Free Radical Research, June 2006; 40(6): 637–645

α-Tocopherol amplifies benzoyl peroxide free radical decomposition in a chemical system

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Accepted by Dr E. Niki

(Received 6 December 2005; in revised form 20 February 2006)

Abstract
Benzoyl peroxide is commonly used in the treatment of acne, even though some adverse effects have been reported, probably mediated by the formation of peroxide-derived free radicals and the depletion of antioxidants. In the present work we have studied, in a chemical system, the effect of α-tocopherol on benzoyl peroxide radical decomposition to analyse the presence of an interaction between these two compounds, leading to an enhanced peroxide-cytotoxicity, as we have previously reported. Under our experimental conditions α-tocopherol strongly amplified the peroxide free radical decomposition occurring either in the presence or in the absence of UV irradiation, and lead to the formation of an unknown radical species in addition to benzoyloxy, phenyl and tocopheroxyl free radicals. The results of this study show that the enhancement of benzoyl peroxide toxicity in cells exposed simultaneously to this peroxide and α-tocopherol, is likely due to the generation of the detected radical species.

Keywords: *Benzoyl peroxide, α-tocopherol, electron spin resonance, UV irradiation*

Introduction

Benzoyl peroxide (BPO) is a free-radical generating compound widely used in the food industry as a bleaching agent for cheese and flour, in the chemical industry as an initiator of the polymerisation of rubber and plastic [1–4], and it is one of the compounds most commonly used in the cosmetic industry for the treatment of acne. BPO has demonstrated to cause the onset of irritation and contact dermatitis in about 1% of the treated patients [5], but despite these inconveniences it is still widely used in cosmetics [6,7]. Evidences have been produced demonstrating that BPO can act as a tumor promoter in the mouse skin even though it does not seem to act either as an initiator or as a complete carcinogen [5,8,9]. *In vitro*, BPO-toxicity is probably associated to the formation of BPO-derived benzoyloxy radical, $^{\cdot}O-C(O)-C_6H_5$, which then by decarboxylation generates the phenyl radical $^{\cdot}C_6H_5$ [10]; both these radical species could be responsible for cell membrane damage. Furthermore it is well known that BPO can affect cellular oxidative status. In fact BPO treatment produces the depletion of intracellular reduced glutathione [11], and topical applications of benzoyl peroxide in the mouse skin causes the oxidation of epidermal α-tocopherol (α-Toc) [12]. α-Toc is the form of vitamin E (VitE) with the highest biological activity and it is one of the most effective lipid soluble chain-breaking antioxidant [13]. α-Toc reacting with a free radical species is converted into the quite stable tocopheroxyl radical, α-Toc$^{\cdot}$: α-Toc$^{\cdot}$ can then be reduced back to native α-Toc with the help of vitamin C and GSH [14].

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Taylor & Francis
DOI: 10.1080/10715760600654895

Valacchi et al. have demonstrated that BPO rapidly reacts with vitamin E in the cellular membrane producing VitE oxidation [15], and Liebler et al. showed that the topical application of a single dose of BPO on mouse skin results in the oxidation of dermal α-Toc [12].

In an *in vitro* study, human keratinocytes RHEK-1 were maintained for a number of passages in a medium supplemented with VitE and then treated with BPO: these cells resulted to be more resistant against BPO toxicity [11]. Other investigators have shown similar effects pre-treating murine leukaemia cells with VitE before the treatment with the peroxide [16].

Recently, in our lab, we have demonstrated that the method of delivery of VitE is crucial in determining its antioxidant efficacy in cells exposed to BPO [17]. When α-Toc was distributed in a uniform layer and cells were seeded on it, its uptake was efficient and resulted in an excellent protection against BPO toxicity. On the contrary, when α-Toc was added in the cell medium, 24 h prior BPO treatment, the antioxidant uptake was minimal. Moreover if α-Toc was added in the medium together with BPO, the peroxide toxicity increased, possibly due to an interaction between BPO and α-Toc [17]. In order to evaluate whether any radical-mediated mechanism is responsible for the interaction between this two compounds, leading to an enhanced BPO toxicity, we studied the BPO/α-Toc interaction in a chemical system.

In the present work, we have employed the electron spin resonance (ESR) spectroscopy coupled to the spin trapping technique to analyse the chemistry involved in this reaction: we have obtained the decomposition of 5 mM BPO in different solvents (methanol or benzene) and in the presence of 0.5 or 5 mM α-Toc. Under these conditions the addition of α-Toc to the system strongly amplified the free radical decomposition of the peroxide, occurring either in the presence or in the absence of UV irradiation. The presence of α-Toc resulted also in the detection of an unknown radical species in addition to benzoyloxy, phenyl and tocopheroxyl free radicals.

Materials and methods

Materials

(+)α-Tocopherol was purchased from Sigma-Aldrich (Milan, Italy). N-*t*-butyl-α-phenylnitrone (PBN) was purchased from Alexis Italia (Vinci, Florence, Italy). Benzoyl peroxide and benzene were purchased from BDH (Milan, Italy). Methanol was purchased from J. T. Baker (Milan, Italy). Hydrochloric acid was purchased from Carlo Erba (Rodano, Milan, Italy).

