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Original Contribution

Peroxyl radical reactions with carotenoids in microemulsions: Influence of microemulsion composition and the nature of peroxyl radical precursor

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ABSTRACT

The reactions of acetylperoxyl radicals with different carotenoids (7,7′-dihydro-β-carotene and ζ-carotene) in SDS and CTAC microemulsions of different compositions were investigated using laser flash photolysis (LFP) coupled with kinetic absorption spectroscopy. The primary objective of this study was to explore the influence of microemulsion composition and the type of surfactant used on the yields and kinetics of various transients formed from the reaction of acetylperoxyl radicals with carotenoids. Also, the influence of the site (hydrocarbon phases or aqueous phase) of generation of the peroxyl radical precursor was examined by using 4-acetyl-4-phenylpiperidine hydrochloride (APPHCl) and 1,1-diphenylacetone (11DPA) as water-soluble and lipid-soluble peroxyl radical precursors, respectively. LFP of peroxyl radical precursors with 7,7′-dihydro-β-carotene (77DH) in different microemulsions gives rise to the formation of three distinct transients namely addition radical (λ_{max}=460 nm), near infrared transient1 (NIR, λ_{max}=700 nm) and 7,7′-dihydro-β-carotene radical cation ($77DH^{\bullet +}$, λ_{max}=770 nm). In addition, for ζ-carotene (ZETA) two transients (near infrared transient1 (NIR1, λ_{max}=660 nm) and ζ-carotene radical cation ($ZETA^{\bullet +}$, λ_{max}=730–740 nm)) are generated following LFP of peroxyl radical precursors in the presence of ζ-carotene (ZETA) in different microemulsions. The results show that the composition of the microemulsion strongly influences the observed yield and kinetics of the transients formed from the reactions of peroxyl radicals (acetylperoxyl radicals) with carotenoids (77DH and ZETA). Also, the type of surfactant used in the microemulsions influences the yield of the transients formed. The dependence of the transient yields and kinetics on microemulsion composition (or the type of surfactant used in the microemulsion) can be attributed to the change of the polarity of the microenvironment of the carotenoid. Furthermore, the nature of the peroxyl radical precursor used (water-soluble or lipid-soluble peroxyl radical precursors) has little influence on the yields and kinetics of the transients formed from the reaction of peroxyl radicals with carotenoids. In the context of the interest in carotenoids as radical scavenging antioxidants, the fates of the addition radicals (formed from the reaction of carotenoid with peroxyl radicals) and carotenoid radical cations are discussed.

1. Introduction

Many studies have been reported about the free radical reactions with carotenoids in organic solvents. These studies have shown that carotenoids can efficiently scavenge different types of radicals [1–10]. The mechanism of the reaction depends on the type of reacting radical, the carotenoid and the solvent [1,2,5,8,9,11–15]. For example, in non-polar solvents, carotenoids (CAR) can scavenge peroxyl radicals ($ROO^\bullet$) *via* addition and/or hydrogen abstraction pathways (Eqs. (2) and (3)) [4–7]. However, in polar solvents, the reaction can proceed *via* addition, hydrogen abstraction and electron-transfer pathways (Eqs. (1)–(3)) depending on the type of the reacting free radical and the carotenoid [1–3,5,16,17].

$$CAR + ROO^\bullet \rightarrow CAR^{\bullet +} + ROO^- \quad \text{Electron transfer} \tag{1}$$

$$CAR + ROO^\bullet \rightarrow CAR^\bullet + ROOH \quad \text{Hydrogen abstraction} \tag{2}$$

$$CAR + ROO^\bullet \rightarrow ROOCAR^\bullet \quad \text{Addition} \tag{3}$$

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Time-resolved peroxyl radical studies with carotenoids in micelles and microemulsions are very limited [18,19] although they can be used as simple models for cellular membranes [20–22]. Microemulsions are composed of water, oil (usually a hydrocarbon solvent such as cyclohexane), a cofactor (*e.g.* an alcohol with a relatively long hydrocarbon chain such as 1-pentanol) and surfactant. Microemulsions can be classified according to the water to oil ratio into (1) water-in-oil (W/O) (2) oil-in-water (O/W). However, at intermediate ratios of water to oil, bicontinuous structures are formed where water and oil channels are separated by a monolayer of surfactant molecules [21]. Earlier studies have shown the schematic representations for W/O, O/W and bicontinuous microemulsions [21,23]. Unlike micelles, microemulsions have the advantage of the ability to dissolve large amounts of hydrophobic substances and to accommodate long molecules due to the presence of the oil and cofactor [24].

Hill et al. studied the reactions of trichloromethylperoxyl radicals with different carotenoids in 2% TX-100 micelles [18]. The reaction gives rise to near infrared (NIR) absorbing species (NIR1) and carotenoid radical cation ($CAR^{\bullet +}$), which absorbs at longer wavelengths than that of NIR1. Subsequently, the NIR1 transient decays slowly to form more $CAR^{\bullet +}$ (Scheme 1). In addition, using pulse radiolysis, the reactions of retinoids with trichloromethylperoxyl radicals in Triton X-100 micelles gave rise to the formation of at least two transient products [19b]. One of them was identified as the corresponding retinoid cation radical ($\lambda_{max} \approx 590$ nm). Furthermore, there is only one study, using pulse radiolysis, for the reaction of peroxyl radicals with carotenoids in microemulsions. Adhikari et al. [19a] reported similar reaction mechanisms to that described by Hill (see Scheme 1) for the reactions of halogenated peroxyl radicals with β-carotene (β-CAR) in microemulsions (sodium lauryl sulfate (3.23% w/v), cyclohexane (75% v/v), water (6.45% v/v) and 1-pentanol (15.32% v/v)) (Fig. 1).

