

One-electron reduction potentials of dietary carotenoid radical cations in aqueous micellar environments

Marc Burke^a, Ruth Edge^a, Edward J. Land^{b,1}, David J. McGarvey^{a,*}, T. George Truscott^a

^a*School of Chemistry and Physics, Lennard-Jones Laboratories, Keele University, Keele, Staffordshire ST5 5BG, UK*

^b*CRC Drug Development Group, Paterson Institute for Cancer Research, Christie Hospital, NHS Trust, Manchester M20 4BX, UK*

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Abstract The one-electron reduction potentials of the radical cations of five dietary carotenoids (β -carotene, canthaxanthin, zeaxanthin, astaxanthin and lycopene) in aqueous micellar environments have been obtained from a pulse radiolysis study of electron transfer between the carotenoids and tryptophan radical cations as a function of pH, and lie in the range of 980–1060 mV. These values are consistent with our observation that the carotenoid radical cations oxidise tyrosine and cysteine. The decays of the carotenoid radical cations in the absence of added reactants suggest a distribution of exponential lifetimes. The radicals persist for up to about 1 s, depending on the medium. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Dietary carotenoid; Radical cation; One-electron reduction potential; β -Carotene; Lycopene; Tryptophan

1. Introduction

The chemistry of carotenoid/radical reactions is generating wide current interest. In photosynthesis, there is much debate on the role of β -carotene in the reaction centres as an electron carrier [1–5]. Furthermore, several dietary carotenoids may act as radical scavengers in vivo and hence their radical chemistry may be linked to disease prevention. Numerous epidemiological studies have suggested a link between diets rich in carotenoids and a lower incidence of several serious diseases including cancer, atherosclerosis, age-related blindness and even ageing itself [6–8]. However, the situation has changed somewhat due to recent epidemiological findings that have shown no health benefits from dietary supplementation with β -carotene, and even a possible deleterious effect in some sub-populations, such as heavy smokers [9,10]. These sub-groups are often deficient in anti-oxidants such as ascorbic acid and this has led to an interest in the interactions between carotenoids and such anti-oxidants. Indeed, we have shown a synergistic protection of human lymphocytes from damage by the nitrogen dioxide radical and the peroxyxynitrite anion by combinations of carotenoids with such anti-oxidants [11].

Previous studies by ourselves [12] and others [13–17] have established the relative order of the reduction potentials of several carotenoid radical cations in organic solvents and lipo-

somes. In this paper we report the results of studies of the one-electron transfer between five carotenoids and tryptophan radicals as a function of pH, which have allowed us to estimate absolute one-electron reduction potentials of the carotenoid radical cations in an aqueous micellar environment. In addition, the results of studies of the reactions of carotenoids with the radical obtained by one-electron oxidation of the dipeptide L-tryptophyl-L-tyrosine as a function of pH [18,19] are reported and these support the reduction potentials obtained.

2. Materials and methods

The carotenoids were supplied by Hoffmann-La Roche (Basle, Switzerland) and used as supplied, all were found to be >99% pure by high-performance liquid chromatography. Tryptophan (TrpH) and tyrosine (TyrOH) from Fluka and cysteine (CySH) and the dipeptide L-tryptophyl-L-tyrosine (TrpH-TyrOH) from Sigma were purified by recrystallisation from ethanol. A long-wavelength 'tail' in the absorption spectrum of TrpH showed evidence of impurities, which were removed by recrystallisation from ethanol. Water was doubly distilled over alkaline permanganate. Triton X-100 (TX-100) and 405 (TX-405), sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) were obtained from Aldrich, BDH and Sigma, respectively. Nitrous oxide (N_2O) was obtained from the British Oxygen Company, potassium bromide from Sigma, and components for phosphate buffer from BDH. All pH measurements were made on a PHM 82 (Radiometer) meter. Incorporation of carotenoids into Triton X micelles was achieved by dissolving the carotenoid in benzene and then mixing this solution with neat TX-100. Rotary evaporation at 40°C under reduced pressure was used to remove the benzene, leaving a detergent-carotenoid film on the walls of the flask. Water (or 0.01 M phosphate buffer) was then added such that the TX-100 concentration was 2% w/v. For mixed TX-100/TX-405 micelles the procedure was the same except that the detergent concentrations were 3% w/v TX-405 and 1% w/v TX-100. For SDS and CTAB micelles a solution of the carotenoid in benzene was added to a 2% w/v aqueous solution of the detergent and the benzene removed by rotary evaporation at reduced pressure and then at high vacuum. The pH was adjusted by addition of small quantities of concentrated HCl or NaOH.

