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First Direct Observation of Reversible Oxygen Addition to a Carotenoid-Derived Carbon-Centered Neutral Radical

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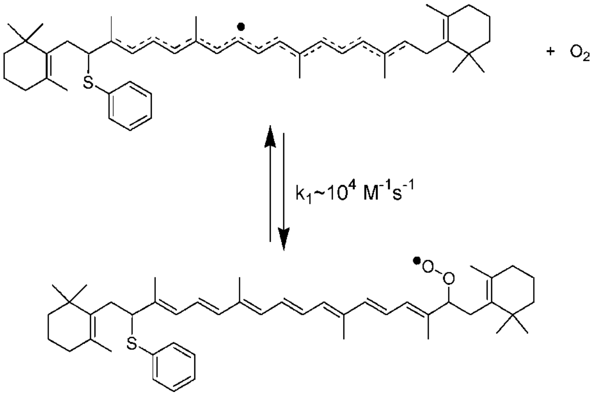
ABSTRACT



Direct observation of reversible oxygen addition to a carotenoid-derived carbon-centered neutral radical is reported for the first time. The influence of temperature on the observed reaction kinetics has been used to obtain kinetic and thermodynamic parameters relating to the reversible addition of oxygen to the carotenoid radical obtained from reaction of 7,7′-dihydro-*β*-carotene (77DH) with phenylthiyl radical ($PhS^•$) in benzene. In addition, the rate constant for oxygen addition to the equivalent *β*-carotene (*β*-CAR) derived radical is also reported.

The reactions of carbon-centered free radicals with oxygen have been the subject of numerous studies,[1] and an understanding of such reactions is important for elucidation of the mechanisms of free-radical-mediated oxidations in biological and polymer systems, as well as for understanding the effectiveness of the protection against such oxidations afforded by radical-scavenging chain-breaking antioxidants.

Recent work by Scaiano,[2] and others[3] has highlighted various structural influences on the reactivity toward oxygen of a number of radicals derived from synthetic organic antioxidants. In addition, Porter et al.[4] have reported the results of detailed experimental and theoretical studies of the kinetics of lipid peroxidation, where it is clear that the dynamics of the reversible addition of oxygen to conjugated lipid-derived carbon-centered radicals is important in accounting for peroxidation rates and product distributions.

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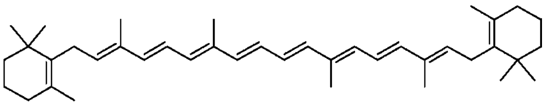
In 1984, Burton and Ingold[5] suggested that β-CAR scavenges peroxyl radicals by addition to the conjugated system of double bonds, yielding a resonance-stabilized carbon-centered radical ($\mathrm{ROOCAR}^{\bullet}$). They presented evidence that β-CAR functions as a very effective chain-breaking antioxidant, but as oxygen pressure is increased the effectiveness of β-CAR as an antioxidant is reduced. The reversible reaction of resonance-stabilized radicals (e.g., $\mathrm{ROOCAR}^{\bullet}$) with oxygen to generate potential chain-carrying peroxyl radicals ($\mathrm{ROOCAROO}^{\bullet}$) was postulated (Scheme S1, Supporting Information).

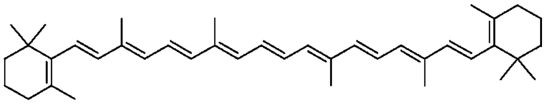
Effective chain-breaking antioxidants (e.g., α-tocopherol) are characterized by potent radical-scavenging properties coupled with poor chain-carrying properties of the antioxidant-derived radical that reflect its lack of reactivity toward oxygen.[6] The radical scavenging properties of carotenoids in the context of their antioxidant properties have been the subject of numerous studies.[7] However, the reaction of carotenoid-derived carbon-centered neutral radicals (e.g., $\mathrm{ROOCAR}^{\bullet}$) with oxygen has not previously been directly observed. However, reported rate constants for the reaction of oleate ($n = 1$) and linoleate ($n = 2$) radicals with oxygen are 1 and $0.3 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, respectively, suggesting that the rate constant for the reaction with oxygen decreases as the number (n) of conjugated double bonds in the free radical increases.[1b]

Previously we studied the influence of oxygen on the decay of carotenoid-derived carbon-centered neutral radicals generated by reaction of the carotenoid with acylperoxyl radicals.[7a] However, the carotenoid-derived radicals formed in such reactions undergo unimolecular $\mathrm{S_H}$i (epoxidation) processes with rate constants around $4 \times 10^3\ \mathrm{s}^{-1}$, and we were not able to detect any influence of oxygen on the decay of such radicals using oxygen concentrations up to $\sim 10^{-2}$ M, suggesting that rate constants for reaction with oxygen do not exceed $10^5\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ for these carotenoid addition radicals.