Sample preparation

All reactions were performed under a nitrogen stream to reach anaerobic conditions, as previously reported [18]. In a eppendorf tube, methanol (or benzene) was added to 5 mM BPO; when indicated, α-Toc (0.5 or 5 mM) was also added. PBN (85 mM) was finally included and mixed. The reaction mixture was transferred into a glass capillary tubing (1 mm diameter) that was sealed at both ends with critoseal and irradiated. Finally the capillary tubing was inserted in a 3 mm i.d. quartz tube and run by ESR. All stock solutions were prepared in methanol or in benzene depending on the experimental requirements.

Sample irradiation

Samples were irradiated using a TORIN TA 450 500 W Hg UV lamp that, after ignition, developed 12 Å and 18 V. The lamp was centered on the sample tubing and kept at a distance of 20 cm from it; samples were irradiated for a total of 5 s, when performing the reaction in methyl alcohol, and 10 s when using benzene instead of methanol. The absorption of UV rays by the glass capillary tubing used to contain the samples was also measured: all UV-A resulted to pass through the glass, while all the UV-C, and most part of the UV-B, were absorbed.

Electron spin resonance measurements

ESR spectra were recorded using a Bruker EMX spectrometer (Billerica, MA, USA) operating at 9.3 GHz with a modulation frequency of 100 kHz and a TM_{110} cavity. Spectrometer conditions were: modulation amplitude, 1 G; microwave power, 20 mW; time constant, 41 ms; conversion time, 164 ms; scan time, 168 s; scan range, 50 G; and receiver gain, 1×10^5. Spectra were recorded on an IBM-compatible computer, interfaced to the spectrometer. The radical species trapped by PBN were identified based on the hyperfine coupling constants of the corresponding PBN radical adducts. ESR spectra were simulated using a computer program available through the Internet (http://epr.niehs.nih.gov/). The details of the program have been published [19].

Results

The ESR spin trapping technique was employed to analyse the effect of vitamin E on the free radical decomposition of benzoyl peroxide under anaerobic conditions. When PBN was dissolved in methanol a weak background signal was detected as shown in Figure 1A.

Sample irradiation increased signal intensity and resulted in the detection of two PBN radical adducts (Figure 1B). On the bases of the hyperfine coupling constants, the detected radical species were assigned as follows: PBN/$^{\cdot}OCH_3$ ($a^N = 14.65$ and $a^H_\beta = 2.74$) (80%) and PBN/$^{\cdot}CH_2OH$($a^N = 15.10$ and $a^H_\beta = 3.71$) (20%). The calculated hyperfine coupling constants were consistent with previously reported values for the same radical species [20,21]. The

formation of the above-mentioned radical species has been demonstrated during methanol radiolysis [22] and also after UV irradiation of methanol obtained at 77 K [23].

When benzoyl peroxide (5 mM) was reacted with 85 mM PBN, in methanol, a weak ESR signal was detected (Figure 1C). Upon irradiation of the reaction mixture, the intensity of the resulting ESR signal strongly increased (Figure 1D); the corresponding computer simulation, superimposed on the experimental signal as a dashed line, is shown in Figure 2A; the individual simulation of each radical species present in the composite spectrum, and the chemical structure of the detected spin adducts, are also shown.

Under these conditions, a mixture of two radical adducts was detected. On the basis of computer simulation and hyperfine coupling constant calculation, one of the detected radical adducts, accounting for the 18% of the total radical concentration, was assigned as follows: phenyl radical adduct PBN/$^{\cdot}C_6H_5$ ($a^N = 15.10$ and $a_\beta^H = 3.19$). The calculated hyperfine splittings have been previously reported for the same radical species [24]. A second unknown PBN radical adduct, accounting for the 82% of the total radical concentration was also detected, this species was characterized by the following hyperfine coupling constants: $a^N = 13.93$ and $a_\beta^H = 2.01$. To identify the above-mentioned radical species we have performed the irradiation of an identical reaction system, where, instead of methanol, benzene was used as solvent (Figure 1E). Figure 2B shows the corresponding computer simulation, superimposed