Pulse radiolysis measurements were made using a 9–12 MeV Vickers linear accelerator, as described previously [20], with pulses of 10–100 ns duration and doses of 1–10 Gy. Quartz flow-through cells were used with a monitoring optical path length of 2.5 cm. Absorption spectra were recorded using a Perkin-Elmer Lambda-2 UV/Vis spectrophotometer. The structures of the carotenoids and the amino acid derivatives are given in Fig. 1.

3. Results

Pulse radiolysis, coupled with UV-NIR (ultraviolet near-infrared) transient absorption detection, of aqueous N_2O -saturated micellar solutions of 10 mM TrpH containing 0.1 M KBr leads to the formation of the one-electron oxidised TrpH

*Corresponding author. Fax: (44)-1782-712378.
E-mail: d.j.mcgarvey@chem.keele.ac.uk

¹ Senior Research Fellow, Keele University.

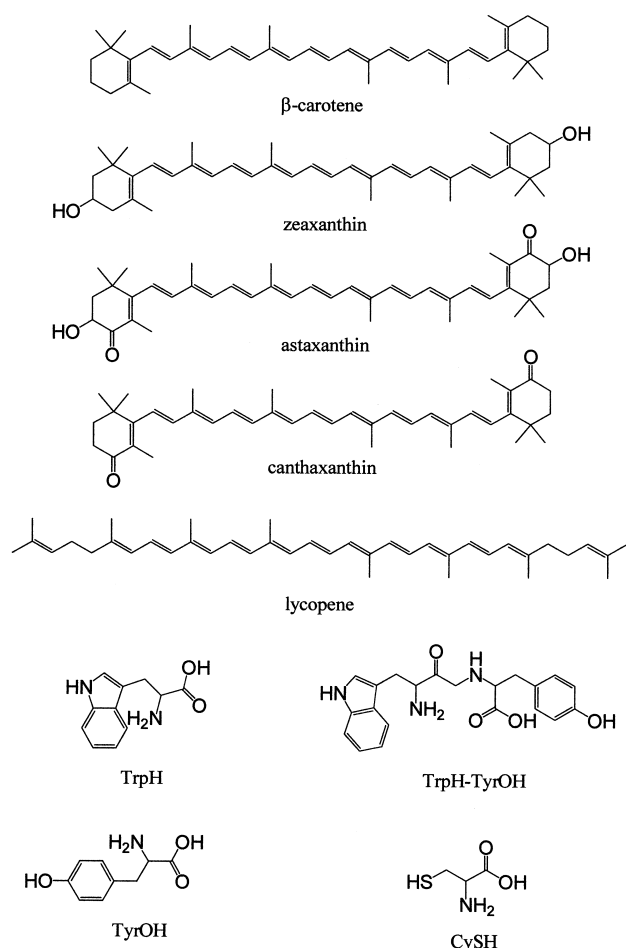


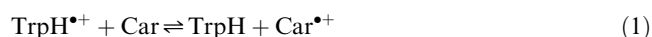
Fig. 1. Structures of the carotenoids and amino acid derivatives.

radicals from the reaction of TrpH with $\text{Br}_2^{\cdot-}$. For the results reported in Fig. 2, the concentration ratio TrpH/carotenoid was always greater than 100 so that virtually no carotenoid radical is generated via direct reaction with the $\text{Br}_2^{\cdot-}$. When the micelles contain carotenoids (TrpH is water-soluble and so is predominantly in the aqueous phase), the TrpH radical oxidises the carotenoids to their respective radical cations in a pH-dependent process.

The apparent relative equilibrium yields of the radical cations from the one-electron oxidation by $\text{TrpH}^{\cdot+}/\text{Trp}^{\cdot}$ in 2% aqueous (w/v) TX-100, and for β -carotene and lycopene, in a detergent mixture of 3% aqueous (w/v) TX-405 and 1% (w/v) TX-100 (lycopene is not sufficiently soluble in TX-100 alone) were obtained as a function of pH over the range of 2.0–7.0. In addition, the reaction of the one-electron oxidised TyrOH radical of the dipeptide TrpH–TyrOH with β -carotene in TX-100 was also studied over the same pH range. Fig. 2a shows the known variation in reduction potential [21,22] for TrpH, TyrOH and CySH as a function of pH. The results for electron transfer from the five carotenoids studied to one-electron oxidised TrpH (based on the relative equilibrium yield of the carotenoid radical cation measured) are shown in Fig. 2b–g (together with the theoretical relative concentration of the $\text{TrpH}^{\cdot+}$ compared to the deprotonated Trp^{\cdot} based on a $\text{p}K_a$ of 4.2). The corresponding results for β -carotene and the dipeptide are shown in Fig. 2h. Since, as discussed below, the

one-electron reduction potentials vary with pH when a hydrogen ion is involved in the half reaction, these electron transfer measurements allow an estimation of the absolute radical cation reduction potentials of the carotenoids in a micellar environment. In all cases, except for lycopene, the $\text{Car}^{\cdot+}$ is formed efficiently at pH 2.0 and not at all at pH 6.0, leading to a range of 1130 ± 50 mV for the carotenoid radical cation reduction potentials. For lycopene, there is some evidence of radical cation at pH 6.0 and therefore possibly its reduction potential is about 60 mV less.