In this letter we report the results of laser flash photolysis experiments in which we have studied the influence of oxygen on the decay rates of carotenoid-derived carbon-centered neutral radicals generated from reaction of the carotenoids with phenylthiyl radicals ($\mathrm{PhS}^{\bullet}$) in benzene. Such carotenoid radicals do not undergo $\mathrm{S_H}$i decay and thus are much longer lived, lasting tens to hundreds of milliseconds rather than hundreds of microseconds as in the case of carotenoid radicals derived from acylperoxyl radical addition to carotenoids. The carotenoids studied are shown in Figure 1. Studies of the addition reactions of thiyl radicals with carotenoids have been reported previously; however, the influence of oxygen concentration on the decay kinetics of the addition radicals has not been investigated.[8]



7,7'-Dihydro-β-carotene (77DH)



β-Carotene (β-CAR)

Figure 1. Structures of carotenoids used in this study.

Laser photolysis (355 nm) of phenyl disulfide (PhS-SPh) in benzene forms phenylthiyl radicals ($\mathrm{PhS}^{\bullet}$), which exhibit a broad absorption band in the 400−500 nm region and decay by second-order kinetics (see Figures S1 and S2 and eq 1) with a first half-life of $\sim 50\ \mu$s at room temperature under our experimental conditions (i.e., for a laser energy of ~ 1 mJ). The decay of $\mathrm{PhS}^{\bullet}$ is not influenced by oxygen[9] in the range of oxygen concentrations used in this study (up to ~ 0.01 M).

$$\mathrm{PhS{-}SPh} \xrightleftharpoons{h\nu} 2\ \mathrm{PhS}^{\bullet} \qquad (1)$$

Laser photolysis (355 nm) of benzene solutions of ca. 10^{-3}−10^{-2} M PhS-SPh in the presence of $\sim 2 \times 10^{-5}$ M 77DH leads to the formation (on a microsecond time scale) of an exceptionally intense transient visible absorption band around 470 nm (Figure S3) attributed to $\mathrm{PhS\text{-}77DH}^{\bullet}$, which is spectrally similar to the transient absorption band observed for addition radicals formed in the reaction of 77DH with acylperoxyl radicals (465 nm in benzene),[7a] suggesting a common site of attack for acylperoxyl and phenylthiyl radicals. Although a number of addition sites are possible for $\mathrm{PhS}^{\bullet}$ addition to carotenoids, terminal addition is likely to be favored as a result of the extensive conjugation within

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the resulting resonance-stabilized radical.[10] No transient absorption features were observed at longer wavelengths in the visible or near-infrared regions. The rate constant for the reaction of PhS$^\bullet$ with 77DH was determined to be 1.32 ± 0.06 × 10^9 M^{-1} s^{-1} (Figure S3 inset). Rate constants for the addition of PhS$^\bullet$ to alkenes and conjugated dienes ranging from ~10^3 to 10^7 M^{-1} s^{-1} have been reported previously.[9]

Under our experimental conditions, in the absence of oxygen, the transient absorption signal at 470 nm decays over hundreds of milliseconds by second-order kinetics, and its decay is relatively insensitive to temperature (Figure S4). In the presence of oxygen at room temperature, the rate of decay of PhS-77DH$^\bullet$ increases (Figure 2) and follows pseudo-first-order kinetics.

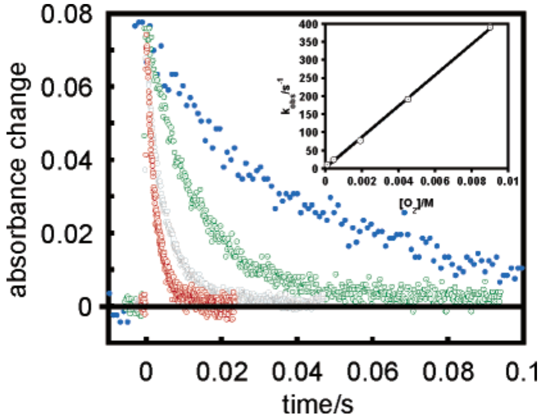


Figure 2. Kinetic absorption profiles for the decay of PhS-77DH$^\bullet$ at 470 nm in benzene at various oxygen concentrations (5%, 21%, 50%, 100%) formed following 355 nm laser photolysis (laser energy ~1.2 mJ) of PhS-SPh (~8 × 10^{-3} M) in the presence of 77DH (~2 × 10^{-5} M) in benzene. The amplitudes of the traces have been normalized for clarity. The inset shows a plot of the room-temperature pseudo-first-order rate constant (k_{obs}) for the decay of PhS-77DH$^\bullet$ (at 470 nm) in benzene versus the oxygen concentration. The intercept of the plot is 1.3 ± 3.3 s^{-1}.

