Role of various carotenoids in mediating electron transfer sensitized by chlorophyll and pheophytin

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Received 28 April 1982

1. INTRODUCTION

Numerous studies demonstrating the photoconductive properties of bilayer lipid membranes containing chlorophyll have appeared [1]. The conjugated bond system found in carotenoids may play a major role in mediating photoconductivity [2,3]. Here we investigate the role of the functional groups on the ionone ring of carotenoids in modifying the photosensitivity of chlorophyll bilayer membranes.

While all the carotenoids used here enhance the photosensitivity of chlorophyll bilayers, the dihydroxy-carotenoids give rise to much greater photocurrents than either the carotenes (α and β) or the diketo-carotenoids. Bilayers formed using the Mg^{2+}-free chlorophyll derivative, pheophytin, produce negligible photocurrents regardless of the carotenoids used. The role of nucleophilic interactions between carotenoids and Mg^{2+} in vivo is discussed.

2. MATERIALS AND METHODS

Planar bilayer membranes were formed solvent free from monolayers as in [4,5]. The membranes were composed of a lipid mixture of lecithin and cholesterol, plus either chlorophyll a or pheophytin a, plus one of several carotenoids. The mole ratio of cholesterol/lecithin was held constant at 1:5 (w/w). The molar ratio of carotenoid/chlorophyll, was 1:4, unless otherwise specified. The molar ratio of total pigment/lipid was always kept at 1:4; this ratio was found to give the greatest degree of stability to the bilayer [6].

In all experiments the bathing solution on each side of the membrane consisted of 10 mM potassium ferrocyanide (reducing side) and 10 mM potassium ferricyanide (oxidizing side). Both phases contained 10 mM KCl buffered at pH 7.8 (10 mM potassium phosphate). For illumination (632.8 mm) a 5 mW He–Ne laser (Spectra Physics, model 150) was used.

Chlorophyll a, chlorophyll b and pheophytin were prepared and purified as in [7–9]. Lecithin (type II), α-carotene, β-carotene and cholesterol were all purchased from Sigma (St Louis, MO). The xanthophylls [lutein (3,3'-dihydroxy-α-carotene), zeaxanthin (3,3'-dihydroxy-β-carotene), astaxanthin (3,3'-dihydroxy-4,4'-diketo-β-carotene) and canthaxanthin (4,4'-diketo-β-carotene)] were all the generous gift of Hoffmann-La Roche (Basel). The carotenoids were grouped as follows: I, carotenes; II, dihydroxy-carotenoids; III, dihydroxy-keto-carotenoids; IV, keto-carotenoids (structures I–IV).

3. RESULTS

Carotenoid free bilayers consisting of pure chlorophyll a or b and lipid, produces negligible photocurrents when illuminated with a He–Ne laser.

3.1. *Group 1 and group 2 carotenoids*

Addition of either β-carotene or the oxygenated derivative zeaxanthin to the chlorophyll bilayer, improves the stability of the bilayer and results in a photocurrent. A maximum photocurrent is reached at a carotenoid mole fraction of 0.2 (carotenoid/chlorophyll + carotenoid) (fig.1). At this mole fraction the photocurrents for bilayers containing β-carotene or zeaxanthin are 25 ± 5 or 85
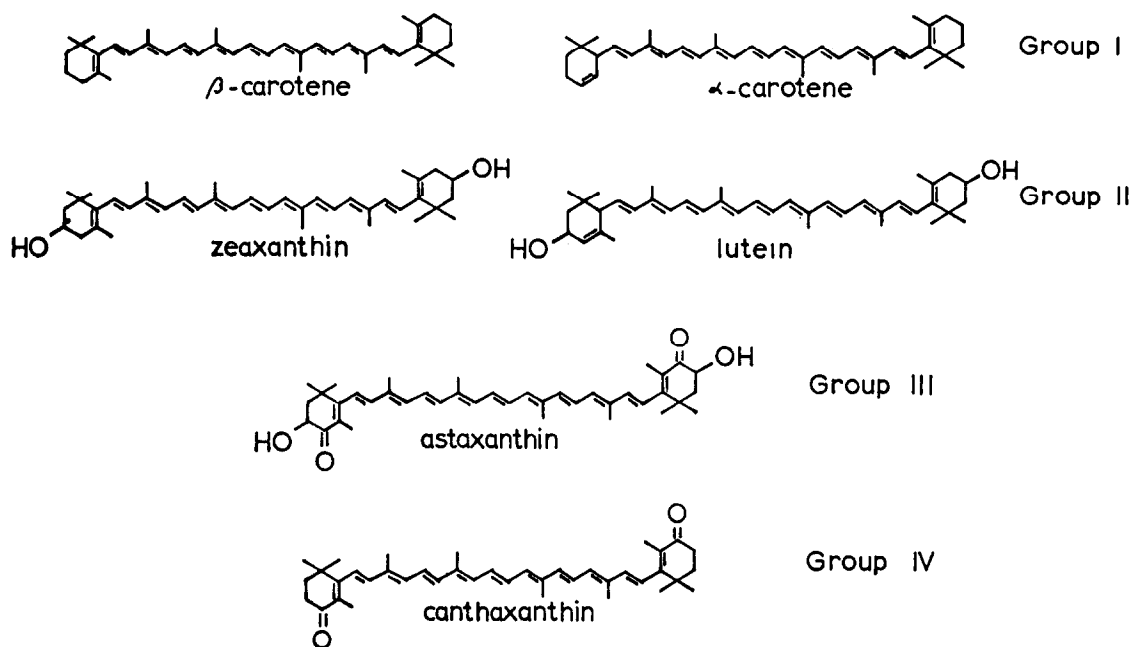
Structures I–IV: I, carotenes; II, dihydroxy-carotenes; III, diketo-carotenes; IV, dihydroxy-diketo-carotenes.