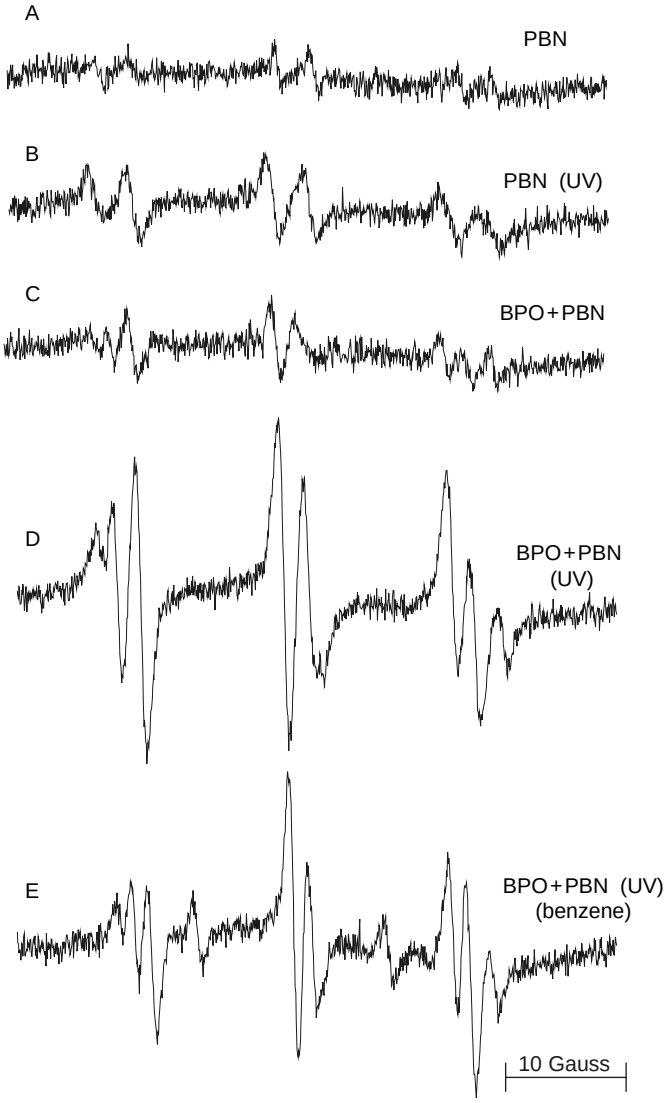

Figure 1. ESR spectra of PBN radical adducts detected after the irradiation of benzoyl peroxide in methanol (A–D) and benzene (E). Spectrum A is the ESR spectrum obtained from a methanol solution of PBN (85 mM). Spectrum B was obtained after the irradiation of 85 mM PBN. Spectrum C resulted from a reaction mixture containing 5 mM BPO and 85 mM PBN. Spectrum D derived from the irradiation of a reaction mixture containing 5 mM BPO and 85 mM PBN. Spectrum E, same as D beside reaction mixture obtained in benzene and not in methanol. Spectral Y scale amplified three times compared to that of the ESR spectra shown in Figures 3 and 5 to better illustrate spectral characteristics.

on the experimental signal as a dashed line; the individual simulations of each radical species present in the composite spectrum, and the chemical structure of the detected spin adducts, are also shown. Under these conditions, we have detected three radical species: the PBN oxidation product $^{\cdot}$PBNOX ($a^{N} = 7.95$), as minor species, phenyl radical adduct PBN/$^{\cdot}C_6H_5$ ($a^{N} = 14.43$ and $a^{H}_{\beta} = 2.21$) and benzoyloxy radical adduct PBN/$^{\cdot}O-C(O)-C_6H_5$ ($a^{N} = 13.26$ and $a^{H}_{\beta} = 1.49$) as major species. All the calculated hyperfine splittings have been previously reported for the same radical adducts [25,26]. Based on the free radical species detected in benzene, we have assigned the unknown radical species, detected during the irradiation of BPO in methanol, to the benzoyloxy PBN radical adduct (Figure 2A).

We have then analysed the free radical generation during the interaction between BPO and α-tocopherol in 10:1 and 1:1 ratios, to better understand the chemistry involved in the interaction occurring between BPO and α-Toc. When 5 mM BPO was reacted with 0.5 mM α-Toc a composite ESR signal was detected (Figure 3C).

The signal differed in composition and was about 7 times more intense compared to the one obtained in the absence of α-Toc (Figure 1C). The irradiation of the reaction mixture did not change signal composition while it increased its intensity (Figure 3D). Figure 4B shows the computer simulation superimposed on the experimental signal as a dashed line; the individual simulations of each radical species present in the composite spectrum, and the chemical structure of the detected spin adducts, are also shown. Two of the detected radical species corresponded to the species obtained in the absence of α-Toc: in fact the composite signal consisted for the 12% of

