SUPEROXIDE ANIONS DO NOT REACT WITH HYDROPEROXIDES

Wolf BORS, Christa MICHEL and Manfred SARAN

Abteilung für Strahlenbiologie, Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, 8042 Neuherberg, FRG

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1. Introduction

It is now well established that the reaction of O_2^- with H_2O_2, the so-called Haber-Weiss reaction [1]:

$$O_2^- + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^- + O_2 \qquad (1)$$

is too slow to account for the formation of ·OH radicals in biological systems [2,3]. Yet, after generating O_2^- either in organic solvents from KO_2/crown ether [4] or by the enzymatic reaction of xanthine oxidase with xanthine or acetaldehyde [5], it was proposed that organic hydroperoxides are capable of reacting with O_2^- [4,5]. The data in [4] further suggest that the rate of formation of the alkoxy radical depends on the alkyl chain length:

$$O_2^- + ROOH \longrightarrow RO^{\cdot} + OH^- + O_2 \qquad (2)$$

Should this reaction occur and the produced alkoxy radical, RO·, react with organic substrates, it would elegantly explain some aspects of the toxicity of O_2^- [6] without invoking the generation of ·OH radicals themselves [7,8].

In a study on the formation and detection of organic oxygen radicals (in preparation), we re-investigated the reactivity of O_2^- with hydroperoxides, derived from both *t*-butanol and linoleic acid. O_2^-, in our case, was generated by pulse radiolysis, which is a far more specific source than biochemical generation methods. We found no reaction of O_2^- with *t*-butyl hydroperoxide ($(CH_3)_3C-OOH$ or *t*-BOOH) and linoleic acid hydroperoxide (13-LOOH), neither by direct spectroscopic observation nor by indirect assay via bleaching of *p*-nitrosodimethylaniline (*p*-NDA) or the carotenoid crocin.

2. Materials and methods

t-Butyl hydroperoxide was bought from Merck (Darmstadt) and was 80% with di-*t*-butyl peroxide present. 13-LOOH was prepared enzymatically [9] and was a gift from Dr Grosch, Dt. Forschungsanstalt für Lebensmittelchemie (Garching). *p*-NDA was bought from Roth (Karlsruhe) and crocin was isolated from saffron [10] (donated by Dr Elstner, Technische Universität München).

The pulse-radiolytic equipment was described in [11]. Spectral plots are represented only as initial transients, whereas the time traces represent a composite of various pulses at different observation periods.

3. Results and discussion

Our first doubts on the reactivity of O_2^- with organic hydroperoxides arose from the fact that the transient spectra observed with 13-LOOH or *t*-BOOH in the presence of O_2^- (in irradiated oxygenated formate solutions) represented the latter species exclusively. This is exemplified by a comparison with an authentic O_2^- spectrum (fig.1).

The calculated molar absorbtivities in these three systems (O_2^- exclusively, O_2^- in the presence of 13-LOOH, O_2^- in the presence of *t*-BOOH) were very similar (2100, 2060, 1970 $M^{-1}.cm^{-1}$), yet somewhat lower than the latest value for O_2^- of 2350 $M^{-1}.cm^{-1}$ [12]. A very close coincidence was found for the decay rates at pH 7.0: 5.6×10^5, 2.4×10^5, 7.5×10^5, all expressed as $M^{-1}.s^{-1}$. This relates to the theoretical value of 4.9×10^5 $M^{-1}.s^{-1}$ for the decay of O_2^- at this pH value [12].

