Biochimica et Biophysica Acta, 973 (1989) 205–211
Elsevier

BBA 42936

Reconstitution of electrochromically active pigment-protein complexes from *Rhodobacter sphaeroides* into liposomes

Wim Crielaard, Klaas J. Hellingwerf * and Wil N. Konings

Department of Microbiology, University of Groningen, Haren (The Netherlands)

(Received 26 August 1988)

Key words: Carotenoid band shift; Pigment-protein complex; Reaction center; Light harvesting complex; Reconstitution; (*Rb. sphaeroides*)

Light-harvesting complex II (B800/850, LHC II complexes) and reaction centers with both light-harvesting complex I and II (B875 and B800/850; i.e., RC LHC I-LHC II complexes) have been isolated from *Rhodobacter sphaeroides*. Both complexes have been incorporated into liposomes made from phospholipids purified from *Escherichia coli*. The electrochromic band shift of carotenoids, present in these complexes, could still be observed in these liposomes upon generation of a potassium diffusion potential. For RC LHC I-LHC II complexes the characteristics of the liposomes were studied in more detail: the extent of the absorbance changes increased with the amount of pigment protein complex incorporated and was maximal at a ratio of 70 nmol bacteriochlorophyll per mg lipid. Higher amounts of incorporated complexes led to a decrease in carotenoid signal due to an increasing membrane leakage. The carotenoid absorbance change at 503–487 nm showed a linear dependency on the diffusion potentials both in the negative as well as in the positive potential range. The spectrum of the absorbance changes at a fixed diffusion potential for RC LHC I-LHC II liposomes had a similar shape as the spectrum found for chromatophores of *Rb. sphaeroides*, however, with shifted maxima and minima. The spectrum found for LHC II liposomes was the inverted spectrum of the RC LHC I-LHC II liposomes. Electron micrographs of both type of liposomes showed distinct protein particles with a diameter of 15 nm for RC LHC I-LHC II and 11 nm for RC LHC II complexes.

Introduction

The electrochromic behaviour of carotenoids has been widely used to determine the electrical potential difference ($\Delta\psi$) across phototrophic membranes, like chromatophores [1], bacterial cells [2,3] and chloroplasts [4,5]. The carotenoid absorbance change has the advantage over other methods for recording the $\Delta\psi$ that the response is rapid. This method therefore allows measurements of actual changes of the $\Delta\psi$ which is not possible with the widely used distribution procedure of lipophilic ions, since the diffusion of these probes across the membrane is not fast enough (see, e.g., Ref. 3). Other advantages of the carotenoid band shift as $\Delta\psi$ indicator are: (i) the method is non-invasive, since carotenoids are natural components of the photosynthetic membrane and (ii) the linear relationship between the $\Delta\psi$ and the measured absorbance change [1], which makes it possible to read directly the $\Delta\psi$ change in vivo without corrections. The major disadvantages of the carotenoid band shift as a $\Delta\psi$ indicator (see, e.g., Ref. 3) are primarily that a calibration of the $\Delta\psi$-dependent bandshifts is not always possible in the experimental system used [3], and secondly that also other compounds can cause absorbance changes in the carotenoid region.

Holmes et al. [6] and Webster et al. [7] have shown that the carotenoids which are responsible for the large linear membrane potential response in photosynthetic bacteria are only those located in the light-harvesting complex II (LHC II) of the photosynthetic apparatus. Exploration of the carotenoid band shift as a $\Delta\psi$ probe in other membrane systems like liposomes and fused membranes therefore requires the reconstitution of pigment protein complexes which contain electrochromically active LHC II complexes in these membrane systems.

* Present address: Department of Microbiology, University of Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands.

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; RC LHC I (II), reaction center light-harvesting complex I (II); BChl, bacteriochlorophyll; diSC(3)5, 3,3'-dipropylthiadicarbocyanine iodide.

Correspondence: W.N. Konings, Laboratorium voor Microbiologie, Rijksuniversiteit Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands.