In the work reported here, the influence of microemulsion composition on the reactions of acetylperoxyl radicals with 77DH and ZETA (see Fig. 1) and of the resulting intermediates has been investigated to explore the influence of microenvironment polarity on the transients formed. Also, water-soluble and lipid-soluble peroxyl radical precursors have been used in order to examine the influence of the site of peroxyl radical generation [25]. The carotenoids used in this study (77DH and ZETA) are selected because their radical spectra are well-resolved. Also, the different compositions of microemulsions used in this study and the pseudoternary phase diagram for the four components (SDS, 1-butanol, water and cyclohexane) have been reported by Martinez et al. [21].

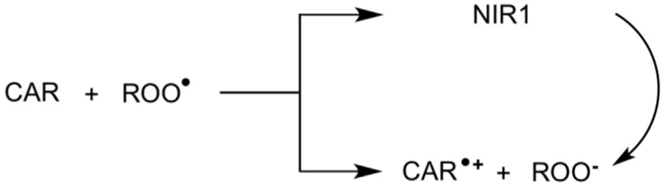
2. Materials and methods

2.1. Materials

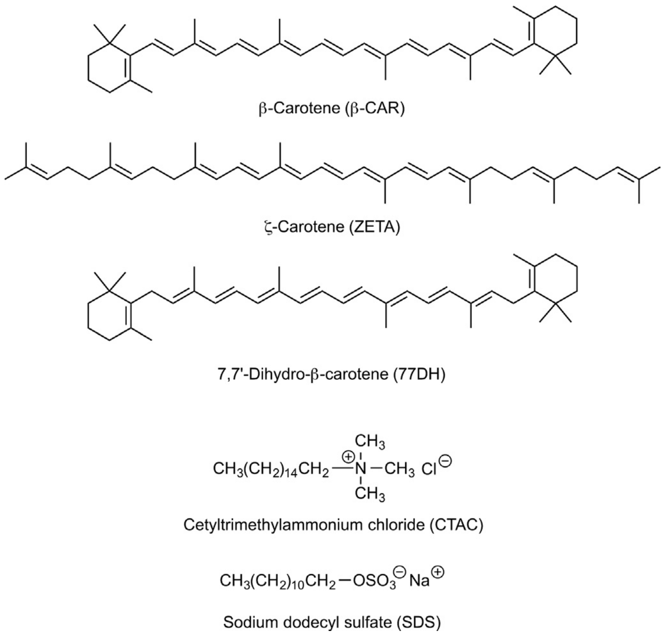
1-Butanol (BDH), sodium dodecyl sulfate (BDH), cyclohexane (Aldrich, HPLC grade), 4-acetyl-4-phenylpiperidine hydrochloride (Aldrich), benzene (sigma-Aldrich, spectroscopic grade), 1, 1-diphenylacetone (Lancaster), and cetyltrimethylammonium chloride (Acros) were used as received. The carotenoids, 7,7′-dihydro-β-carotene (77DH) and ζ-carotene (ZETA), were kindly supplied by DSM Nutritional Products and used as received. For microemulsion solutions, ultra-pure water (conductivity < 0.067 μS/cm, Elga UK) was used.

2.2. Laser flash photolysis

The details of the laser flash photolysis system have been described previously [26a,b]. Unless otherwise stated, 266 nm laser energies were in the range 2–4 mJ pulse^{-1} with a beam diameter of ~4 mm. Quartz sample cells (2 mm excitation pathlength × 10 mm monitoring pathlength) fitted with vacuum taps (where necessary) were employed for the laser flash photolysis measurements. If necessary (for example, during acquisition of transient absorption spectra), fresh solution was introduced into the sample cell following each exposure to the laser.



Scheme 1. Pathways proposed for the reaction of $CCl_3O_2^{\bullet}$ with different carotenoids [18].



$CH_3(CH_2)_{14}CH_2-\overset{\oplus}{N}(CH_3)_3\ Cl^{\ominus}$

$CH_3(CH_2)_{10}CH_2-OSO_3^{\ominus}Na^{\oplus}$

Fig. 1. Structures of the carotenoids and surfactants.

2.3. Preparation of microemulsions

Stock solutions of carotenoids and peroxyl radical precursors were prepared in benzene and methanol respectively. Then volumes containing the required carotenoid and peroxyl radical precursor concentrations were evaporated using a rotary evaporator. The film of carotenoid and peroxyl radical precursor was dissolved in 1-butanol and cyclohexane (calculated as weight percentage of microemulsion composition, see Table 1).

Table 1
Composition (as wt%) of different microemulsions used in this study [21].

Microemulsion	Surfactant[a]	BU[a,b]	W[b]	CH[b]
A (W/O)	8	16	7	69
B[c]	10	20	23	47
C[c]	10	20	39	31
D[c]	10	20	55	15
E (O/W)	8	16	72	4

[a] The ratio of surfactant to BU in different microemulsion is 1:2.
[b] BU = 1-butanol; W = water; CH = cyclohexane.
[c] The microemulsions B, C and D are bicontinuous microemulsions.