Furthermore, studies of mixtures of 0.1 mM carotenoids, alone and in the presence of varying amounts of TrpH (< 20 mM), indicate the transient absorption due to the formation of $\text{Car}^{\cdot+}$ is consistent with equilibria of the type



being established, thus enabling equilibrium constants (K) to be measured. Typical values of K for β -carotene at pH 2.0 are 101, 130 and 116 for concentrations of TrpH of 5, 10 and 20 mM with a β -carotene concentration of 0.1 mM. From the equilibrium constants, the differences in reduction potential between TrpH and the carotenoid radical cations have been obtained (980 mV for lycopene and a mean of near 1040 mV for all the other carotenoids studied) at both pH 5.0 and 2.0 (and also at pH 4.0 for β -carotene). Table 1 shows the one-electron reduction potentials of the carotenoid radical cations, calculated using the well-established TrpH radical reduction potentials [21,22] (1135 and 1185 mV versus NHE at pH 5.0 and 2.0, respectively).

The radicals of TyrOH and CySH do not oxidise the five carotenoids studied in TX-100 or mixed micelles at pH 7.0. Indeed, using pulse radiolysis to generate the radical cation of the carotenoids, only the reverse reaction was observed, with rate constants of $\sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $\sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$, respectively, for TyrOH and CySH.

The inherent lifetimes of the carotenoid radical cations in a micellar environment are of importance in understanding their reactivity. In an attempt to gain information on this we have studied carotenoid radical cation lifetimes in three such environments: the neutral, positively and negatively charged micelles of TX-100 (mixed TX-405/TX-100 micelles for lycopene), CTAB and SDS, respectively. These decays were measured as a function of radiation dose. No effect of dose on the lifetime is observed suggesting no contribution from second-order processes. However, in all cases, the decays are not single exponentials. All the carotenoid radical cations display kinetics that probably represent a distribution of exponential decays, and the radicals persist for milliseconds to seconds in the environments studied. As an example, making the simplifying assumption that the decay kinetics follow two exponentials, lifetimes in TX-100 and CTAB of 7–16 ms and 2–6 ms, respectively, for the short-lived component, with 40–100 ms and 10–30 ms for the longer-lived component, respectively, were obtained. In SDS even longer lifetimes (up to 600 ms for canthaxanthin) arise from the biexponential approximation.

4. Discussion

We have used the one-electron oxidation of carotenoids by amino acid radicals to estimate the reduction potentials of