Matsuura and Nishimura [8] have shown that it is possible to reconstitute solubilized chromatophore extracts which still show electrochromic behaviour, demonstrating that such a procedure does not destroy these characteristics. This conclusion can also be drawn from the fusion studies of Carcia et al. [9].

Previously, we have reported protonmotive force generation in liposomes containing reaction centers and antenna pigments from *Rhodopseudomonas palustris* [10]. In this study a modified procedure of Varga and Staehelin [11] was used to purify pigment-protein complexes from *Rhodobacter sphaeroides*. Electrochromic absorbance changes have been more extensively described in intact cells and chromatophores from this organism than in corresponding samples from *Rhodopseudomonas palustris*. Reconstitution of either reaction centers with both antenna complexes attached (RC LHC I-LHC II complexes) or isolated (RC LHC II complexes) in liposomes was achieved. In these liposomes the electrochromic carotenoid band shifts in response to an imposed $\Delta\psi$ were studied.

Materials and Methods

Growth of Rb. sphaeroides and preparation of chromatophores

Rb. sphaeroides, strain 2.4.1., was grown anaerobically at low light intensity (equidistant from two 60 W tungsten lamps placed 50 cm apart) in 1 l bottles in the medium described by Sistrom [12] at 30 °C. Cells were harvested at an A_{660} of approx. 3, washed twice in 50 mM potassium phosphate (pH 7.6), 50 mM KCl, 8 mM $MgCl_2$, 10% sucrose and resuspended in this buffer.

Chromatophores were prepared from these cells by two successive passages through a French pressure cell at 125 MPa, 0 °C. Debris was removed from the preparation by low spin centrifugation (30 min, 20000 × *g*, 0 °C). Chromatophores were collected by ultracentrifugation (1 h, 200000 × *g*, 4 °C) and resuspended in 50 mM potassium phosphate (pH 7.6), 50 mM KCl, 8 mM $MgCl_2$, 10% sucrose, to 1–1.5 mM bacteriochlorophyll. The chromatophores were kept on ice until further use.

Isolation of pigment-protein complexes

The pigment-protein complexes were isolated from the chromatophores according to a modified procedure from Varga and Staehelin [11] as described by Molenaar et al. [10], but using cholate instead of *n*-octyl-β-D-glucopyranoside (octylglucoside). The complexes were extracted from the membranes with 1% cholate (RC LHC I-LHC II complexes) or a mixture of 1% cholate and 2% deoxycholate (LHC II complexes), in 50 mM potassium phosphate (pH 7.6), 50 mM KCl, 8 mM $MgCl_2$, 10% sucrose plus 20 mM potassium EDTA (ethylenediaminetetraacetate) for 1 h at 0 °C. The mixture was vortexed every 5 min during solubilization. Non-solubilized material was removed by centrifugation (2 min in an Eppendorf centrifuge). The supernatant (0.3 ml) was layered on a 9 ml sucrose gradient with 10–50% sucrose (w/v) in 50 mM potassium phosphate (pH 7.6), 50 mM KCl and either 1% cholate or 1% cholate plus 2% deoxycholate, for RC LHC I-LHC II or RC LHC II isolation, respectively. Gradients were centrifuged in a Beckman SW 41 Ti rotor (19 h, 35000 r.p.m., 6 °C). Pigmented bands were recovered from the gradient, analyzed spectrophotometrically and kept on ice until further use.