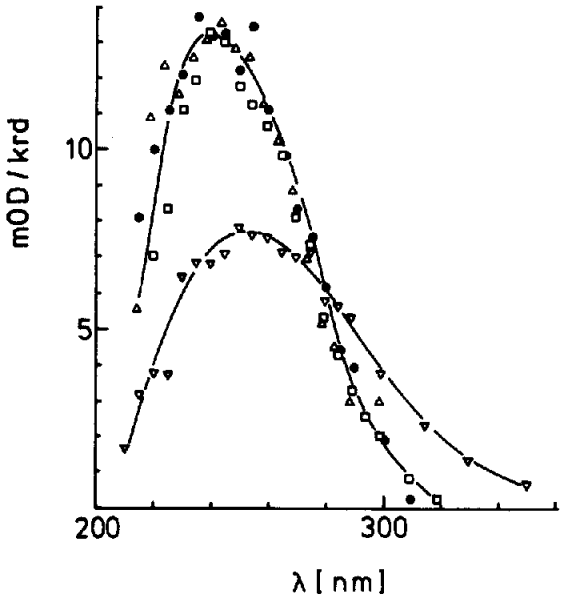

Fig.1. Transient spectra of O_2^- in the presence of hydroperoxides and of the peroxy radical of *t*-butanol. Initial absorption 5 μs after the pulse in neutral solutions, dose-normalized. (•) O_2^- alone: 10 mM $HCOO^-$, O_2; (□) O_2^- + 13-LOOH (10 μM); (△) O_2^- + *t*-BOOH (0.5 mM); (▽) *t*-BOO·: 10 mM *t*-butanol, N_2O/O_2 gas mixtures.

The plot depicted in fig.1 also contains the transient spectrum of the peroxy radical, obtained from ·OH-attack at *t*-butanol and subsequent O_2-attachment [13]:

$$(CH_3)_3COH + \cdot OH \longrightarrow (CH_3)_2C(OH)CH_2^\cdot \quad (3)$$

$$(CH_3)_2C(OH)CH_2^\cdot + O_2 \longrightarrow (CH_3)_2C(OH)CH_2OO^\cdot \quad (4)$$

It is evident that this species closely resembles O_2^- if one compares the absorption maximum, yet it has a smaller molar absorbtivity (1350 $M^{-1}.cm^{-1}$) and it decays much faster than O_2^- itself, also in a second-order reaction ($2k = 1.8 \times 10^8$ $M^{-1}.s^{-1}$). It has to be emphasized, however, that this peroxy radical is different from the one derived by H-abstraction from *t*-BOOH. The latter radical cannot be obtained by radiolytic methods.

The fact of close spectroscopic identity of O_2^- and the peroxy radical ROO· (RO· shows only a minor end absorption with very low molar absorbtivity even at 230 nm) led us to investigate the reactions by indirect methods, i.e., the use of specific assay compounds for the organic oxygen radicals. We generated the oxygen radicals in question, RO· and ROO·, by alternative methods:

(i) Alkoxy radicals, RO·, derived from *t*-BOOH and 13-LOOH, were generated by reactions with hydrated electrons (e_{aq}^-) in nitrogenated solutions with 10 mM *t*-butanol as ·OH scavenger:

$$ROOH + e_{aq}^- \longrightarrow RO^\cdot + OH^- \quad (5)$$

or supposedly by reaction with O_2^- (R.(2)) in oxygenated solutions containing 10 mM sodium formate.

(ii) The peroxy radical derived from *t*-butanol was prepared by the method in [14,15], used [13], in solutions saturated with gas mixtures of N_2O and O_2 (see R.(3) and (4)).

As detection substances we selected *p*-nitrosodimethylaniline, used [16] to evaluate the presence of ·OH radicals, and the water-soluble carotenoid crocin. Both substances are unreactive towards O_2^-, *p*-NDA according to [17] (see however fig.2b) and crocin as determined in our laboratory (Bors et al., submitted).

By comparing the bleaching of these compounds with e_{aq}^- and O_2^- proper, as well as with RO· generated by attack of e_{aq}^- (R.(5)) or O_2^- (R.(2)) on *t*-BOOH and 13-LOOH, we are able to demonstrate that O_2^- is unable to react with these organic hydroperoxides (fig.2a,b).

