The reduction potential of the β-carotene•+/β-carotene couple in an aqueous micro-heterogeneous environment

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Abstract There is a resurgence of interest in the role of electron transfer reactions involving β-carotene in photosynthesis. There is also current debate on the health benefits of dietary carotenoids and the possible deleterious effects on certain sub-populations such as smokers. The impact of dietary carotenoids on health may well be also related to radical reactions. A key parameter in biological systems is therefore the one-electron reduction potential of the carotenoid radical cation, now reported for the first time in a model biological aqueous environment. The value obtained is 1.06 ± 0.01 V and is sufficiently high to oxidise cell membrane proteins, but is low enough to repair $P_{680}^{\bullet+}$ in the photosynthetic reaction centre.

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1. Introduction

There is considerable recent interest in the molecular mechanisms underpinning carotenoid/radical reactions. As well as the established photo-protective and antenna roles for carotenoids in photosynthesis, there has been much recent interest in electron transfer processes involving β-carotene. Hanley et al. (1999) [1] propose that β-carotene acts as an electron carrier between the most oxidising species in photosystem II (PS II) ($P_{680}^{\bullet+}$ with an $E_m \approx 1.1$ V) and monomer chlorophyll, and that the carotenoid may be a branch point in electron transfer from cytochrome b_{559} and monomer chlorophyll. Vrettos et al. (1999) [2] have observed the β-carotene radical cation in a manganese depleted *Synechocystis* PS II complex and speculate that only $P_{680}^{\bullet+}$ and not $P_{870}^{\bullet+}$ in bacterial reaction centres is capable of oxidising the carotenoid. In addition, there is current interest in the structure of PS II [3], and Moore et al. [4,5] have shown that photo-excitation of a triad model (carotenoid–porphyrin–quinone) of reaction centre complexes leads to charge separation and the formation of the carotenoid radical cation.

Deleterious [6,7] and protective effects [8] of dietary β-carotene may well also be related to electron transfer reactions and the subsequent reactivity of carotenoid radicals generated by such electron transfer reactions when β-carotene reacts with an oxy-radical. Previous electrochemical measurements in halogenated organic solvents have mainly measured the two-electron reduction potentials [9,10] and one of us [11] has reported a one-electron reduction potential for β-carotene via cyclic voltammetry in dichloromethane as solvent at 10°C. However, these electrochemical measurements are somewhat difficult to interpret and certainly do not refer to a biologically relevant environment. Furthermore, He and Kispert [12] have reported that it is not possible to oxidise β-carotene in TX-100 micelles by cyclic voltametry, and propose that this is due to the hydrophobic barrier of the micelle.

We now report, for the first time in a model biological aqueous environment, the one-electron reduction potential of the β-carotene radical cation. This was obtained using ns pulse radiolysis techniques coupled with kinetic, near infra-red absorption measurements, of the β-carotene radical cation reacting with amino acids. In the case of tryptophan a pH dependent equilibrium was established.

2. Materials and methods

β-Carotene was supplied by Hoffmann-la Roche (Basel) and used as supplied. TrpH and TyrOH were obtained from Fluka and cysteine was obtained from Sigma. A long wavelength 'tail' in the absorption spectrum of TrpH showed evidence of impurities which were removed by recrystallisation from ethanol. The water was doubly distilled over alkaline permanganate. The detergent, Triton X-100 was obtained from Sigma and the N_2O from British Oxygen Company.

Pulse radiolysis measurements were made using a 9–12 MeV Vickers linear accelerator as described previously [13,14] with pulses of 10–100 ns duration and doses of between 1 and 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 relative yield of the radical cation of β-carotene, following one-electron oxidation by tryptophan radicals in 2% aqueous (w/v) Triton X-100, was obtained as a function of pH over the range 2–7. The reactions between β-carotene radical cation and tyrosine and cysteine were monitored by observing the increased rate of decay of the carotenoid radical cation as a function of the amino acid concentration.

3. Results

The variations in the reduction potential for tryptophan, tyrosine and cysteine as a function of pH are well established [15–18]. The results for electron transfer from β-carotene to one-electron oxidised tryptophan (based on the relative absorbance at 950 nm of the carotene cation radical measured) are shown in Fig. 1, together with the theoretical concentration of $trpH^{\bullet+}$ compared with the deprotonated $trp^{\bullet}$.