Incorporation of pigment protein complexes into reconstituted liposomes

Acetone-washed *Escherichia coli* phospholipids (type IX, mainly L-α-phosphatidylethanolamine, Sigma Chem. Comp., St. Louis, MO, U.S.A.), dispersed in 50 mM potassium phosphate (pH 7.6), 50 mM KCl and 1% cholate, was sonicated to clarity under a constant stream of nitrogen gas at 0 °C using a probe type sonicator (MSE Scientific Instruments, West Sussex, U.K.) at an output of 2 μm. After the addition of pigment-protein complexes (at the desired BChl/lipid ratio) the mixture was dialyzed at 4 °C for 20 h against a 1000-fold volume of 50 mM potassium phosphate (pH 7.6), 50 mM KCl (three changes). After dialysis the liposomes were stored in 1 ml aliquots in liquid nitrogen. Before use the liposomes were thawed slowly at room temperature and sonicated twice for 3 s at 0 °C with the probe-type sonicator, at an output of 2 μm. The liposomes were concentrated in 50 mM potassium phosphate (pH 7.6), 50 mM KCl by ultracentrifugation (1 h, 200000 × *g*, 4 °C) and again sonicated two times for 3 s (0 °C, 2 μm) to remove aggregates.

Potassium-diffusion potentials

Potassium-diffusion potentials, inside negative, were induced in chromatophores and concentrated liposomes by diluting the samples in 50 mM sodium phosphate (pH 7.6), 50 mM NaCl (supplemented with 10% sucrose and 8 mM $MgCl_2$ in the case of chromatophores) and adding 100 nM valinomycin. The potassium-diffusion potentials were varied by varying the external Na^+-buffer/K^+-buffer ratio.

For applying potassium-diffusion potentials, inside positive, the liposomes were washed once in 50 mM sodium phosphate (pH 7.6), 50 mM NaCl in the presence of 250 nM valinomycin. Membranes were recovered by ultracentrifugation and resuspended in the same buffer. The potentials were induced by KCl pulses and varied by changing the final KCl concentration.

Absorbance changes

Dual-beam absorbance changes were measured with a chopped, dual-wavelength Aminco DW-2a spectro-

photometer. Spectra were recorded and analyzed using a single-beam Philips PU8700 series UV/VIS spectrophotometer; spectra were plotted using the medium smoothing mode. All experiments were performed at 20 °C in a 3 ml quarts cuvette with continuous stirring.

Electron micrographs

Freeze-fracture electron micrographs of reconstituted liposomes were prepared from freon-frozen liposomes in 50 mM potassium phosphate (pH 7.6), 60 mM KCl, 10% glycerol. For freeze-fracturing a Balzer's freeze-etch unit was used according to the method described by Moor [13]. The replicas were examined in a Philips EM 300 electron microscope.

Analytical procedures

Bacteriochlorophyll was estimated at 772 nm in acetone methanol extracts according to Clayton [14].

Results

Functional reconstitution of membrane proteins is strongly affected by the type of detergent used for solubilization. In order to reconstitute electrochromically active RC LHC I-LHC II or RC LHC II complexes several types of detergent were tested. The use of ionic detergents (like 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) or cholate) during solubilization and sucrose gradient centrifugation yielded more active preparations than non-ionic detergents (like, e.g., octylglucoside; data not shown). Since cholate (1%) gave the best preparations we have used this detergent throughout our experiments. The addition of deoxycholate (2%) was needed for breaking the coupling between the LHC II complexes and the RC LHC I core. With this modification of the procedure of Varga and Staehelin [11] we obtained preparations from *Rhodobacter sphaeroides* with a similar degree of purity and pigment/protein ratios as described previously for *Rhodopseudomonas palustris* [10,11].

Potassium-diffusion potentials in reconstituted liposomes

After reconstitution of the isolated pigment-protein complexes the absorbance change of the reconstituted carotenoids was measured at 503–487 nm (a wavelength pair which is commonly used to measure carotenoid absorbance changes in 'green mutants', see Ref. 15) upon the induction of a potassium-diffusion potential. Fig. 1 shows time-courses of absorbance changes in liposomes reconstituted with RC LHC I-LHC II complexes, loaded with either K^+- or Na^+-buffer. The K^+-loaded liposomes showed, after dilution in Na^+-buffer, an increase in absorbance at 503–487 nm upon the addition of valinomycin. This increase could be abolished by the subsequent addition of nigericin, which leads to uncoupling of the membrane (Fig. 1A). When