It is evident that all systems containing O_2^- show only a marginal bleaching, demonstrating the poor reactivity of O_2^- with the hydroperoxides as well as with the assay compounds. The non-reactivity of O_2^- with *p*-NDA [17] had to be qualified, however, as it was determined only for steady-state radiolysis. In pulse radiolysis a minor reversible bleaching is apparent for periods of <100 ms. The gradual reconstitution of the initial absorption in the case of *p*-NDA, furthermore, points to a dismutative decay of the radicals formed by the attack of RO· or ROO· at *p*-NDA. Taken together the data reveal some important aspects for the organic oxygen-centered radicals:

(1) Alkoxy radicals are more efficient in bleaching both compounds than the peroxy radical;

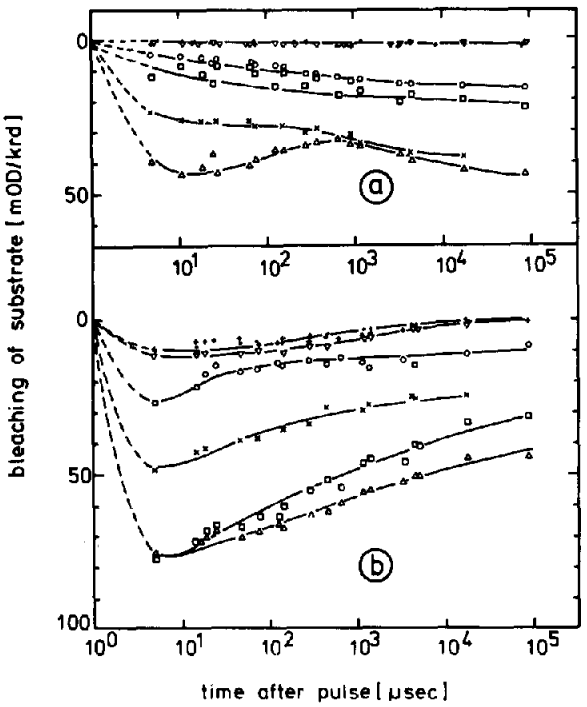

Fig.2. Kinetics of the bleaching of crocin (a) and *p*-nitrosodimethylaniline (b) after pulse-radiolytic generation of various radicals. Crocin was 10 μM, *p*-NDA 50 μM; both were observed at 440 nm in neutral solutions. Start of the reactions arbitrarily set at 1 μs after the pulse, first observation point at 5 μs. (△) 7.8 μM 13-LOOH, 10 mM *t*-butanol, N_2 (formation of 13-LO· from e^-_{aq}-attack); (×) 0.5 mM *t*-BOOH, 10 mM *t*-butanol, N_2 (formation of *t*-BO·); (□) 10 mM *t*-butanol, N_2 (e^-_{aq} alone); (○) 10 mM *t*-butanol, N_2O/O_2 gas mixtures (formation of *t*-BOO·). Solutions containing: (•) only O_2^- (10 mM $HCOO^-$, O_2): O_2^- alone; (▽) O_2^- in the presence of 7.8 μM 13-LOOH; (+) of 0.5 mM *t*-BOOH.

(2) The biologically relevant alkoxy radical derived from linoleic acid hydroperoxide (13-LO·) induces a higher initial bleaching than *t*-BO·. The complex kinetic behaviour of LO· with crocin (fig.2a) implies sequential reactions of LO· and crocin-derived radicals. The reaction is under further investigation.

(3) Crocin is a more specific assay for organic oxygen radicals as compared to *p*-NDA. The latter compound exhibited the highest bleaching rate with e^-_{aq} and an appreciable albeit reversible effect of O_2^-, which was not the case with crocin.

In conclusion we could show that:

(i) Organic alkoxy and peroxy radicals can be generated selectively by radiolytic methods;

(ii) O_2^- does not react with organic hydroperoxides, to the extent that supposedly formed alkoxy radicals could not be detected by sensitive assay compounds, such as *p*-NDA and crocin.

The latter finding does not support the proposal [4,5] that O_2^- reacts with organic hydroperoxides and, that the reaction of O_2^- with pre-formed LOOH is responsible for the lipid peroxidation, initiated by xanthine oxidase [5]. Yet it corroborates the data [18] questioning this reaction in aprotic solvents, thereby contradicting the proposal in [19]. It also supports our contention of the generally poor reactivity of O_2^- and points out the unspecificity of xanthine oxidase as 'exclusive' source of O_2^-, as presumably other species' respective reactions were causing the effect observed in [4,5]. In addition it highlights the advantage of using highly specific radiolytic sources of radicals.

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