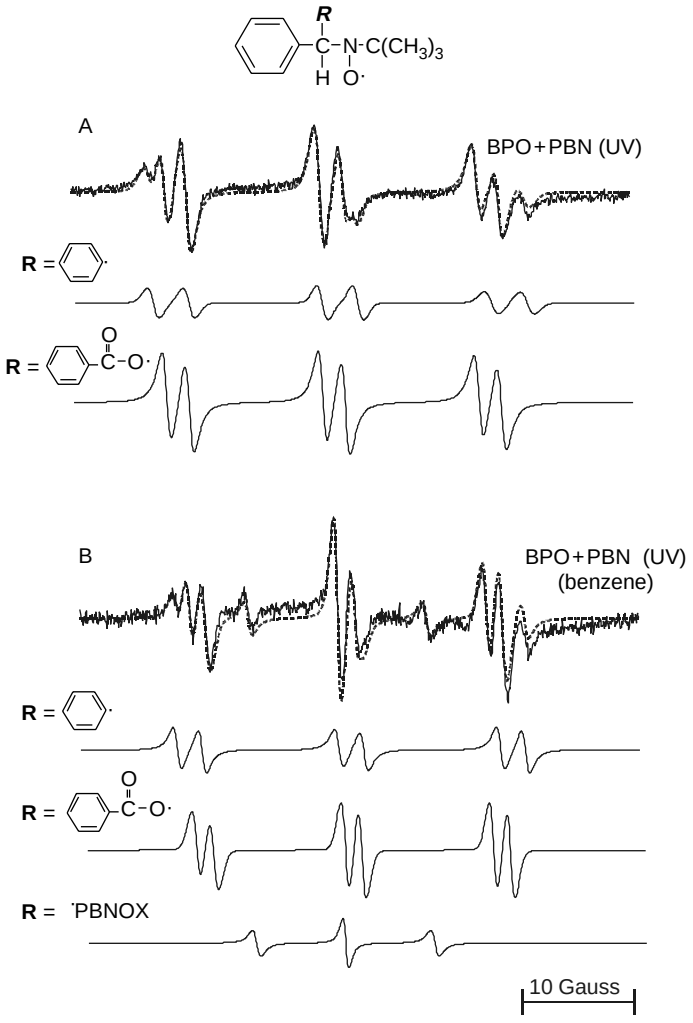

Figure 2. Computer simulation and deconvolution of the ESR spectra obtained after the irradiation of a reaction mixture containing BPO and PBN. Spectrum A is the computer simulation (dashed line) superimposed on the experimental spectrum obtained irradiating a reaction mixture containing 5 mM BPO and 85 mM PBN in methanol; following are shown the simulations and the chemical structure of each species present in the composite spectrum. Spectrum B is the computer simulation (dashed line) superimposed on the experimental spectrum obtained irradiating a reaction mixture containing 5 mM BPO and 85 mM PBN in benzene; following are shown the simulations and the chemical structure of each species present in the composite spectrum.

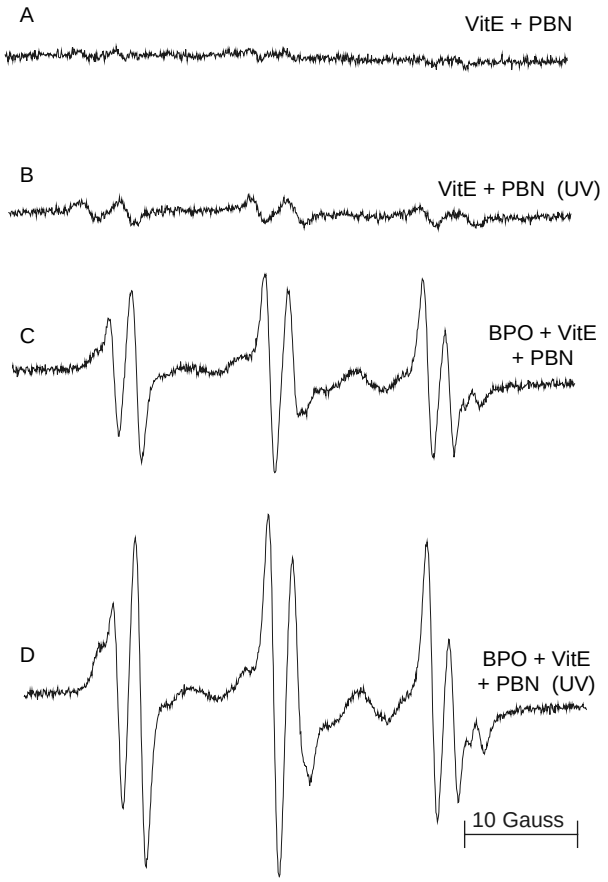

Figure 3. ESR spectra of PBN radical adducts detected after the irradiation of benzoyl peroxyde in the presence of 0.5 mM α-tocopherol, in methanol. Spectrum A is the ESR spectrum of α-Toc (0.5 mM) in the presence of PBN (85 mM). Spectrum B was obtained irradiating 0.5 mM α-Toc in the presence of 85 mM PBN. Spectrum C resulted from a reaction mixture containing 5 mM BPO, 0.5 mM α-Toc and 85 mM PBN. Spectrum D derived from the irradiation of 5 mM BPO and 0.5 mM α-Toc in the presence of 85 mM PBN.