$\pm$ 15 nA/cm^2, respectively. At greater carotenoid concentrations the photocurrent decreases. Lutein and α-carotene exhibit behavior similar to their respective isomers, β-carotene and zeaxanthin. The maximum photocurrents observed with these carotenoids are shown in table 1.

3.2. Group 3 and group 4 carotenoids

Canthaxanthin the keto-derivative of β-carotene produces a lower photocurrent (18 $\pm$ 5.5 nA/cm^2) (table 1). Astaxanthin yields a photocurrent of 60 $\pm$ 10 nA/cm^2, approximately equal to the mean value for zeaxanthin and canthaxanthin.

3.3. Pheophytin

To evaluate the role of magnesium in electron-transfer mechanisms, bilayers were formed using pheophytin a, the Mg^{2+}-free derivative of chlorophyll a. Negligible photocurrents are observed with bilayers containing pheophytin and the various carotenoids (table 1).

3.4. Chlorophyll b

Chlorophyll b differs from chlorophyll a in that it contains a CHO group instead of a CH$_3$ group, in ring II of porphyrin. At the wavelength of illumination (632.8 nm) the absorbance of chlorophyll b is 3.3-times greater than that of chlorophyll a. If chlorophyll b and chlorophyll a behave similarly, the photocurrent for chlorophyll b/carotenoid bilayers should be 3.3-times greater for comparable chlorophyll a bilayers. Chlorophyll b is found to behave in this manner, except with carotenoids containing a keto-group in the ionone ring (group 3 and 4). The photocurrent generated by chlorophyll a bilayers containing the group 4 carotenoid canthaxanthin is 1.5-times greater than the photocurrent from comparable chlorophyll b bilayers.

4. DISCUSSION

Since the common structural feature of all carotenoids is that of a conjugated double-bond system, the observed photoconductivity of chlorophyll-carotenoid bilayers appears to be via the π-bond configuration [2,3]. Differences between the photocurrents of carotenes and their oxygenated derivatives, suggest an active role for the functional groups of the ionone ring.

Interactions between Mg^{2+} of chlorophyll and

Table 1

Summary of maximum photocurrents in chloro-phyll—carotenoid bilayers

	Chl-a (nA/cm^2)	Chl-b (nA/cm^2)	Pheo-a (nA/cm^2)
Lutein	95 ± 20	330 ± 55	2.0 ± 1.0
Zeaxanthin	85 ± 15	295 ± 70	2.0 ± 0.5
Astaxanthin	60 ± 10	90 ± 10	1.5 ± 0.5
β-Carotene	25 ± 5	80 ± 15	1.0 ± 0.5
α-Carotene	25 ± 5	75 ± 15	1.0 ± 0.5
Canthaxanthin	18 ± 5	30 ± 5	1.0 ± 0.5

The mole ratio of chlorophyll:carotenoid was kept at 4:1. The mole ratio of lipid:pigment was kept at 4:1. Bathing solutions were as in fig.1