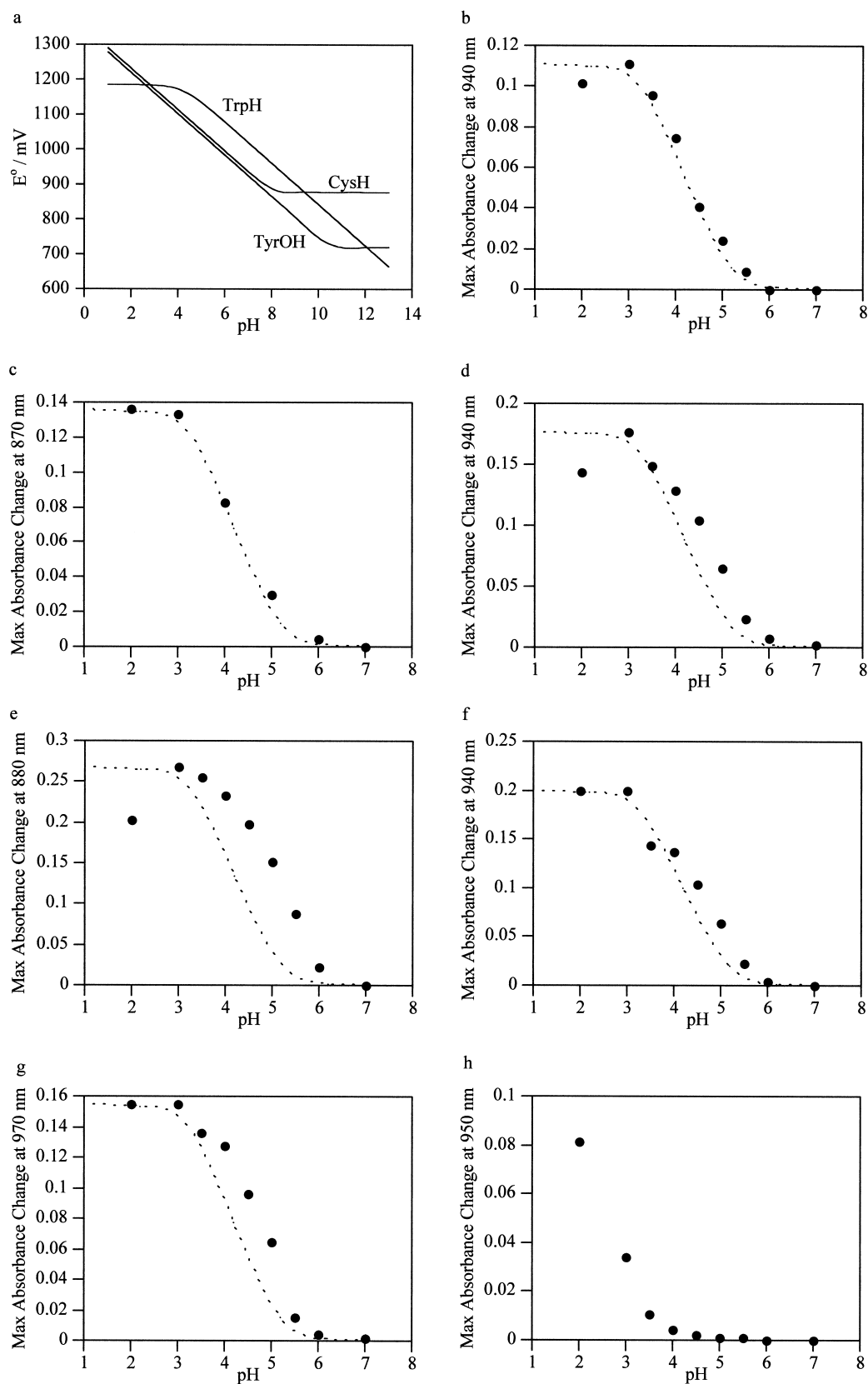


Fig. 2. Data associated with the measurement of reduction potentials of carotenoid radical cations in aqueous environments. a: Known reduction potentials of amino acids as a function of pH [21,22]. Yields of radical cation of carotenoids following oxidation by TrpH radicals in aqueous micellar solution: (b) β -carotene in TX-100, (c) canthaxanthin in TX-100, (d) zeaxanthin in TX-100, (e) astaxanthin in TX-100, (f) β -carotene in TX-100+TX-405, and (g) lycopene in TX-100+TX-405 with pK_a curves superimposed. h: Yield of β -carotene radical cation following oxidation by radicals from the dipeptide TrpH-TyrOH in TX-100 as a function of pH.

Table 1

One-electron reduction potentials for carotenoid radical cations, calculated from the equilibrium with TrpH^{*+}

Carotenoid radical cation	$E^0/\text{mV} \pm 25 \text{ mV}$
β -Carotene in TX-100	1060
Canthaxanthin in TX-100	1041
Zeaxanthin in TX-100	1031
Astaxanthin in TX-100	1030
β -Carotene in TX-405/TX-100	1028
Lycopene in TX-405/TX-100	980

carotenoid radical cations. The oxidised radical of TrpH may be TrpH^{*+} and/or neutral Trp^{\bullet} , depending on the pH. The reduction potential is independent of pH at low pH values (< 4.0), because no H^+ is involved in the half reaction ($\text{TrpH}^{*+} + e^- \rightleftharpoons \text{TrpH}$) and has the value 1185 mV (calculated from the data of Harriman [21]). However, at higher pH values, a proton is involved in the half reaction ($\text{Trp}^{\bullet} + \text{H}^+ + e^- \rightleftharpoons \text{TrpH}$), and the one-electron reduction potential varies by 59.1 mV for each unit of pH. This range for TrpH is from an E^0 of about 665 mV at pH 13.0 to 1185 mV at pH 4.0 (see Fig. 2a). Providing the reduction potential of a carotenoid radical cation is between these limits, it is possible to estimate the value of E^0 for $\text{Car}^{*+}/\text{Car}$, by monitoring the efficiency of electron transfer as a function of pH. As can be seen from Fig. 2, all of the carotenoid radical cations are efficiently produced from TrpH^{*+} , showing that the reduction potentials of the carotenoid radical cations are less than 1185 mV. Comparing the two hydrocarbon carotenoids, β -carotene and lycopene (Fig. 2f,g), there is evidence of more oxidation of the lycopene at pH 4.0–5.0 than from the TrpH^{*+} alone, i.e. some oxidation due to Trp^{\bullet} , suggesting that the reduction potential of the radical cation of lycopene is lower than that of β -carotene. This is in agreement with the relative values for these two hydrocarbon carotenoids reported previously [12–17].

