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Electrochromic responses of bacteriochlorophyll absorbance bands in purified light-harvesting complexes of *Rhodobacter capsulatus* reconstituted into liposomes

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Purified light harvesting complexes I and II (LHI and LHII) from *Rhodobacter capsulatus* were purified and separately incorporated into liposomes. Electrochromic absorbance changes of bacteriochlorophyll bands in the proteoliposomes in response to K^+-diffusion potentials were recorded. In LHII proteoliposomes the application of positive-inside potentials led to red shifts in the bacteriochlorophyll absorbance bands centred at 377, 801 and 858 nm. Negative-inside potentials caused blue shifts of these bands. Electrochromism of the 590 nm band was too small to detect. The band at 858 nm was considerably more electrochromic than that at 801 nm. Electrochromic absorbance changes measured at 865–850 nm were linear with the applied diffusion potential. In LHI proteoliposomes positive-inside diffusion potentials caused red shifts of the bands centred at 374 nm and 880 nm. At 880 nm the response was linear with the applied diffusion potential and was equivalent in amplitude to that of the 858 nm band in LHII proteoliposomes. If it is assumed that the permanent dipole moment differences between the ground state and excited state of B800, B850 and B870 are similar and that polarisability effects are negligible, it follows that (a) the plane of the bacteriochlorin ring of B850 is more perpendicular to the membrane plane than that of B800 and (b) the orientations of B850 and B870 relative to the membrane plane are similar.

Introduction

Certain light-induced changes in the bacteriochlorophyll absorbance bands of chromatophores from photosynthetic bacteria are electrochromic – they arise in response to the electric potential generated either *across* the membrane as a result of photosynthetic electron transport or *locally* due to charge separations within proteins [1–5]. The spectra of the bacteriochlorophyll electrochromic absorbance changes in chromatophore membranes have been described [3] and it has been shown that the response is linear with the applied membrane potential [4]. Reich and colleagues [1,6,7] made an extensive study of the equivalent chlorophyll electrochromic absorbance changes in thylakoid membranes from green plants. Using the theory developed by Labhart [8] and Liptay [9], and reviewed [1,10], they were able to predict the orientation of chlorophyll *a* and *b* relative to the plane of the membrane [7].

Natural photosynthetic membranes are complicated by the fact that they contain a number of different chlorophyll- or bacteriochlorophyll-containing protein complexes. Therefore the effects of electric fields on different pigments in different environments will all contribute to the electrochromic spectrum. In this report we describe the effects of transmembrane electric fields on the absorbance spectra of liposomes incorporated with purified light harvesting complexes from *Rhodobacter capsulatus*. In this case we are dealing with a well-defined system with only a small number of pigment environments.

For small wavelength shifts it can be shown [1,10] that the absorbance change ($\Delta\epsilon$) induced by an electric field is given by

$$\Delta\epsilon = \frac{|\mu_e - \mu_g|}{hc}\cos\Theta' F\frac{\delta\epsilon}{\delta\nu} + \frac{1}{2}\frac{\alpha_e - \alpha_g}{hc}F^2\frac{\delta\epsilon}{\delta\nu} + \frac{1}{2}\frac{|\mu_e - \mu_g|^2}{h^2c^2}\cos^2\Theta' F^2\frac{\delta^2\epsilon}{\delta\nu^2}$$

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Abbreviations: LHI and LHII, light-harvesting complexes I (B870) and II (B800–850), respectively; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone.

where μ_g and μ_e are the permanent dipole moments of the ground state and excited state, α_g and α_e are the polarisabilities of the ground state and excited state, Θ' is the angle between $\mu_g - \mu_e$ and the direction of the external electric field, F is the external electric field, ν is the wavenumber, h is the Planck constant and c is the velocity of light. Molecules such as bacteriochlorophyll have a large permanent dipole moment. Therefore, the first term of the equation is expected to dominate when bacteriochlorophyll, bound within a protein that is oriented across a membrane, is subjected to a transmembrane potential giving field strengths in the region of 10^5–10^6 V·cm^{-1}. Hence, it is predicted that the electrochromism of individual bacteriochlorophyll molecules will be linear with the applied potential and proportional to the first derivative of the absorbance spectrum [1]. The factors then determining the strength of the electrochromic response will be Θ' and $\mu_g - \mu_e$. Thus, in principle, from the electrochromic changes of the well-separated near infra-red absorbance bands of the bacteriochlorophylls, the relative orientations of B800, B850 and B870 with respect to the membrane can be assessed. The conclusions can be compared with the predictions of pigment orientation based on the linear dichroism of dried chromatophore films [11] and on the fluorescence polarisation of detergent solubilised LHII.

