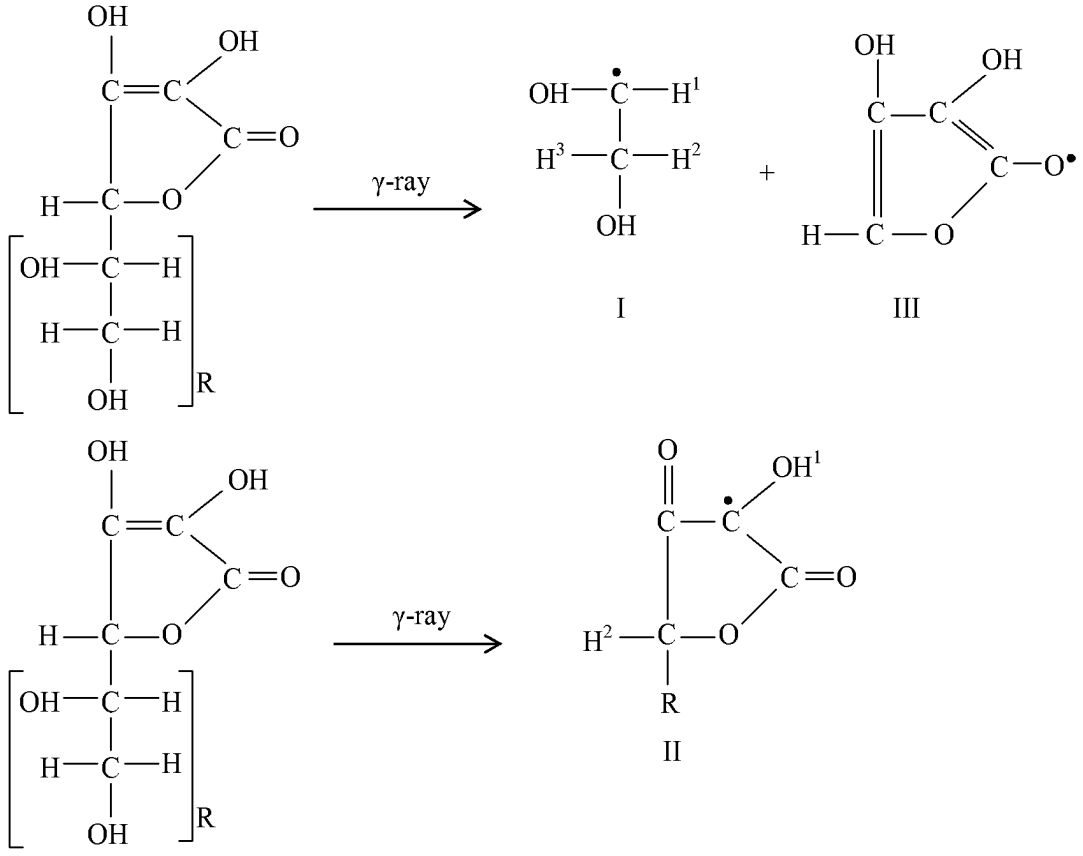

Fig. 7. Radicals produced

References

1. D. L. MADHAVI, S. S. DESPANDE, D. K. SALUNKHE, Food Antioxidants, Marcel Dekker, New York, 1995, p. 406.
2. A. J. ALISTE, N. L. DEL MASTRO, Phsycochem. Eng. Aspects, 249 (2004) 131.
3. B. S. N. RAO, Radiat. Res., 5 (1962) 683.
4. X. T. FARI, D. W. THAYER, J. Food Prot., 61/10 (2001) 1574.
5. N. GETOFF, I. PLATZER, C. WINKELBAUER, Rad. Phys. Chem., 55 (1999) 699.
6. I. YAMAZAKI, H. S. MASON, L. PIETT, J. Biol. Chem., 235 (1960) 2444.
7. W. M. VAUGHAN, J. I. HENRY, B. COMMONER, Biochim. Biophys. Acta, 329 (1973) 159.
8. P. KENNY, B. COMMONER, Nature, 223 (1969) 1229.
9. G. R. BUETTNER, K. P. KIMINYO, J. Biochem. Biophys. Meth., 24 (1992) 147.
10. H. N. REXROAD, W. GORDY, Proc. Nat. Am. Soc., 45 (1959) 256.
11. J. P. BASLY, I. LONGY, M. BERNARD, Pharm. Res., 14 (1997) 1186.