From measurements of the equilibrium constants for the one-electron transfer processes between TrpH and carotenoids, the difference in reduction potential between TrpH^{*+} and the carotenoid radical cations is obtained. Using the established reduction potential for TrpH radicals (see Fig. 2a) leads to values of E^0 for each carotenoid, as presented in Table 1.

Fig. 2a also shows that in the pH range 3.0–12.0, the one-electron reduction potential of the Trp^{\bullet} radical is more positive than that of TyrO^{\bullet} . Thus, in order to confirm our estimates of the reduction potential for β -carotene, we attempted to study the one-electron oxidation by the radicals derived from TyrOH. Unfortunately, this experiment was not feasible due to the inefficient rate of oxidation of TyrOH compared to TrpH by $\text{Br}_2^{\bullet-}$ (relative rate constants 2×10^7 and $7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for TyrOH and TrpH, respectively) [23]. However, this problem was overcome by the study of the dipeptide TrpH–TyrOH. Here it is established that the initial radical product is the TrpH^{*+} moiety and, between pH 3.0 and 12.0, an intramolecular electron transfer occurs [18] so that the one-electron oxidised radical from the TyrOH moiety is formed. In the presence of β -carotene, the carotenoid radical cation is efficiently formed at pH 2.0, where the oxidising radical TrpH^{*+} has a reduction potential of 1185 mV. At pH 3.5 the amount of carotenoid radical cation formed is reduced and to a smaller extent, it can even be detected at pH 4.0. However, at

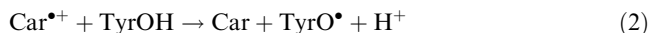
higher pH values, where only TyrOH phenoxyl radicals arise, there is no detectable carotenoid oxidation in agreement with the potential derived above.

As can be seen from Table 1, the E^0 values for all five carotenoids studied are similar, lying in the range 1020 ± 40 mV. Although in the present work, we cannot distinguish between the one-electron reduction potentials of all five carotenoids studied, in hexane they are distinct [12–15], as complete electron transfer between several pairs could be observed with rate constants of $> 10^9 \text{ M}^{-1} \text{ s}^{-1}$ [12]. These values of 1020 ± 40 mV are markedly higher than reported by Skibsted and co-workers [24] for the carotenoid glycoside crocin. These workers used an electrochemical method (cyclic voltammetry) and obtained a value of 700 mV in water.

In micelles, we propose that the terminal oxygenated substituent on the xanthophylls lies near to the micellar/water interface and interacts (e.g. via hydrogen bonding) with water, and that this interaction cannot arise with the hydrocarbon carotenoids. Indeed, Gabrielska and Gruszecki [25] have suggested an analogous explanation to understand the differing effects of zeaxanthin and β -carotene on membrane rigidity. He and Kispert [26] have also reported that it is not possible to oxidise β -carotene in TX-100 micelles by cyclic voltammetry, and propose that this is due to the hydrophobic barrier of the micelle.

The values we report here suggest that all the carotenoid radical cations studied are able to oxidise both TyrOH and CySH at physiological pH, where the reduction potentials of these two amino acids are quite close (see Fig. 2a).

A mean value of 1044 mV for the E^0 of β -carotene radical cation is just below that of P_{680}^{*+} (1100 mV) in the photosystem II (PSII) reaction centre, indicating that β -carotene may reduce P_{680}^{*+} and may, in turn, be reduced by TyrOH. As previously noted [27], the electron transfer processes in PSII involve P_{680}^{*+} reduction by TyrOH with the oxidised TyrOH being reduced by the manganese cluster. However, if such electron transfer reactions are blocked, various other processes may arise, e.g. Car^{*+} formation. The efficiency of electron transfer processes in the PSII reaction centre will depend on the rigid geometry imposed on the photosynthetic system. Our results suggest that it is possible that the primary molecule that reduces P_{680}^{*+} is β -carotene and the β -carotene radical cation thus formed is, in turn, reduced by TyrOH.



Of course, the detection of Car^{*+} within intact photosynthetic systems depends, amongst other factors, on the steady-state concentration of the Car^{*+} .

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