Methods

The preparation of LHI and LHII, their incorporation into liposomes and the methods for measuring electrochromic absorbance changes in response to K$^+$-diffusion potentials are described in [13]. In all cases the absorbance changes were corrected for the dilution artifacts resulting from addition of either the valinomycin or KCl solution. The electrochromic character of the absorbance changes was confirmed by performing two types of control experiment: (a) with NaCl replacing the KCl and (b) in the presence of 160 μM FCCP to increase the proton permeability of the membrane and thus prevent the build-up of the K$^+$-diffusion potential.

Results

The near infra-red spectrum of electrochromic absorbance changes generated with K$^+$-diffusion potentials in chromatophores from *Rb.capsulatus* is shown in Fig. 1. It is very similar to the light-dark difference spectrum of *Rb. capsulatus* [14]. Apparent band shifts to longer wavelengths are centred at isosbestic points of approx. 803 nm and 857 nm.

The application of a positive-inside diffusion potential to proteoliposomes containing pure LHII complexes gave rise to absorbance changes in the near i.r. as shown in Fig. 2B. The absorbance spectrum of the proteoliposomes in the equivalent region of the spectrum is shown in Fig. 2A. Within the scatter on the data, the electrochromic absorbance changes at long wavelengths can be modelled by a shift of 0.15 nm of the entire Q_y absorbance band of B850 (Fig. 2C). Similarly a homogeneous shift of the B800 Q_y absorbance band (of somewhat less than 0.1 nm) might account for the changes in the 790–810 nm region of the spectrum but the signal/noise ratio is too small to conclude this with confidence. For equivalent oscillator strength B850 is about 3 times as sensitive to membrane potential as B800.

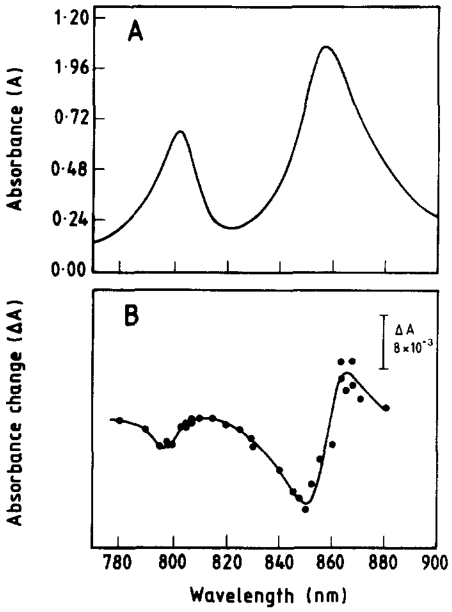

Fig. 1. Electrochromic changes in bacteriochlorophyll bands of chromatophores in response to K$^+$-diffusion potentials. (A) Absorbance spectrum of chromatophores in the near i.r. (bacteriochlorophyll 10.8 μM). (B) Electrochromic absorbance changes (measured relative to a reference of 825 nm) resulting from the application of a positive-inside K$^+$-diffusion potential.

Upon application of a negative-inside diffusion potential the electrochromic absorbance changes were the mirror image of those generated by positive-inside potentials (Fig. 2), indicating a blue shift of the absorbance band. The dependence of the B850 absorbance changes (measured at the peak and trough of the electrochromic spectrum) upon the value of the diffusion potential was linear from approx. −106 to 105 mV (Fig. 3). The kinetics of the decay of the B850 absorbance changes (at 865–850 nm) after application of a diffusion potential were similar to the kinetics of decay of the electrochromic carotenoid absorbance changes [13] recorded in parallel experiments and were similarly sensitive to FCCP.