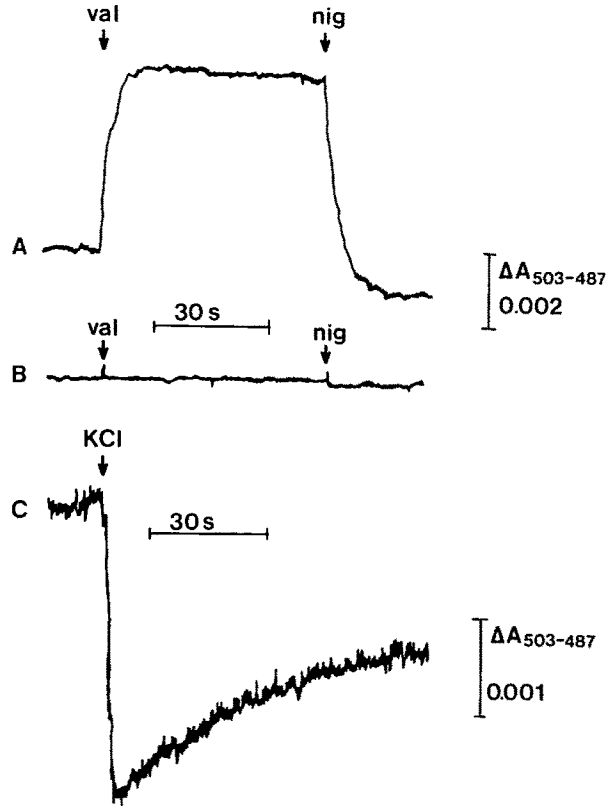

Fig. 1. Carotenoid absorbance changes at 503–487 nm induced by K^+-diffusion potentials in liposomes reconstituted with RC LHC I-LHC II complexes (70 nmol BChl per mg lipid). Liposomes loaded with K^+ (A, B) or Na^+ (C) were diluted 67-fold in 2.0 ml 50 mM sodium phosphate (pH 7.6)/50 mM NaCl (A, C) or 50 mM potassium phosphate (pH 7.6)/50 mM KCl (B). Where indicated valinomycin (val) and nigericin (nig) were added to give final concentrations of 100 nM and 250 nM, respectively. KCl was added from a 3M stock solution to give a final K^+ concentration of 295 mM. The final BChl concentration in all experiments was 6.6 μM

these liposomes were diluted in K^+-buffer no absorbance changes could be detected upon the addition of valinomycin or nigericin (Fig. 1B). A potential with the opposite polarity, induced by the addition of external KCl to Na^+-loaded liposomes, resulted in a decrease in absorbance at 503–487 nm (Fig. 1C). No significant absorbance band shifts were observed in liposomes reconstituted with RC LHC I complexes.

Spectra of the absorbance changes

In intact bacterial cells and chromatophores the carotenoid absorbance changes show a characteristic spectrum, which can be explained by a shift (to higher or lower wavelengths) of the absorbance maxima of a part of the carotenoids in all three carotenoid peaks [16,17]. In order to find out whether such a characteristic spectrum could also be observed in the reconstituted liposomes the spectral changes in the carotenoid region were measured of liposomes which contained RC LHCI-LHC II or RC LHC II complexes and were loaded with K^+-buffer. For comparison also the spec-

