The use of carotenoids and oxonol VI as probes for membrane potential in proteoliposomes

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Received 8 March 1985

Carotenoids present in lipids extracted from the cyanobacterium *Synechococcus* 6716 indicate trans-membrane potential in proteoliposomes reconstituted from these lipids and the ATPase complex isolated from the same organism. A carotenoid absorbance band shift to a longer wavelenght is obtained with valinomycin-induced potassium ion diffusion potentials, irrespective of the polarity of the potassium gradient. In contrast to this, the (externally added) probe oxonol VI only shows an absorbance band shift when the external potassium ion concentration is higher than the internal one. In liposomes without ATPase complex, no carotenoid absorbance band shifts were observed.

Proteoliposome *Membrane potential* *Diffusion potential* *Carotenoid shift* *Oxonol VI*

1. INTRODUCTION

Native carotenoids have widely been used as intrinsic membrane potential-indicating probes in chromatophores [1] and chloroplasts [2,3]. By means of valinomycin-induced potassium ion diffusion potentials the carotenoid response in these systems has been calibrated [1,4,5]. In addition to the use of the carotenoid absorbance band shift in whole photosynthetic membranes we have reported a carotenoid shift induced by the hydrolysis of ATP in ATPase proteoliposomes reconstituted from the ATPase complex and lipids from the thermophilic cyanobacterium *Synechococcus* 6716 [6,7]. In this system the native carotenoids are present in the lipid mixture extracted from the cyanobacterium. To explore the possibilities to use natural carotenoids as probes for trans-membrane potentials in reconstituted systems, we studied the carotenoid response in this reconstituted system upon induction of ion diffusion potentials of different polarities. Moreover, we compared the response of the intrinsic probe with that of the externally added probe oxonol VI. This latter probe was also used earlier to monitor trans-membrane potentials (outside negative) in submitochondrial particles [8], chromatophores [9,10] and in chloroplasts [11,12]. It is also of great interest to investigate whether the carotenoids are oriented in the liposome membrane in a way that allows discrimination between membrane potentials of opposite polarity.

2. MATERIALS AND METHODS

Unilamellar ATPase proteoliposomes were prepared by dialysis according to Van Walraven et al. [6]. The protein to lipid ratio was about 0.01 (w/w). The lipids used were isolated as described [13]. The reconstitution medium contained 10 mM Na-Tricine (pH 7.5, unless otherwise indicated), 2.5 mM $MgCl_2$, 1 mM dithioerythritol, 10 mM KCl and 90 mM LiCl. The reaction medium had the same composition, but KCl and pH varied as indicated in the legends to the figures. At KCl concentrations of 100 mM or lower, osmolarity of the reaction medium was kept constant with LiCl; total salt concentration (KCl + LiCl) was then 100 mM. Final lipid concentration was 0.25 $mg \cdot ml^{-1}$.

Absorbance spectra were recorded with an Aminco DW-2a spectrophotometer in a multi-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

purpose cuvette (1.8 ml) [14] thermostatted at 50°C. The spectrophotometer was equipped with a PDP 11/03 microprocessor (Digital Equipment Co.) and connected on-line to a disc-based mini computer (Hewlett-Packard 21 M-E, with RTE IV-B).

Oxonol VI (bis(3-propyl-5-oxoisoxazol-4-yl)pentamethine-oxonol) was synthesized and kindly provided by Professor W.G. Hanstein (Ruhr Universität, Bochum, FRG), S-13 (5-chloro-3-*t*-butyl-2'-chloro-4'-nitrosalicylanilide) was a kind gift from Dr P.C. Hamm (Monsanto Co., St. Louis, MO). Valinomycin was obtained from Boehringer (Mannheim, FRG).

3. RESULTS AND DISCUSSION

Fig.1, spectrum A, shows the carotenoid absorbance shift observed upon addition of valinomycin to ATPase proteoliposomes with 10 mM K^+ inside, and 100 mM K^+ outside. At 50°C this results in a diffusion potential of 65 mV (negative outside). The spectrum indicates a shift to longer wavelengths of all three absorbance maxima of the carotenoids in the *Synechococcus* lipid mixture [13]. In this experiment the situation is similar to that of chloroplasts and chromatophores: the diffusion potential applied is the result of K^+ influx. The absorbance change is stable for at least 30 min after addition of valinomycin, and may be abolished by the addition of the uncoupler S-13 (not shown).