PBN/$^{\cdot}C_6H_5$ ($a^N = 15.10$ and $a^H_\beta = 3.18$) and for the 28% of PBN/$^{\cdot}O-C(O)-C_6H_5$ ($a^N = 13.92$ and $a^H_\beta = 1.95$). The presence of α-Toc resulted in the appearance of two additional radical species: the tocopheroxyl free radical α-Toc$^{\cdot}$ ($a^H_{5CH_3} = 5.25$ and $a^H_{7CH_3} = 4.6$) (18%) whose calculated hyperfine splittings are in good agreement with previously reported values [27], and an unknown species, PBN/$^{\cdot}$X, accounting for the 28% of the radical concentration and characterized by the splittings $a^N = 14.47$ and $a^H_\beta = 3.07$. When 0.5 mM α-tocopherol was reacted with 85 mM PBN a very weak background signal was detected (Figure 3A). A weak ESR signal was obtained upon irradiation of the reaction mixture (Figure 3B). Based on computer simulation (shown in Figure 4A) and hyperfine splitting calculation, the signal resulted to consist of PBN/$^{\cdot}OCH_3$ ($a^N = 14.54$ and $a^H_\beta = 2.72$) (52%) and PBN/$^{\cdot}CH_2OH$ ($a^N = 15.18$ and $a^H_\beta = 3.61$) (48%) deriving from the irradiation of methanol.

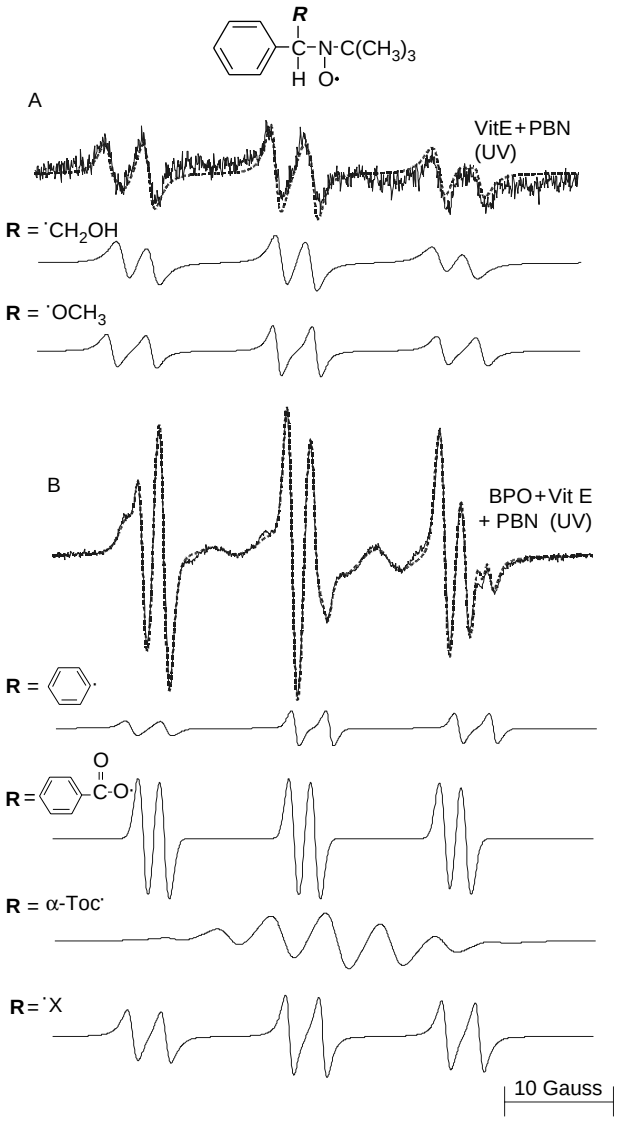

Figure 4. Computer simulation and deconvolution of the ESR spectra obtained in methanol, by the irradiation of a reaction mixture containing BPO and 0.5 mM α-Toc in the presence of PBN. Spectrum A is the computer simulation (dashed line) superimposed on the experimental spectrum obtained by irradiating a reaction mixture containing 0.5 mM α-Toc and 85 mM PBN; following are shown the simulations and the chemical structure of each species present in the composite spectrum. Spectrum B is the computer simulation (dashed line) superimposed on the experimental spectrum obtained by irradiating a reaction mixture containing 5 mM BPO, 0.5 mM α-Toc and 85 mM PBN; following are shown the simulations and the chemical structure of each species present in the composite spectrum.

Finally, we have analysed the formation of free radical species when using BPO and α-Toc in a 1:1 ratio. When 5 mM BPO was reacted with 5 mM α-Toc the composite ESR signal, shown in Figure 5C, was detected.

The signal was more intense when compared to the one obtained in the presence of 10 times less α-Toc, and consisted of the same radical species,

although present in different ratios (Figure 5C). The irradiation of the reaction mixture did not change either signal composition, nor signal intensity (Figure 5D). Figure 6B shows the computer simulation superimposed on the experimental signal as a dashed line; the individual simulation of each radical species present in the composite spectrum, and the chemical structure of the detected spin adducts, are also shown. Based on computer simulation and hyperfine coupling constant calculation, the detected radical species were assigned as follows: PBN/$^{\cdot}C_6H_5$ ($a^N = 15.16$ and $a^H_\beta = 3.20$) (29%); PBN/$^{\cdot}O-C(O)-C_6H_5$ ($a^N = 13.99$ and $a^H_\beta = 1.91$) (8%); α-Toc$^{\cdot}$ ($a^H_{5CH_3} = 5.38$ and $a^H_{7CH_3} = 4.50$) (38%) and PBN/$^{\cdot}$X ($a^N = 14.39$ and $a^H_\beta = 3.04$) (25%).