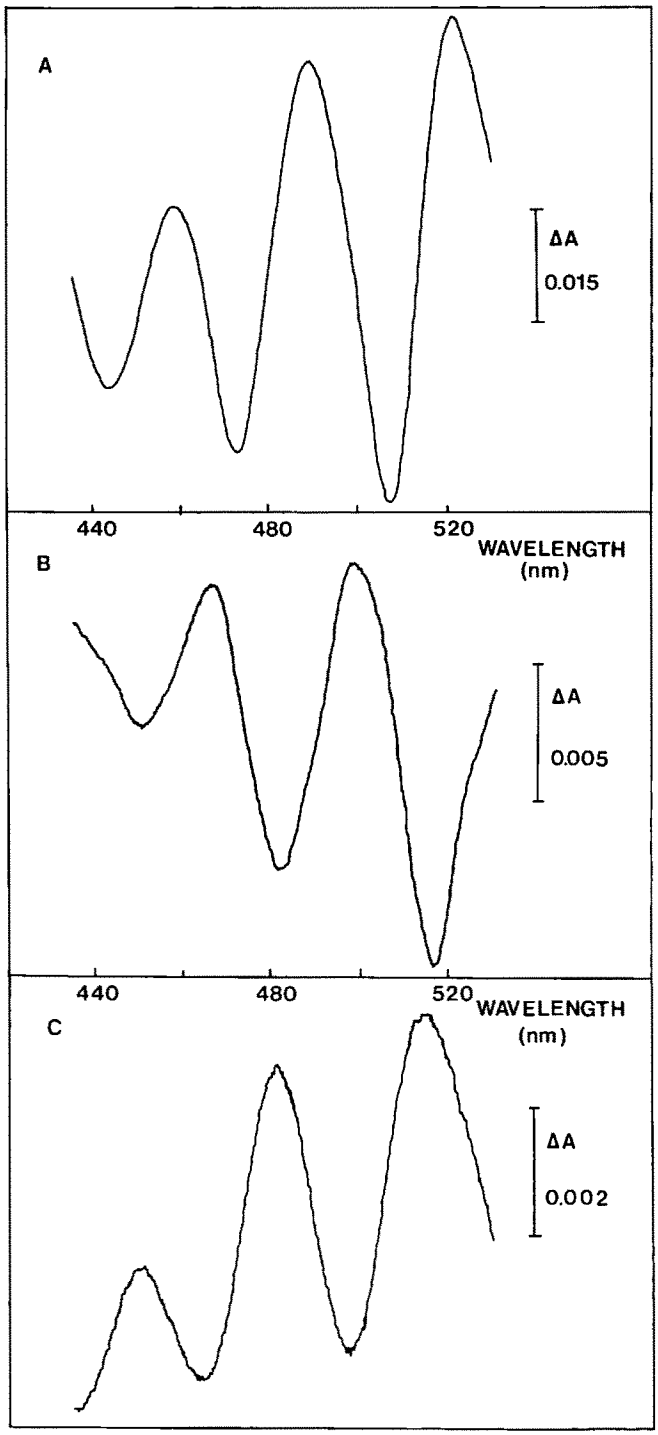

Fig. 2. Spectra of carotenoid absorbance changes induced by K^+-diffusion potentials in chromatophores of *Rb. sphaeroides*, liposomes containing RC LHC I-LHC II complexes (70 nmol BChl per mg lipid) and liposomes containing LHC II complexes (60 nmol BChl per mg lipid). K^+-loaded chromatophores and liposomes were diluted 50-fold into 2 ml 50 mM potassium phosphate (pH 7.6) 50 mM KCl (supplemented with 10% sucrose and 8 mM $MgCl_2$ in the case of chromatophores). The figures show the difference between the spectra before and after the addition of 100 nM valinomycin. (A) Chromatophores: final BChl concentration, 36 μM; (B) RC LHC I-LHC II liposomes: final BChl concentration, 8.8 μM; (C) RC LHC II liposomes: final BChl concentration, 6.4 μM.

tral changes in the same region were recorded of *Rb. sphaeroides* chromatophores from which the pigment-protein complexes were isolated. Fig. 2 shows the result of these analyses. Chromatophores showed a diffusion-potential-induced difference spectrum with maxima at 521, 488 and 458 nm and minima at 506, 472 and 442 nm (Fig. 2A). Liposomes containing RC LHC I-LHC II complexes showed a similarly shaped difference spectrum (Fig. 2B), however, with different maxima (499 and 467 nm) and minima (517, 482 and 451 nm). An inverted difference spectrum with respect to the RC LHC I-LHC II liposomes was found in liposomes containing LHC II complexes (Fig. 2C): maxima at 517, 482 and 451 nm and minima at 499 and 467 nm. No changes in the absolute spectra of the different preparations were observed in this spectral region (data not shown).