Electrochromic absorbance changes were also detectable in the Soret bands of the bacteriochlorophylls of LHII proteoliposomes (Fig. 4) but they were weaker

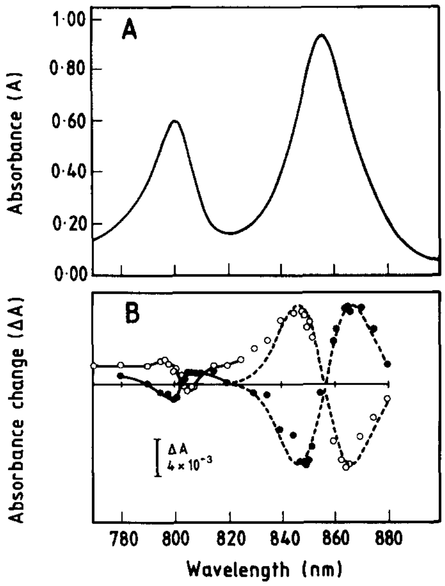

Fig. 2. (A) Absorbance spectrum and (B) electrochromic and modelled spectra of LHII proteoliposomes in the near i.r. Bacteriochlorophyll concentration, 10.8 μM. In part (B) the *closed points* are for positive-inside diffusion potentials and the *open points* are for negative-inside diffusion potentials. The *solid lines* are empirical curves for the data for B800 electrochromism. The *dashed lines* are modelled curves for the B850 electrochromism; for positive-inside and for negative-inside diffusion potentials. The former was obtained by shifting the best Gaussian fit of the B850 absorbance band 0.15 nm to longer wavelengths and subtracting the original; the latter is similar but applying a 0.15 nm shift to shorter wavelengths.

than those in the Q_y region. The Soret absorbance changes had the character of a band shift. Within error they can be attributed to a homogeneous shift to longer wavelengths by 0.025 nm/105 mV of the main absorbance band. However, the B_x and B_y bands of both B800 and B850 are difficult to resolve in this region of the spectrum and the origin(s) of the electrochromic response can not be determined. The application of diffusion potentials in chromatophores led to electrochromic absorbance spectra in the Soret region which were very similar to those observed in LHII-liposomes (data not shown).

The Q_x bands of the bacteriochlorophylls around 600 nm in chromatophores are thought to be susceptible to membrane potential [15] but this could not be confirmed in the proteoliposome experiments. This is probably because in chromatophores the electro-

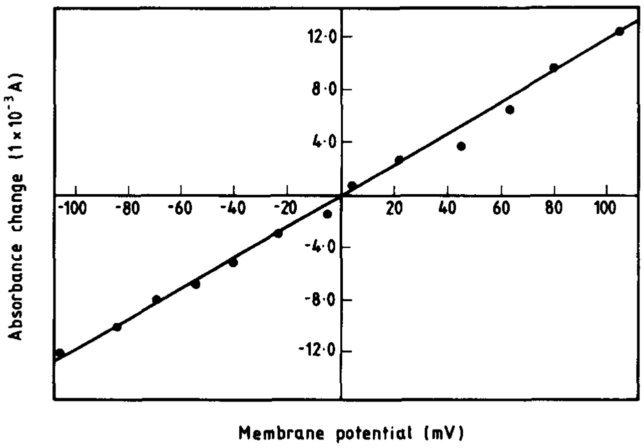

Fig. 3. Dependence of B850 absorbance changes on the value of the diffusion potential. Absorbance changes were recorded at 865–850 nm using the procedures and calculations described [13].

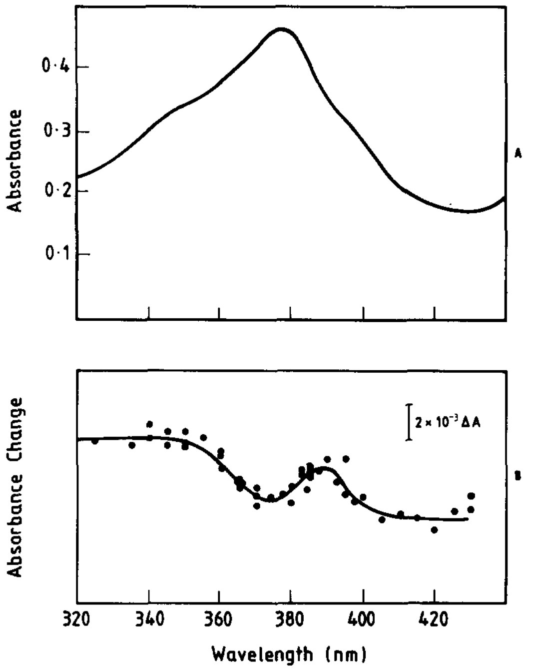

Fig. 4. (A) Absorbance spectrum and (B) electrochromic spectrum of LHII proteoliposomes in the Soret region. Only the response to positive-inside diffusion potentials is shown.