When 5 mM α-Toc was reacted with 85 mM PBN, a very weak background signal was detected (Figure 5A). The weak ESR signal detected when the reaction mixture was irradiated (Figure 5B), has been assigned, upon computer simulation (shown in Figure 6A) and hyperfine splitting calculation, to the following PBN radical adducts: PBN/$^{\cdot}OCH_3$ ($a^N = 14.58$ and $a^H_\beta = 2.80$) (38%) and PBN/$^{\cdot}CH_2OH$ ($a^N = 15.23$ and $a^H_\beta = 3.63$) (62%).

The latter free radical species derives from the irradiation of methanol. All the hyperfine coupling constants calculated for the PBN radical adducts detected from the reaction mixtures prepared in methanol are summarized in Table I.

When the reaction mixture containing BPO and α-Toc was analyzed by direct ESR only, the signal corresponding to α-Toc$^{\cdot}$ was detected. The resultant signal was characterized by the same hyperfine splittings obtained when using PBN with no appearance of any other radical species detectable directly (data not shown). Based on these results, we can exclude the possibility of having detected an alternative α-Toc$^{\cdot}$ resonance structure.

The addition of tocopherol to BPO in 1:10 ratio, when using benzene instead of methanol, resulted in a ESR signal slightly more intense compared to that obtained in the absence of α-Toc. When α-Toc and BPO where present in a 1:1 ratio the resulting signal further increased (it was nearly 4 times more intense with respect to the signal obtained in the absence of the antioxidant). Moreover, in this reaction the benzoyloxy radical adduct disappeared. UV irradiation resulted in the simple amplification of signal intensities (data not shown).

The kinetic of the reactions performed in benzene demonstrated some differences compared to those in methanol, both when reacting BPO and α-Toc, and when using BPO alone. The $^{\cdot}$PBNOX, for instance, was detected only in benzene, the signal intensity was very weak, greatly increasing upon irradiation. These differences are probably due to the different chemical characteristics of the solvents employed for the reaction, and also to the longer irradiation time used in the reaction performed in benzene.

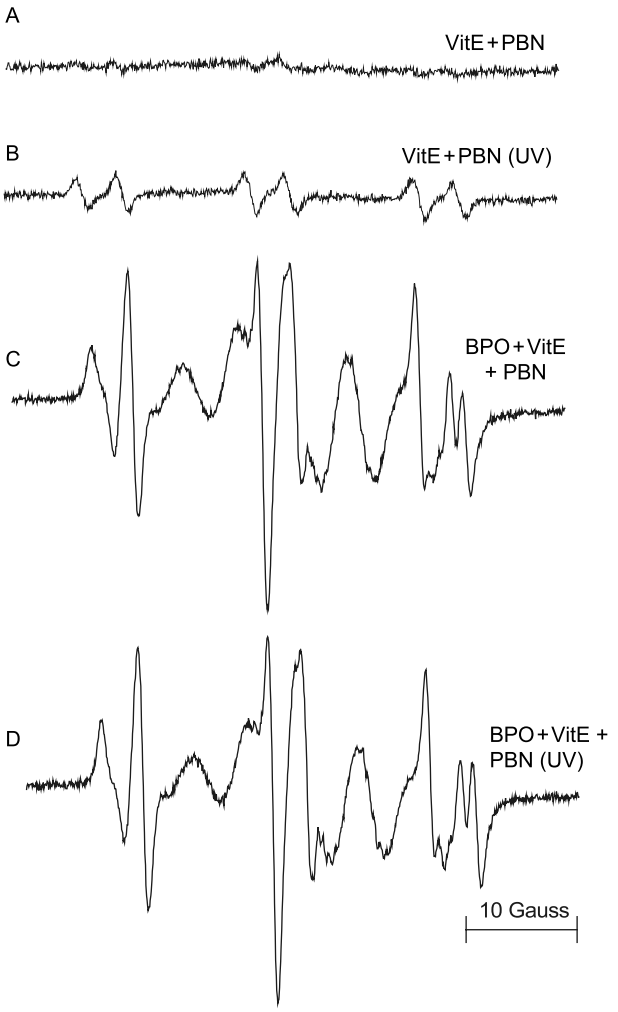

Figure 5. ESR spectra of PBN radical adducts detected after the irradiation of benzoyl peroxide in the presence of 5 mM α-tocopherol in methanol. Spectrum A is the ESR spectrum obtained from a solution containing α-Toc (5 mM) and PBN (85 mM). Spectrum B was obtained irradiating 5 mM α-Toc in the presence of 85 mM PBN. Spectrum C resulted from a reaction mixture containing 5 mM BPO, 5 mM α-Toc and 85 mM PBN. Spectrum D derived from the irradiation of a reac tion mixture containing 5 mM BPO, 5 mM α-Toc and 85 mM PBN.