Protein-to-lipid ratio

The magnitude of the carotenoid band shift in the reconstituted liposomes was expected to increase with the amount of probe present in the membrane and therefore with increasing pigmented protein-to-lipid ratios. However, an increase of the protein-to-lipid ratio will also decrease the tightness of the membrane and therefore increase the ion leakage through the membrane. The optimal protein-to-lipid ratio was determined by varying the amount of reconstituted RC LHC I-LHC II in the liposomal membrane and measurement of the carotenoid signal after imposing a

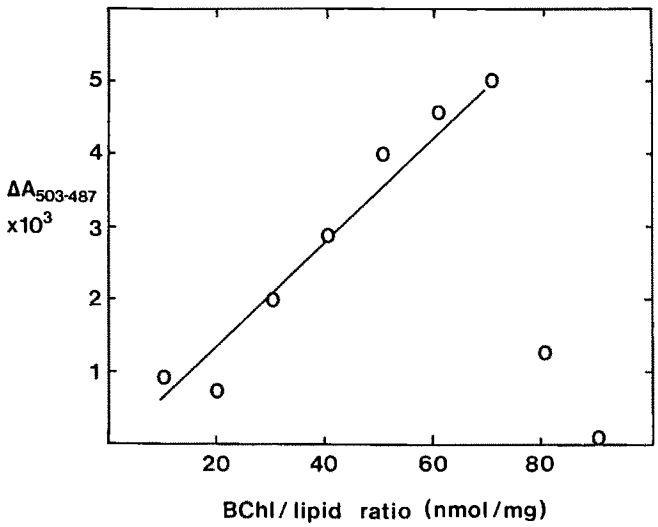

Fig. 3. The effect of increasing amounts of RC LHC I-LHC II incorporated into liposomes on the carotenoid signal at 503–487 nm. Liposomes reconstituted with varying amounts of RC LHC I-LHC II (but a constant lipid concentration), loaded with 50 mM potassium phosphate (pH 7.6), 50 mM KCl (as described in Materials and Methods) were diluted 67-fold in 2.0 ml 50 mM sodium phosphate (pH 7.6)/50 mM NaCl. Potassium-diffusion potentials were induced by adding 100 nM valinomycin. Carotenoid-absorbance changes were recorded at 503–487 nm. The maximal absorbance changes (upon the addition of valinomycin) are plotted.

constant (−110 mV) potassium-diffusion potential. As can be seen in Fig. 3 the carotenoid signal increased proportionally with increasing amounts of protein incorporated in the liposomes, up to 70 nmol BChl per mg lipid for RC LHC I-LHC II complexes. Above this ratio the signal dropped steeply and at 90 nmol BChl per mg lipid no absorbance change could be observed. Separate membrane potential measurements (with the cyanine dye 3,3′-dipropylthiadicarbocyanine iodide (diSC(3)5)) showed that at this ratio no valinomycin-induced potential could be generated (data not shown), indicating an increased ion permeability of the liposomal membrane. A ratio of 70 nmol BChl per mg lipid was therefore used in all further experiments.