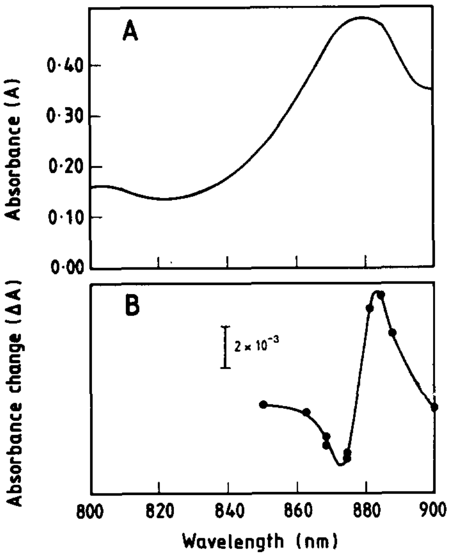

Fig. 5. (A) Absorbance spectrum and (B) electrochromic spectrum of LHI proteoliposomes in the near i.r. Only the response to positive-inside diffusion potentials is shown. Bacteriochlorophyll concentration, 5 μM.

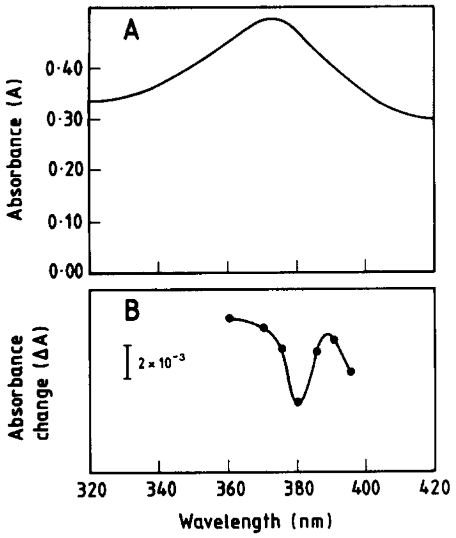

Fig. 6. (A) Absorbance spectrum and (B) electrochromic spectrum of LHI proteoliposomes in the Soret region. Only the response to positive-inside diffusion potentials is shown. Bacteriochlorophyll concentration, 5 μM.

chromic absorbance changes were detected at high resolution [15] by averaging the light-induced signals, a technique that is not feasible for diffusion potential-induced events.

Although the carotenoid bands of LHI proteoliposomes were not detectably electrochromic [13], the bacteriochlorophyll bands of this complex at 880 nm and 374 nm did respond to membrane potential (Figs. 5 and 6). As with B850 in LHII proteoliposomes, the electrochromism of B870 was linear and could be modelled by a homogeneous shift of the entire absorbance band (note that there is a contribution from the light scattering of the proteoliposomes to the spectra shown in Figs. 5A and 6A). The extent of the electrochromic response was very similar for B850 and B870 (for a diffusion potential of 105 mV, the former was shifted by 0.15 nm and the latter by 0.18 nm). Electrochromic absorbance changes of the 590 nm band were not observed in LHI proteoliposomes.

Discussion

From a comparison of Figs. 1 and 2 it can be seen that the electrochromic absorbance changes of chromatophores in the near i.r. arise mainly from bacteriochlorophylls associated with LHII. The bacteriochlorophyll band at 880 nm in LHI is responsive to membrane potential (Fig. 5), but its contribution to the electrochromic spectrum of chromatophores is less due to the relatively low concentration of this complex in membranes of cells grown at moderate light intensities [16].

It was concluded on the basis of the electrochromic absorbance changes of carotenoids [13] that in LHII liposomes the protein adopts predominantly the same orientation as in chromatophores. This is fully supported by the data shown in Figs. 1 and 2 of this report. The application of a positive-inside diffusion potential led to a red shift of the B850 absorbance band in both chromatophores and the proteoliposomes. We can be less sure about the protein orientation of LHI in proteoliposomes where the carotenoids are not detectably electrochromic. However, the fact that the electrochromic spectra indicate band shifts to longer wavelengths in the Soret region and in the near-i.r. of both LHI and LHII suggests a similar orientation.

