REACTION CENTRE CAROTENOID BAND SHIFTS

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Received 2 May 1977
Revised version received 10 June 1977

1. Introduction

Reversible red shifts in the absorption spectrum of carotenoids ('the carotenoid band shift'), induced either by light or oxygenation, have been observed in both *Rhodospirillum rubrum* [1] and *Rhodopseudomonas sphaeroides* [2]. Since then there have been several attempts to explain the mechanism of this phenomenon (for a recent review see [3]), and most recently attention has focused upon the idea that the carotenoid band shift represents an electrochromic response of the carotenoids to a transmembrane potential [4,5]. A similar explanation has also been proposed to account for the so called '515 shift' in chloroplasts [6].

However, when the behaviour of the in vivo carotenoid band shift is compared to that which might be predicted from the theory of electrochromism [7–9] the agreement is not very exact [10,11]. This is probably due to an oversimplification; even if the type of carotenoid is restricted [11] the hetereogeneities of carotenoid environments will result in a band shift whose nature will be a complex mixture.

If, however, one accepts that the carotenoid band shift is an electrochromic phenomenon, there still remains the question of whether it is induced solely by a transmembrane potential or whether changes in other factors, such as local fields arising from charges on or within the membrane, contribute to the magnitude of the observed band shift.

It is possible to isolate and purify photochemical reaction centres from carotenoid containing strains of *Rps. sphaeroides* [12,13]. Each of the purified centres retains 1 mol of a specific carotenoid that appears to be bound in a specific site [14]. In this study we describe the effect of the local electric fields within the reaction centre complex upon the absorption spectrum of the reaction centre carotenoid.

2. Materials and methods

Cells of *Rps. sphaeroides* strains 2.4.1 (wild type), Ga and GIC (green mutants) and R26 (carotenoidless mutant) were grown anaerobically in the light, harvested and chromatophores prepared from them as previously described [15]. Reaction centres were then isolated from the chromatophores by treatment with the detergent lauryldimethylamine-*N*-oxide (LDAO) and then purified by ammonium sulphate precipitation [12,13,16]. The purified reaction centres were dialysed to remove the LDAO, which was replaced by 2% cholate.

Spectra were measured using a computer-linked spectrophotometer as previously described [17].

The concentration of reaction centres was determined from their absorbance at 802 nm using the extinction coefficient of 288 mM^{-1} cm^{-1} [18].

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3. Results

In *Rps. sphaeroides* the photochemical redox reaction involves the oxidation of the primary donor (a special pair of bacteriochlorophyll molecules called P870) and the reduction of the primary electron acceptor (which is most probably an iron-ubiquinone complex) [19].

$$P870\,(Fe\text{-}UQ) \xrightarrow{Light} P870^{+}\,(Fe\text{-}UQ)^{-}$$

This charge separation occurs quite readily when the isolated reaction centres are illuminated.

Trace A in fig.1 shows a typical light-dark difference spectrum for reaction centres from *Rps. sphaeroides* R26 between 400 and 590 nm. It illustrates the well known absorbance changes in this region of the spectrum due to the primary photochemical reactions, a broad peaked increase in absorbance around

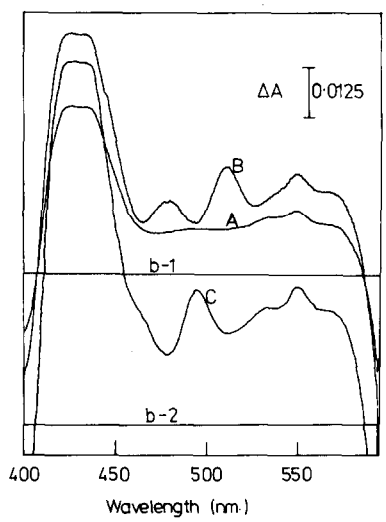

Fig.1. Light-Dark difference spectra of reaction centres from *Rps. sphaeroides*. Trace A: The cuvette contained 3.3 μM reaction centres from mutant R26 in 20 mM Tris–HCl, and 0.13% cholate at pH 7.5. Trace B: The cuvette contained 4.7 μM reaction centres from strain 2.4.1 in the same buffer as Trace A. Trace C: The cuvette contained 13 μM reaction centres from mutant Ga in the same buffer as Trace A. The spectra were all recorded while the cuvette was illuminated with far red light provided by light from a 12 V, 55 W quartz iodine bulb filtered through a Wratten 87A filter. Traces A and B share baseline b-1; baseline b-2 refers to Trace C.