Relation between the electrical membrane potential and the carotenoid signal

In bacterial systems a linear relationship instead of the theoretically predicted quadratic relationship has been found between the carotenoid absorbance change at a given wavelength pair and the membrane potential [1,18]. It has been suggested that this linear relationship is caused by a permanent electric field [6], resulting from charged amino acids in the neighbourhood of the field-sensitive carotenoids. Purification and/or reconstitution could lead to a change of this permanent field and thereby change the relationship between the electric field and the induced absorbance change. Fig. 4 shows that this is not the case in RC LH I-LHC II liposomes. The absorbance change at 503–487 nm was propor-

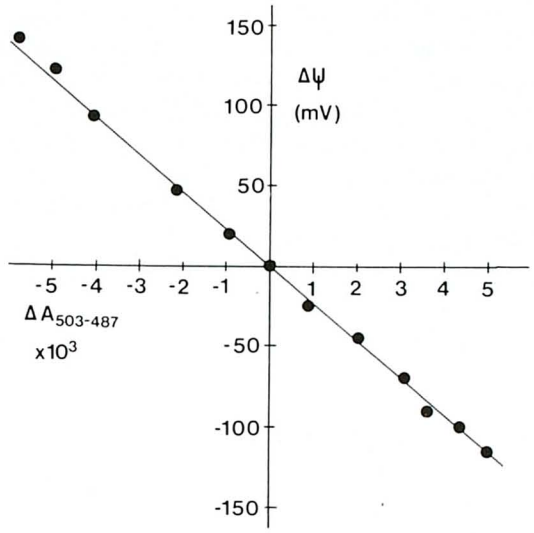

Fig. 4. Relation between the carotenoid absorbance changes at 503–487 nm and the membrane potential in RC LHC I-LHC II liposomes (70 nmol BChl/mg lipid). K^+- or Na^+-loaded liposomes were diluted 67-fold in 50 mM sodium phosphate (pH 7.6)/50 mM NaCl. Potentials negative inside (negative $\Delta\psi$) were induced by adding 100 nM valinomycin to the K^+-loaded liposomes. The potential was varied by varying the K^+-buffer-to-Na^+-buffer ratio. Potentials inside positive (positive $\Delta\psi$ (= the transmembrane potential difference, defined as the electrical potential inside minus the electrical potential outside)) were induced by the addition of KCl to the Na^+-loaded liposomes. KCl was added from a stock solution of 3 M. The potential was varied by varying the amount of KCl added. A correction was made for dilution. The potentials were calculated after the determination of the internal K^+-concentration by extrapolation to zero bandshift. The final BChl concentration was in all cases 6.6 μM.