No shift is observed in the absence of a potassium ion concentration gradient (fig.1B). However, also in the opposite (10 mM K^+ inside, 1 mM K^+ outside) valinomycin induces a carotenoid absorbance band shift to the red (fig.1C). In this case efflux of K^+ results (at 50°C) in a diffusion potential of −65 mV (positive outside). Fig.1, spectrum D, shows that this opposite shift is also abolished with the uncoupler S-13.

As opposed to the carotenoids, the externally added probe oxonol VI only detects a diffusion potential that is negative outside (fig.1E), but no absorbance shift in the oxonol spectrum is observed in the absence of a concentration gradient (fig.1F) or with a diffusion potential of opposite polarity (fig.1G). In the presence of 0.5 μM oxonol VI, the carotenoid absorbance shift was abolished

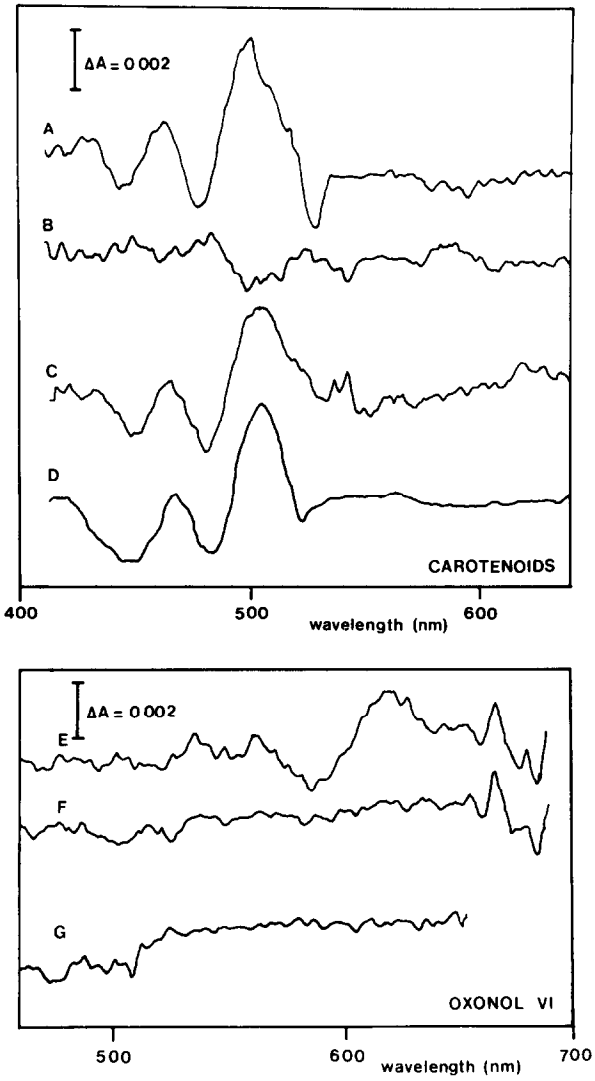

Fig.1. Diffusion potential-induced spectral changes of carotenoids and oxonol VI. Shown are difference spectra (A–C and E–G, 100 nM valinomycin present minus no additions; D, 100 nM valinomycin present minus valinomycin together with 1 μM S-13 present). In E–G, 0.5 μM oxonol VI is also present. The external $[K^+]$ is as follows: A, 100 mM; B, 10 mM; C, 1 mM; D, 1 mM; E, 100 mM; F, 10 mM and G, 1 mM. Internal $[K^+]$ is 10 mM in all cases.

(cf. fig.1E), due to the energy-dissipating character of the probe response (cf. [15], for the same effect of oxonol VI on chloroplasts).

Thus, carotenoids show an absorbance band red shift in response to diffusion potentials, irrespec-

tive of the polarity of the potentials. Quantitative use of the probe signal, however, is problematic; at diffusion potentials larger than about 40 mV the extent of the probe signal is highly variable. Fig.2A illustrates this for S-13 induced H^+ diffusion potentials. In fig.2B the response of oxonol VI is plotted as a function of diffusion potential (both valinomycin-induced K^+ influx and S-13 induced H^+ influx). In this case a reasonable calibration line is obtained.

The presence of the ATPase complex in the reconstituted system is essential for the occurrence of the carotenoid absorbance changes. Liposomes without protein did not show valinomycin-induced carotenoid absorbance changes under the conditions used; also, preliminary experiments with proteoliposomes prepared from carotenoid-containing lipids and beef heart cytochrome *c* oxidase were without success (unpublished).