The detected unknown radical species, X$^{\cdot}$, appeared when reacting BPO and α-Toc in methanol, was independent from sample irradiation and was not detected when performing the reaction in benzene. This implies a role of methyl alcohol in its formation. The use of $FeSO_4$ in methanol, instead of UV irradiation, resulted in the detection of X$^{\cdot}$, both in the presence and in the absence of α-Toc (data not shown). Hyperfine coupling constant values similar to those calculated for PBN/X have been reported for the formyl radical adduct PBN/$^{\cdot}$CHO obtained, in benzene, after hydrogen abstraction by tert-butoxyl free radicals or irradiation of formaldehyde [28].

Table I. Summary of the hyperfine coupling constants for the free radical species detected, in methanol, in the presence of PBN. a^N, a^H_β values for PBN radical adducts and $a^H_{5CH_3}$, $a^H_{7CH_3}$ values for the directly detected α-Toc·

System	Radical	$a^N/a^H_{5CH_3}$	$a^H_\beta/a^H_{7CH_3}$	Reference
Methanol, UV	$^{\cdot}OCH_3$ OH	14.65	2.74	[20]
	$^{\cdot}CH_2OH$	15.10	3.71	[21]
BPO	$^{\cdot}C_6H_5$	15.09	3.10	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.92	1.99	This work
BPO, UV	$^{\cdot}C_6H_5$	15.10	3.19	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.93	2.01	This work
α-Toc (0.5 mM), UV	$^{\cdot}OCH_3$ OH	14.54	2.72	[20]
	$^{\cdot}CH_2OH$	15.18	3.61	[21]
BPO, α-Toc (0.5 mM)	$^{\cdot}C_6H_5$	15.10	3.19	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.92	1.95	This work
	$^{\cdot}X$	14.47	3.10	This work
	α-Toc·	5.37	4.69	[27]
BPO, α-Toc (0.5 mM) UV	$^{\cdot}C_6H_5$	15.10	3.18	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.92	1.95	This work
	$^{\cdot}X$	14.47	3.07	This work
	α-Toc·	5.32	4.64	[27]
α-Toc (5 mM), UV	$^{\cdot}OCH_3$ OH	14.58	2.80	[20]
	$^{\cdot}CH_2OH$	15.23	3.63	[21]
BPO, α-Toc (5 mM)	$^{\cdot}C_6H_5$	15.13	3.19	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.98	1.94	This work
	$^{\cdot}X$	14.38	3.04	This work
	α-Toc·	5.38	4.50	[27]
BPO, α-Toc (5 mM), UV	$^{\cdot}C_6H_5$	15.16	3.20	[24]
	$^{\cdot}O-C(O)-C_6H_5$	13.99	1.91	This work
	$^{\cdot}X$	14.39	3.04	This work
	α-Toc·	5.38	4.50	[27]

The oxidation of the detected hydroxymethyl radical $^{\cdot}CH_2OH$, by iron or by α-Toc·, could easily lead to formaldehyde formation and consequently to the formyl radical detection.

Discussion

In the present work, we have employed the ESR spin trapping technique to analyse the effect of α-Toc on the free radical decomposition of BPO obtained, in methanol, under anaerobic conditions and in the presence or in the absence of UV irradiation.

When BPO was reacted with the spin trap PBN, a weak ESR signal, consisting of BPO derived benzoyloxy and phenyl radical adducts was detected (Figure 2A). UV irradiation resulted in the detection of the same radical species, though much more intense (Figure 1C and D). It is well known that the peroxyl bond in the BPO molecule is easily breakable with the formation of benzoyloxy free radicals, that upon decarboxylation produces phenyl radicals. Several years ago these radical species have been identified in the reaction of BPO and PBN in benzene at room temperature [29]. Here, the novelty is represented by the effect of vitamin E on BPO radical decomposition. To analyse the chemistry involved when reacting BPO in the presence of α-Toc, we have performed BPO decomposition, in methanol or benzene, in the presence of two different concentrations of tocopherol. When radical decomposition of BPO occurred in methanol, in the presence of α-Toc, either in 10:1 or in 1:1 ratio, free radical formation increased several times. UV irradiation did not change radical composition; it increased ESR signal intensity, when using the lower α-Toc concentration (Figures 1D and 3D), and it showed almost no effect when α-Toc was present in 1:1 ratio with BPO (Figure 5D). The employment of benzene instead of methanol resulted in a ESR signal significantly more intense only upon the addition of tocopherol to BPO in 1:1 ratio, with the disappearance of the benzoyloxy radical adduct. Also in this system UV irradiation resulted in the simple amplification of signal intensities (see above). The kinetic of the reactions performed in benzene demonstrated some differences compared to that of BPO decomposition in methanol. This is probably due to the different chemical characteristics of the two solvents employed.

These data demonstrate the central role of the solvent in affecting the chemical interaction between BPO and α-Toc.

The enhancement of free radical formation, induced by the presence in the chemical system of α-Toc, could be due to hydrogen abstraction from α-Toc, operated by BPO-derived free radicals.