Fig.2. Photocurrent as a function of mole fraction of carotenoid (carotenoid/chlorophyll + carotenoid) for bilayers containing chlorophyll b. The carotenoids shown are β-carotene (○) and zeaxanthin (△). The bathing solutions are the same as in fig.1.
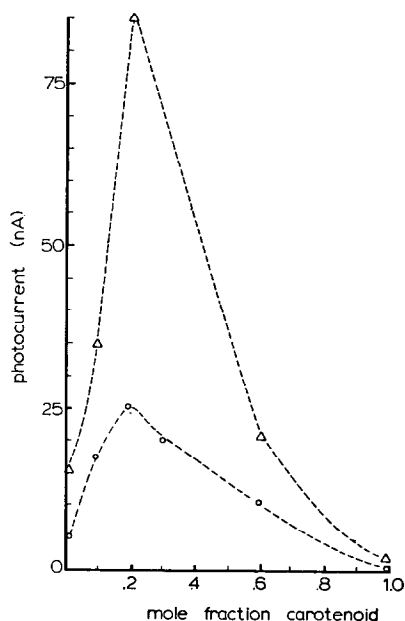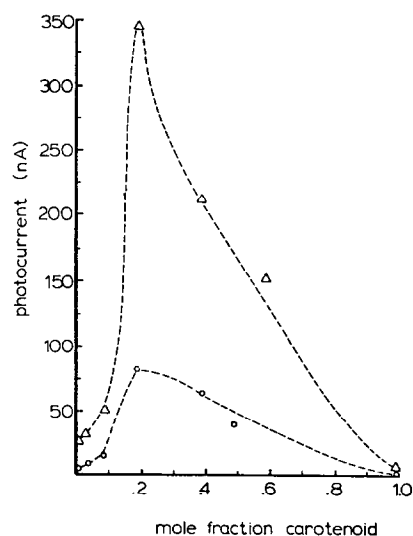

Fig.1. Photocurrent as a function of the mole fraction of carotenoid (carotenoid/chlorophyll + carotenoid), for bilayers formed containing chlorophyll a. The carotenoids shown are β-carotene (○) and zeaxanthin (△). The aqueous phases consisted of 10 mM potassium ferrocyanide and 10 mM potassium ferricyanide in 10 mM solution of KCl buffered at pH 7.8. The bilayer area is 2×10^{-4} cm^2.

the OH$^-$ groups of lutein have been implicated as the cause of electrochromic shifts in chloroplasts [10]. Coordination interactions using nuclear magnetic resonance, indicates that β-carotene does not interact with the chlorophyll macrocycle. On the other hand, there is an interaction between chlorophyll and oxygenated carotenoids such as lutein and β-carotene epoxide [11]. The higher photocurrent for chlorophyll bilayers containing dihydroxy-carotenoids (group 2) may thus be a result of a nucleophilic interaction between the OH$^-$ of carotenoid molecules and Mg^{2+} of chlorophyll. This interaction allows for the formation of chlorophyll—carotenoid complexes within the lipid domain.

Although it seems plausible that the number 4 keto group of canthaxanthin could act as a nucleophile, the low photocurrent (table 1) indicates the absence of a chlorophyll—canthaxanthin interaction. Alternatively, the keto group on carotenoids such as canthaxanthin may preferentially orient at the lipid—water interface with its polar groups interacting with the aqueous phase. Such an orientation would prevent an efficient bridge between chlorophyll and canthaxanthin molecules.

The negligible photocurrent obtained with pheo-

phytin *a* cannot be accounted for solely by a lower absorbance of pheophytin compared to chlorophyll *a* at the exciting wavelength (632.8 nm). Based on the above, the absence of Mg^{2+} may preclude interactions between pheophytin and carotenoids and consequent photocurrent. The negligible photocurrent in pheophytin bilayers might also be attributed to its inability to oxidize potassium ferricyanide. Pheophytin has been shown to have a more positive oxidation–reduction potential than chlorophyll *a* [12].

We have shown that both Mg^{2+} in chlorophyll as well as the functional groups of the ionone ring of carotenoid play a major role in determining the photocurrent of chlorophyll bilayers. Similar conditions may be occurring in vivo. It is feasible that in vivo, the various forms of chlorophyll may, in part, be determined by the structure of the ionone ring of the carotenoid with which chlorophyll–proteins may be complexed.

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