The observed red shift at potentials of both polarities may be explained in a number of ways. However, any blue shifts occurring must be of minor significance. The first explanation assumes that a single pool of carotenoids is responsible for the observed red shifts upon induction of diffusion potentials of opposite polarity. The second explanation assumes that two types of ATPase-associated carotenoids may be involved. This latter explanation could either mean that there are two pools each associated with ATPase complexes of different membrane orientation, or that there are two pools associated with ATPase complexes of the same orientation. The fact that ATPase complexes in proteoliposomes prepared by reconstitution are oriented nearly completely with the F_1 part outside (see [16] and references cited therein) and that the shifts at different polarities of the potential are of roughly the same size, seems to exclude the first possibility.

Hence, either carotenoids from a single pool, or different carotenoids associated with ATPase complexes in a single orientation, are responsible for

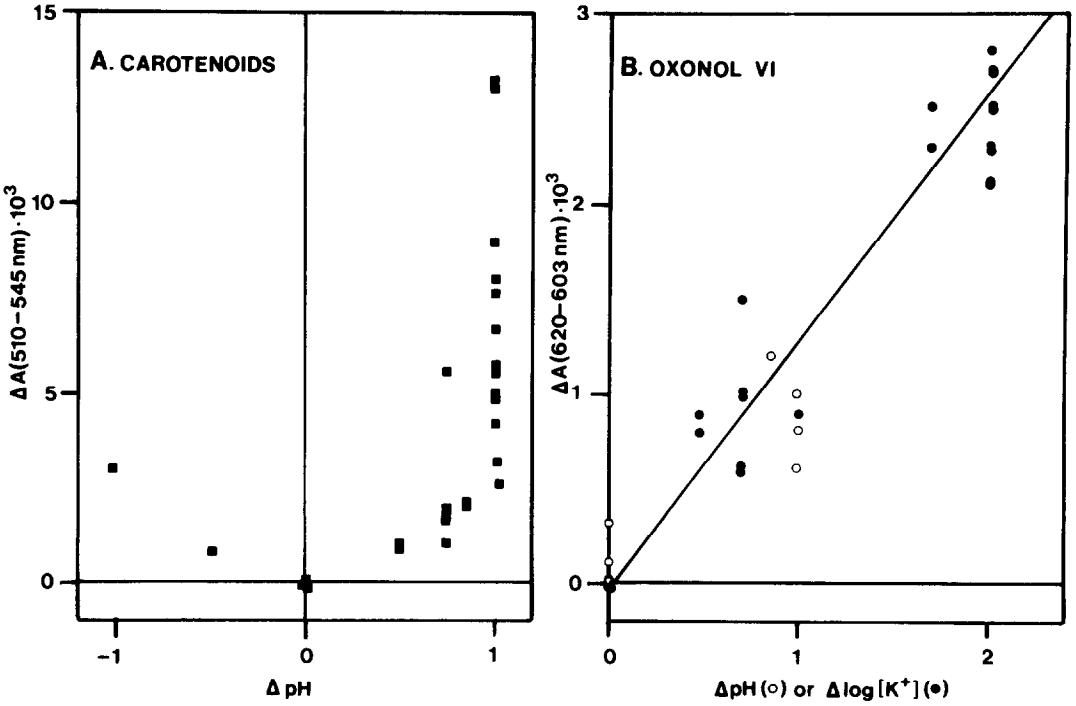

Fig.2. Magnitude of the probe responses in dependence of initial ion gradients. (A) Carotenoid absorbance changes upon addition of 250 nM S-13. The $[K^+]$ is 10 mM (inside as well as outside), and internal pH is 6.5. ΔpH is defined as inside minus outside. (B) Oxonol VI absorbance changes upon addition of 100 nM valinomycin (●) or 250 nM S-13 (○). The oxonol VI concentration is 0.5 μM, the internal $[K^+]$ is 10 mM, and the internal pH 6.5. ΔpH is defined as inside minus outside, $\Delta\log[K^+]$ as outside minus inside.

the red shifts induced by membrane potentials of opposite polarities. It is possible that also in the natural membrane, carotenoids react in the same way.

We conclude that in this reconstituted system carotenoids can be used as qualitative probes for membrane potential, in agreement with our interpretation of the absorbance changes that occur when the ATPase proteoliposomes hydrolyse ATP [6,7]. Also, the results show clearly that the polarity of the trans-membrane potential may be either way. For a quantitative determination of potentials (negative outside) oxonol VI is the tool of choice.

ACKNOWLEDGEMENTS

This work was supported in part by the Foundation for Biophysics with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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