The addition of α-Toc to BPO, not only enhanced free radical decomposition, but also resulted in the appearance of two extra radical species, the α-Toc derived tocopheroxyl radical, α-Toc·, and an unidentified radical species $^{\cdot}X$ (Figures 3C and 5C).

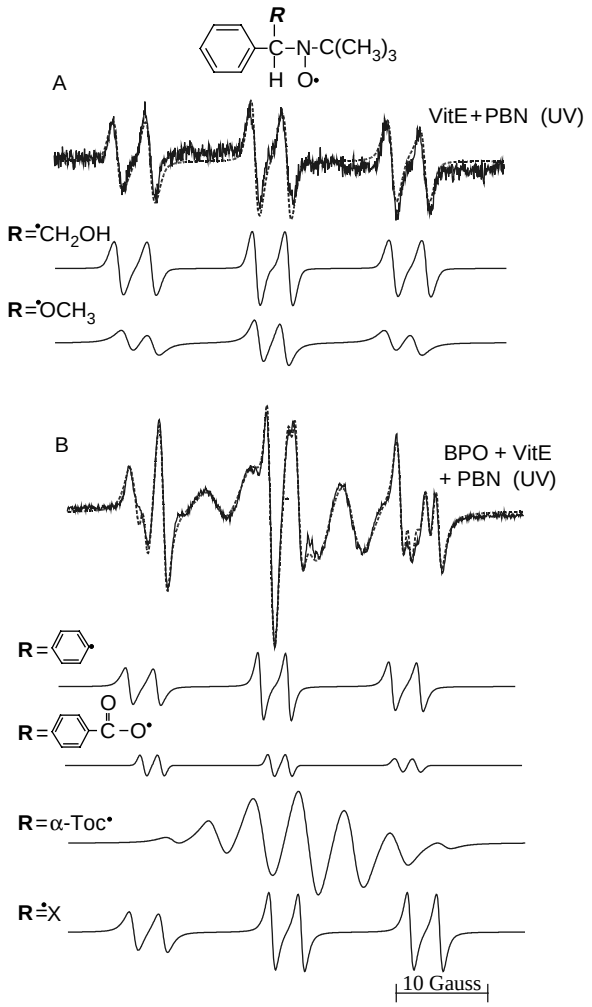

Figure 6. Computer simulation and deconvolution of the ESR spectra obtained by the irradiation a reaction mixture containing 5 mM BPO, 5 mM α-Toc and 85 mM PBN in methanol. Spectrum A is the computer simulation (dashed line) superimposed on the experimental spectrum obtained irradiating a reaction mixture containing 5 mM α-Toc and 85 mM PBN; following are shown the simulations and the chemical structure of each species present in the composite spectrum. Spectrum B is the computer simulation (dashed line) superimposed on the experimental spectrum obtained irradiating a reaction mixture containing 5 mM BPO, 5 mM α-Toc and 85 mM PBN; following are shown the simulations and the chemical structure of each species present in the composite spectrum.

The latter species was never detected when analyzing α-Toc or BPO alone, moreover $^{\bullet}X$ never appeared when analyzing samples by direct ESR, excluding the possibility of having detected an alternative α-Toc$^{\bullet}$ resonance structure.

The detection of this unknown radical species was evident when performing the reaction in methanol while it was never obtained in benzene, demonstrating the importance of the solvent in $^{\bullet}X$ formation. Moreover, when we obtained BPO decomposition using $FeSO_4$ instead of UV irradiation, $^{\bullet}X$ was detected even in the absence of α-Toc. As discussed above, hyperfine coupling constant values similar to those calculated for PBN/$^{\bullet}X$ have been previously reported for the formyl PBN radical adduct $^{\bullet}CHO$ [28]. This radical species could probably derive from formaldehyde produced as a consequence of the iron- or α-Toc$^{\bullet}$-induced oxidation of the hydroxymethyl radical, $^{\bullet}CH_2OH$.

Taken together our data show that the simultaneous presence of BPO and α-Toc in solution can lead to the enhancement of BPO free radical decomposition and that, with some differences, this phenomenon occurs both in polar protic and in apolar solvents. Under these conditions the formation of significant amounts of α-Toc-derived free radicals is also evident.

Some investigators have formerly examined the direct interaction between α-Toc and BPO, but these studies were focused on the formation of non-radical compounds, having different characteristics depending on the solvent employed [30]. In particular, the formation of 8a-alkoxyl-α-tocopherones was demonstrated when BPO oxidation of α-Toc was obtained in the presence of alcohols [31].

Our data set the attention on the possible interactions occurring between benzoyl peroxide and α-tocopherol resulting in the enhancement of free radical formation. This phenomenon needs to be taken in account when designing systems where the aim is the usage of α-tocopherol for protection against benzoyl peroxide or other aryl peroxide toxicity. The present results elucidate the mechanism underlying the enhanced BPO toxicity in cells exposed simultaneously to BPO and α-Toc, as we have previously reported [17].

Acknowledgements

Authors are grateful to Prof. Loris Grossi for helpful suggestions and discussion.

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