All the electrochromic absorbance changes attributable to B800, B850 and B870 in LHII and LHI proteoliposomes are, within error, modelled by small (< 1 nm) homogeneous band shifts to longer wavelengths, indicating that the response is proportional to the first derivative of the absorbance spectrum. Together with the linear relationship between the electrochromic absorbance changes and the value of the diffusion potential (Fig. 3), this indicates that, as predicted for a molecule with a large permanent dipole moment, the first term in equation (i) is predominant. Since the electric fields are generated perpendicular to the liposome membrane, the electrochromic spectra can, in principle, provide information on the orientation of the bacteriochlorophylls relative to the membrane plane. Unfortunately, neither the magnitude nor the direction within the molecule of the permanent dipole moment of bacteriochlorophyll is known. The directions of the dipole moment difference of chlorophyll *a* and *b* have been deduced on the basis of the influence of the polar groups on the chlorin skeleton molecular orbitals [7] – it was thought that the dipole moment differences have approximately the same directions as the corresponding transition dipole moments. We make the minimal assumption for bacteriochlorophyll that the permanent dipole moment difference lies in the plane of the bacteriochlorin ring.

Several models for the structure of the light-harvesting complexes have been proposed [12,17]. Predictions of the orientation of the bacteriochlorophylls relative to the plane of the membrane are based on measurements of chromatophores air-dried on to glass slides – it was assumed that the drying process caused flattening of the chromatophore membranes in the plane of the slide [11]. It then followed from linear dichroism data that the transition dipoles corresponding to the Q_y bands of B800 and B850 are almost parallel to the plane of the membrane. With hindsight, the conclusion that the Q_x transition moments lie more perpendicular to the membrane plane [11] is questionable since linear dichroism around 600 nm will be complicated by the overlapping absorbance contributions from several different bacteriochlorophylls. The relative orientations of the Q_x and Q_y transition moments of B800 and B850 have been analysed by measurements of fluorescence polarisation in isolated LHII. However, those measurements could only be related to the plane of the membrane on the basis of the finding that pressed gels containing isolated detergent-solubilised LHII showed similar linear dichroism to the air-dried chromatophore films. Thus, the proposed orientations of the bacteriochlorophylls cannot be considered definitive. However, the proposals do receive some support from the experiments described in this report. Following the arguments presented above and assuming that the dipole moment differences for the pigments are similar, the fact that the electrochromism of B850 bacteriochlorophyll in proteoliposomes is much stronger than that of B800 (Fig. 2) might suggest that the plane of the bacteriochlorin ring of the former is more perpendicular to the membrane than the plane of the latter, in agreement with current models of LHII (see [12,17]). Incidentally, the small red shift of the B800 band in *Rb. capsulatus* is replaced in *Rb. sphaeroides* by a small blue shift [14,2]. Perhaps in both organisms the B800 bacteriochlorin ring lies approximately parallel to the plane of the membrane but a slight tilt of the ring is oppositely directed in the two organisms.

There is some evidence, however, that electric fields lead to a larger electrochromic response (Stark effect) of B850 than B800 also in an isotropic system, a frozen glycerol/buffer glass of a detergent-solubilised preparation of LHII, and this was taken to indicate a larger intrinsic dipole moment difference, $\mu_g - \mu_e$ in equation (i), for B850 than B800 [18]. Were this to be the case, then the above conclusions about the orientation of the bacteriochlorin rings in our anisotropic system would be untenable. In fact, a puzzling feature of the isotropic glass samples was that the electrochromic spectra included, not only a contribution that was proportional to the second derivative of the absorbance spectrum, as would be predicted from the expected dependence upon the third term of equation (i), but also a first derivative contribution [18]. This might indicate that the second term in equation (i), which includes the polarisation difference ($\alpha_e - \alpha_g$), has a significant influence on electrochromism of LHII in isotropic systems: the larger electrochromic effect from B850 than B800 would then be due to a greater polarisation difference in the former. However, this does not necessarily mean that the second term in equation (i) is also significant in the anisotropic reconstituted system. Indeed the linear, rather than quadratic, dependence on membrane potential (and hence field strength) supports the conclusion (above) that the first term in equation (i) is dominant.

On the basis of amino acid homology in the region of the conserved histidine residues thought to bind B870 in LHI and B850 in LHII it has been suggested [19,17] that these two bacteriochlorophylls might occupy similar environments within their respective proteins. Although there are uncertanties over the direction of insertion of the light-harvesting complexes in liposomes, the fact that the electrochromic sensitivities of B870 in LHI and B850 in LHII are approximately equal (Figs. 5 and 2, respectively) indicates that the factor in the first term of equation (i), which includes the permanent dipole moment difference and the orientation factor, is similar for the two bacteriochlorophylls and supports the view derived from the amino acid sequences.

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