Stern−Volmer analysis of the variation of k_{obs} with $[O_2]$ leads to a rate constant (k_1) for oxygen addition of 4.3 ± 0.07 × 10^4 M^{-1} s^{-1} (Figure 2 inset), which is consistent with the upper limit predicted from our previous studies of addition radicals formed by reactions of carotenoids with acylperoxyl radicals.[7a]

At room temperature there is no clear evidence that the reaction with oxygen is reversible under our experimental conditions, although a small residual baseline offset that is oxygen-concentration-dependent is evident on close inspection (Figure 2).

However, at elevated temperatures and constant oxygen concentration,[11,12] the kinetic profile of the addition radical decay is altered and changes from monoexponential to biexponential (Figures 3 and S5). This behavior is interpreted in terms of the system approaching equilibrium (the first exponential) with the second exponential component representing the decay of the system at equilibrium (Scheme 1).

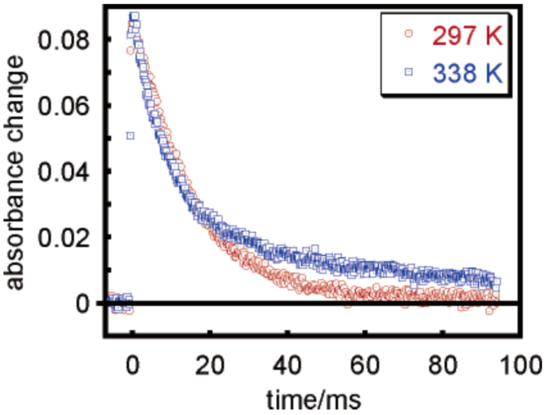
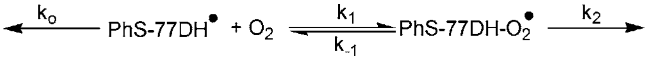


Figure 3. The influence of temperature on the normalized transient profiles, at 470 nm, of PhS-77DH$^\bullet$ following 355 nm laser photolysis (laser energy = 1.3 mJ) of PhS-SPh (~4 × 10^{-3} M) in the presence of 77DH (~2 × 10^{-5} M), in air-saturated benzene solution.

Scheme 1



$$\xleftarrow{k_o} \text{PhS-77DH}^\bullet + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{PhS-77DH-}O_2^\bullet \xrightarrow{k_2}$$

The amplitude of the second exponential increases with increasing temperature, and this is consistent with an equilibrium that has an exothermic forward reaction (Table S1) and thus indicates that the rate constant for the reverse reaction has a larger activation energy than the forward reaction and is therefore more sensitive to temperature. This behavior has been observed previously for reversible oxygen addition to pentadienyl radicals[13] and 1-methylallyl radicals[14] in the gas phase and interpreted along similar lines. The reverse reaction is proposed to be a β-fragmentation. Such reactions in lipid peroxyl radicals have been studied extensively by Porter et al.,[4,15−18] who has shown that oxygen off rates (β-fragmentation) are much more rapid for peroxyl

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radicals derived from nonterminal oxygen addition than for those derived from terminal oxygen addition. We speculate that the reaction we observe results from terminal oxygen addition at either end of the resonance-stabilized addition radical (Figure S6).

Kinetic fitting of the absorption profiles at different temperatures to a biexponential function (eq 2) yields parameters A_1, A_2, $k_{\mathrm{obs}(1)}$ and $k_{\mathrm{obs}(2)}$:

$$A_t = A_1\,\mathrm{e}^{-k_{\mathrm{obs}(1)}t} + A_2\,\mathrm{e}^{-k_{\mathrm{obs}(2)}t} \tag{2}$$