These results suggest a value of near 1 V for the reduction

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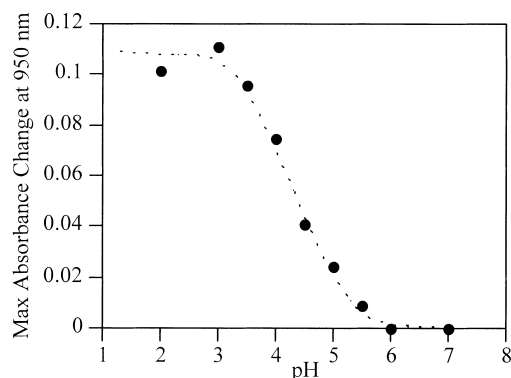
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Fig. 1. Relative yields, as a function of pH, of β-carotene radical cation produced via oxidation of β-carotene by tryptophan radicals, with the pK_a curve for tryptophan radicals superimposed. The tryptophan radicals are produced by pulse radiolysis of N_2O-saturated 10^{-2} M tryptophan, 10^{-4} M β-carotene and 10^{-1} M KBr in aqueous 2% TX-100 micellar solutions.

potential of the β-carotene radical cation. However, to obtain a more precise number we have used the pulsed techniques to determine the apparent equilibrium constant for:

$$trpH^{\bullet+} + \beta\text{-carotene} \rightleftharpoons trpH + \beta\text{-carotene}^{\bullet+}$$
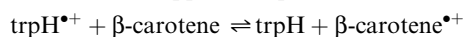
The values, determined at pH 2, 4 and 5, were found to be 120, 70 and 29 and using the Nernst equation, yields the difference in reduction potentials for tryptophan and β-carotene radical cations as 123, 109 and 86 mV, respectively. The established value for tryptophan [18] at pH 7 is 1020 mV, which, using the appropriate equilibrium constants, leads to values of 1185, 1173 and 1135 mV at pH 2, 4 and 5, respectively. This yields a value of E_m for β-carotene of 1.06 ± 0.01 V.

This value of E_m of 1.06 ± 0.01 V is very close to but just below that of the E_m of $P_{680}^{\bullet+}$ (1.1 V) in the PS II reaction centre, but is much too high for β-carotene to be involved in reducing the radical cation arising in PS I. It is generally accepted that the molecular electron transfer processes in PS II involve $P_{680}^{\bullet+}$ reduction by the tyrosine 161 of the D1 protein (known as tyr_z) with the oxidised tyr_z being reduced in its turn by electrons from the manganese cluster. Under conditions which block such tyr_z to $P_{680}^{\bullet+}$ electron transfer, various 'side-path' donation reactions may arise, e.g. involving $Car^{\bullet+}$ formation.

Our measurement of ≈ 1 V for the reduction potential of $Car^{\bullet+}$ suggests that $Car^{\bullet+}$ itself would be reduced by tyrosine and cysteine, we have confirmed this in micellar systems by observing a reaction between $Car^{\bullet+}$ and tyrosine and cysteine to regenerate the parent β-carotene e.g.:

$$Car^{\bullet+} + TyrOH \rightarrow Car + TyrO^{\bullet} + H^+$$
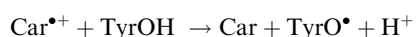
The efficiency of electron transfer processes in the PS II reaction centre will depend critically on the rigid geometry imposed on the system. Our results show that it is at least possible that the primary molecule which reduces $P_{680}^{\bullet+}$ is in fact β-carotene itself and the β-carotene radical cation thus formed is, in turn, reduced by tyrosine.

$$P_{680}^{\bullet+} + Car \rightarrow P_{680} + Car^{\bullet+} \tag{1}$$

$$Car^{\bullet+} + TyrOH \rightarrow Car + TyrO^{\bullet} + H^+ \tag{2}$$

Whether or not $Car^{\bullet+}$ would be detected in intact photosynthetic systems depends on the steady-state concentration of the $Car^{\bullet+}$ and, hence, on the relative rates of these two processes. Where $Car^{\bullet+}$ can be detected it may be that the geometry has been sufficiently disturbed such that process 2 is reduced in efficiency.

Turning to the role of dietary β-carotene as an anti- or pro-oxidant, the particularly high reduction potential value we obtained for $Car^{\bullet+}$ in an aqueous micellar system and the reaction with tyrosine noted above also with cysteine (approximately 100 times faster than with tyrosine) suggests that unless $Car^{\bullet+}$ is removed/repaired, (e.g. by vitamin C) protein damage could result. Hence our finding that E_m ($Car^{\bullet+}$/Car) is greater than that of tyrosine or cysteine at pH 7 does suggest a possible molecular mechanism for the deleterious effects of β-carotene dietary supplementation on smokers, who have particularly low serum ascorbic acid levels. Furthermore, in cell model experiments, using DPPC liposomes, we have shown that vitamin C efficiently repairs $Car^{\bullet+}$ even though the parent hydrocarbon carotenoid must be in the non-polar region of the liposomes (to be reported in detail elsewhere). Presumably, the more polar radical cation, once formed, can reorientate nearer to the aqueous interface.

In conclusion, the one-electron reduction potential of the β-carotene radical cation is 1.06 V in aqueous micellar solution and this is sufficiently high to oxidise tyrosine and cysteine, but low enough to repair P680$^{\bullet+}$ in the PS II photosynthetic reaction centre.

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