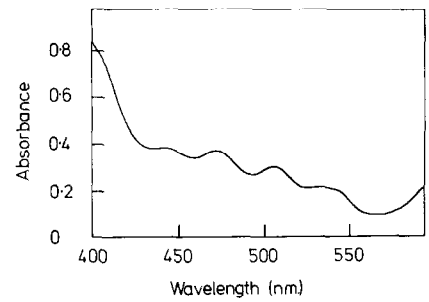

Fig.2. The absolute absorption spectrum of reaction centres from strain 2.4.1. The cuvette contained 4.7 μM reaction centres in 20 mM Tris–HCl and 0.13% cholate at pH 7.5.

430 nm, a small rather featureless increase between about 450 nm and 570 nm, and a decrease above 580 nm.

When a similar light-dark difference spectrum is recorded for 2.4.1 reaction centres (Trace B, fig.1) and compared with light-dark difference spectrum obtained from the R26 reaction centres, a striking difference is apparent in the 450–530 nm region. A comparison of the 2.4.1 reaction centre difference spectrum with the absolute absorption spectrum of the 2.4.1 reaction centres obtained in the dark (fig.2) reveals that illumination has produced a red shift in the absorption spectrum of the reaction centre carotenoid.

Reaction centres from strains *Rps. sphaeroides* Ga and GIC, which contain different carotenoids, where the absorption spectrum is blue shifted compared to strain 2.4.1, show a similar light induced red shift of the carotenoid absorbance spectrum of the reaction centre carotenoid (for example see Trace C, fig.1).

Illumination of the reaction centres produces $P870^{+} \cdot (Fe\text{-}UQ)^{-}$, but it is possible chemically to produce individually either $P870^{+}$ or $(Fe\text{-}UQ)^{-}$. P870 Can be conveniently oxidised with potassium ferricyanide and (Fe-UQ) reduced by sodium dithionite.

Reduction of reaction centres from *Rps. sphaeroides* strain 2.4.1 by addition of sodium dithionite produces the difference spectrum shown in Trace B of fig.3. Again a carotenoid band shift is apparent (R26 reaction centres show none of the peaks and troughs in the dithionite induced difference spectrum between 450 and 530 nm).

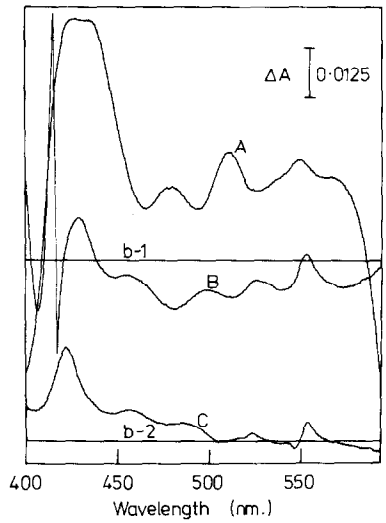

Fig.3. A comparison of the light-dark difference spectrum with those generated by the production of $(Fe\text{-}UQ)^-$ and I^-. Trace A: The Light-Dark difference spectrum. Conditions as for fig.1, Trace B. Trace B: The difference spectrum induced by addition of dithionite in the dark. The cuvette contained 4.7 μM reaction centres from strain 2.4.1 in 20 mM Tris–HCl, 0.13% cholate. Note the trace went off scale below 430 nm and was offset back onto scale. Trace C: The difference spectrum induced by illumination of the reaction centres in the presence of dithionite and cytochrome *c*. Conditions as in Trace B except that 50 μM cytochrome *c* was also added. Illumination was provided by the same light as used in fig.1. Baseline B-1 is the same for Traces A and B, while B-2 refers to Trace C.

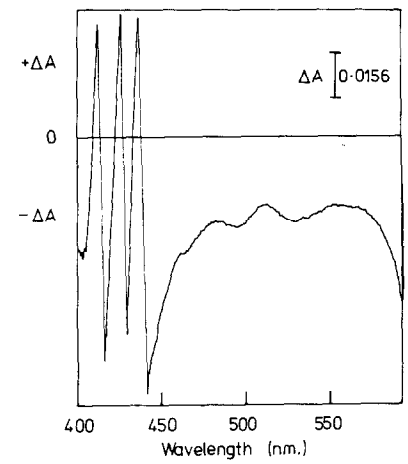

Fig.4. The difference spectrum induced by addition of ferricyanide in the dark. The cuvette contained 4.7 μM reaction centres from strain 2.4.1 and the oxidation was achieved by addition of a few crystals of potassium ferricyanide. The interference due to the strong absorption of ferricyanide is apparent on the left hand side of the figure; note that as the trace goes off scale the computer offsets it automatically.