Assuming only PhS-77DH$^\bullet$ absorbs at the monitoring wavelength (470 nm), then $A_1 \propto [\mathrm{PhS\text{-}77DH{-}OO^\bullet}]_\mathrm{e}$ and $A_2 \propto [\mathrm{PhS\text{-}77DH^\bullet}]_\mathrm{e}$. Thus the equilibrium constant K is given by

$$K = \frac{[\mathrm{PhS} - 77\mathrm{DH} - \mathrm{O_2}^\bullet]_\mathrm{e}}{[\mathrm{PhS} - 77\mathrm{DH}^\bullet]_\mathrm{e}[O_2]_\mathrm{e}} = \frac{A_1}{A_2[\mathrm{O_2}]_\mathrm{e}} = \frac{k_1}{k_{-1}} \tag{3}$$

The first exponential component represents the approach to equilibrium where the observed rate constant ($k_{\mathrm{obs}(1)}$) has been taken as

$$k_{\mathrm{obs}(1)} = k_1[\mathrm{O_2}] + k_{-1} \tag{4}$$

The second exponential component represents the decay of the system at equilibrium and reflects the slow intrinsic decays of PhS-77DH-OO$^\bullet$ and PhS-77DH$^\bullet$.

Using the van't Hoff equation (eq 5), the standard molar enthalpy change ($\Delta H°$) and the standard molar entropy change ($\Delta S°$) for the reaction were calculated as -53.9 ± 1.6 kJ and -99.6 ± 5.0 J K^{-1} mol^{-1}, respectively (Figure S7). Porter's group[4a] suggest that C-OO BDEs approach a limiting value of ~44 kJ mol^{-1} for peroxyl radicals with conjugated π systems and the value of $\Delta H°$ we obtain from the van't Hoff plot is consistent with this.

$$\ln K = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} \tag{5}$$

From eqs 3 and 4, the values of k_1 and k_{-1} can be calculated, and these are given in Table S1 for the different temperatures used.

Using the empirical relationship between log k_β and BDE reported by Pratt et al.,[4a] the β-fragmentation rate constant for peroxyl radicals bearing only extended π systems is predicted to approach 10^5 s^{-1}, suggesting a small equilibrium constant ($K = k_1/k_{-1}$ with $k_{-1} \geq k_1$) with the position of equilibrium lying heavily on the side of the carbon-centered radical under our experimental conditions. However, the β-fragmentation rate constant we have estimated is several orders of magnitude smaller than that predicted by this empirical relationship.

From an Arrhenius plot for the rate constant (k_{-1}) of the β-fragmentation reaction, the activation energy ($E_{\mathrm{a}-1}$), was calculated as 57.4 ± 4.0 kJ mol^{-1} (Figure S8). Therefore, the activation energy for oxygen addition to PhS-77DH$^\bullet$ ($E_{\mathrm{a}1}$) is determined to be 3.6 ± 4.3 kJ mol^{-1}, and this small value is consistent with the observed temperature insensitivity of the forward reaction.

Laser (355 nm) photolysis of benzene solutions of PhS-SPh in the presence of $\sim 3 \times 10^{-5}$ M β-CAR leads to the formation of a transient absorption band around 540 nm (Figure S9), which is much weaker than that observed for 77DH and which decays on millisecond time scales under our experimental conditions (laser intensities were used that were higher than those used for 77DH studies because the transient absorption band is weaker (Figures S9 and S10). This absorption band is attributed to the addition radical PhS-β-CAR$^\bullet$. The decay of PhS-β-CAR$^\bullet$ is oxygen-concentration-dependent, and the observed rate constant for the reaction with oxygen, obtained in the same manner as for 77DH (Figure S10 inset), is $0.64 \pm 0.09 \times 10^4$ M^{-1} s^{-1}, which is smaller by a factor of ~7 than the value for PhS-77DH$^\bullet$. This may reflect the increased conjugated chain length in PhS-β-CAR$^\bullet$ compared with PhS-77DH$^\bullet$.

In conclusion, the results reported in this letter show, for the first time, direct evidence for the reversible addition of oxygen to carotenoid-derived carbon-centered neutral radicals. For these systems, the rate constant for oxygen addition is much lower than for shorter resonance-stabilized radicals such as 1-methylallyl[14] and pentadienyl.[13] We are currently extending these studies to the investigation of oxygen addition to radicals derived from other carotenoids.

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Supporting Information Available: Transient absorption spectra for PhS$^\bullet$ and PhS-β-CAR$^\bullet$. Kinetic absorption profiles for PhS$^\bullet$ and PhS-77DH$^\bullet$ at different temperatures and for PhS-β-CAR$^\bullet$ at various oxygen concentrations. Arrhenius plot for β-fragmentation of PhS-77DH-OO$^\bullet$. Reaction scheme and experimental data for temperature-dependent measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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