However in this case chemical reduction of the primary electron acceptor has produced a blue shift of het carotenoid absorption bands. Similar results were obtained with Ga and GIC reaction centres.

Chemical production of $P870^+$, on the other hand, induces a red shift of the reaction centre carotenoid (fig.4). Due to spectral interference from the ferricyanide, it was only practical to produce a partial oxidation of the P870 present, but it is clear in this case that the shift of the carotenoid absorbance bands is further to the red than the shift induced by the formation of $P870^+(Fe\text{-}UQ)^-$ upon illumination.

There is now a considerable body of evidence that the primary photochemical reaction proceeds by way of an intermediate step prior to the production of $(P870^+)(Fe\text{-}UQ)^-$ [20,21]. The probable sequence is the following:

$$P870 \cdot I \cdot (Fe\text{-}UQ) \xrightarrow{\text{Light}} P870^+ \cdot I^- \cdot (Fe\text{-}UQ)$$
$$P870^+ \cdot I^- \cdot (Fe\text{-}UQ) \longrightarrow P870^+ \cdot I \cdot (Fe\text{-}UQ)^-$$

where *I*, the proposed intermediate, is thought to be a molecule of bacteriopheophytin [22]. Indeed in reaction centres from *Rps. viridis* and *Chromatium* [22,23] it has been possible to 'trap' *I* in its reduced state. The experimental procedure for this has been well described [22]. It is possible approximately to imitate this procedure with reaction centres from *Rps. sphaeroides* by illuminating in the presence of a large excess reduced mammalian cytochrome *c*. Even though this situation is less favourable than that with reaction centres from *Rps. viridis* and *Chromatium*, illumination of reduced 2.4.1 reaction centres in the presence of added cytochrome *c* does trap a proportion of reaction centres in the state I^- (as judged by bleaching in the 760 nm bacteriopheophytin band). Production of I^- induces a blue shift of the reaction centre carotenoid (Trace C fig.3).

4. Discussion

Production of fixed charges within the photochemical reaction centre has been shown to induce carotenoid band shifts. The direction of the shift varied with the nature of the fixed charge, as follows:

$P870^+ \cdot (Fe\text{-}UQ)^-$ (dipole) red shift
$P870^+$ (+ve monopole) red shift
and
$(Fe\text{-}UQ)^-$ or I^- (–ve monopoles) blue shift

In the absence of any evidence in favour of a set of reaction centre conformational changes that might induce these carotenoid shifts, the simplest explanation is that they are electrochromic in origin, being induced by the local electric fields set up within the reaction centre by the fixed charges.

Electrochemic theory predicts that for molecules like the carotenoids which have no permanent dipole moment, the extent of shift will be a quadratic function of the applied field [7–9]. In this case no blue shift would be expected, so that our observation of a blue shift must indicate that some local environmental factor, possibly a permanent fixed charge, displaces the carotenoid spectrum from that at zero field. It seems likely from the direction of the shifts observed (see above) on changing the charge, that the environmental field with respect to the carotenoid is the same as that induced on oxidising P870.

If this electrochromic hypothesis is correct then it is theoretically possible to compute the relative positions of the carotenoid molecule and the primary photochemical reactants. However this calculation will not produce a unique solution until the distance between P870 and (Fe-UQ), the contribution of environmental factors to the field, and the orientation of the long axis of the carotenoid molecule to the $P870^+ \cdot (Fe\text{-}UQ)^-$ dipole are determined. The latter problem might be approached by linear dichroism studies.

The presence of different carotenoids within the reaction centre does not alter the basic pattern of the responses seen, only the magnitude of the change. The shifts are the largest with the 2.4.1 reaction centres.

If the reaction centre carotenoid band shift is induced by changes in a local electric field, what error will this introduce into calculations which use the chromatophore carotenoid band shift as an indicator of transmembrane potential? Assuming the size of the photosynthetic unit of *Rps. sphaeroides* 2.4.1 to be about 100 Bchl/reaction centre [24], the error would be in the order of 2–5%, and so can probably be neglected.

Acknowledgements

We thank Mr Gerry Tierney for except assistance with the computer interfacing, Mrs Janet Fielding and Mr Wallace MacDonald for microbiological assistance, Dr Michael Hipkins for help with the calculations upon where within the reaction centre complex the carotenoid might be located and the Science Research Council for a grant towards equipment.

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