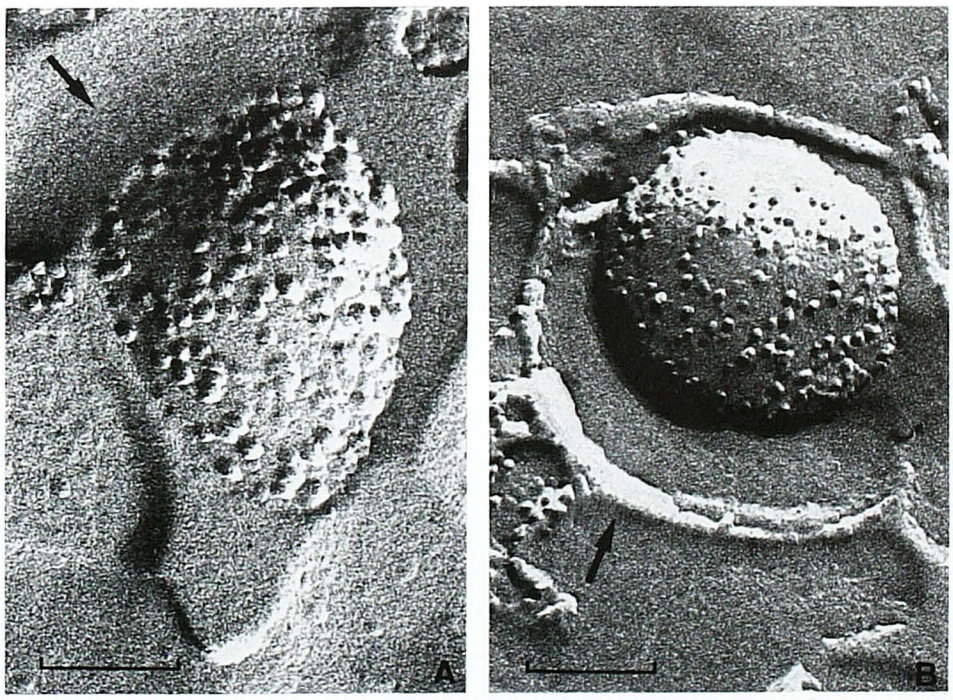

Fig. 5. Freeze-fracture electron micrographs of liposomes reconstituted with pigment-protein complexes from *Rhodobacter sphaeroides*. (A) Liposomes containing RC LHC I-LHC II complexes at 70 nmol BChl per mg lipid; (B) liposomes containing RC LHC II complexes at 60 nmol BChl per mg lipid. The bars equal 100 nm. Electron micrographs were prepared as described in Materials and Methods.

tional to the applied potential in the range from +133 mV (KCl-pulses) to −110 mV (valinomycin pulses).

The internal K^+-concentration of the Na^+-loaded liposomes after washing (1.25 mM), was calculated by extrapolating the induced absorbance change to zero (where the internal $[K^+]$ equals the external $[K^+]$). A correction was made for dilution with KCl (by the addition of an amount of buffer equaling the KCl pulse, after relaxation of the induced potential). Using these calculations a calibration factor for the RC LHC I-LHC II liposomes of $6.6 \cdot 10^{-6}$ $(mV)^{-1}$ per μM BChl can be determined.

Electron micrographs of the reconstituted liposomes

The reconstituted liposomes were further characterized with respect to their structure by freeze-fracture electron microscopy. Fig. 5 shows such micrographs for RC LHC I-LHC II liposomes (Fig. 5A) and RC LHC II liposomes (Fig. 5B). The particles visible in the fractured liposomes are in both cases evenly distributed over the two fracture faces and homogeneously over the liposomes. The size of the liposomes is slightly heterogeneous and ranges in both cases from approx. 75 to 300 nm diameter. The number of particles in the fracture faces ranges from 10 to 200 in the case of RC LHC I-LHC II liposomes and from 0 to 75 in the case of RC LHC II liposomes. The size of these particles is 15 nm for RC LHC I-LHC II complexes and 11 nm for RC LHC II complexes. Few multilamellar membrane structures were observed in these preparations.

Discussion

We report in this study that pigment-protein complexes, isolated from *Rhodobacter sphaeroides* according to a procedure modified from Varga and Staehelin [11], still show membrane-potential dependent carotenoid absorbance changes after reconstitution into *Escherichia coli* lipids. In RC LHC I-LHC II liposomes the potassium-diffusion potential-induced absorbance change can be observed at 503–487 nm (Fig. 1). This absorbance change can be abolished by uncoupling the membranes (by the subsequent addition of nigericin, Fig. 1A). Like in intact photosynthetic membranes [1], the direction of the absorbance change is dependent on the polarity of the applied potential: potentials negative inside lead to an increase in absorbance at 503–487 nm (Fig. 1A), whereas potentials positive inside lead to a decrease (Fig. 1C). Van Walraven et al. [19] showed that in liposomes prepared from *Synechococcus* lipids the carotenoids, still present in these lipids (i.e. not associated with antenna proteins), undergo a red shift irrespective of the polarity of the induced potentials.

The extent of the induced absorbance change at a fixed potential is, up to 70 nmol BChl per mg lipid of RC LHC I-LHC II incorporated, almost linear with the amount of pigmented protein (Fig. 3). Assuming that all (or a fixed ratio) of the added pigment-protein complexes are indeed incorporated so that the BChl/LHC II ratio is constant in the different preparations, these results are comparable with those found by Holmes et al. [6] who showed that in intact cells of *Rb. sphaeroides* the extent of the carotenoid absorbance change increases proportionally with the amount LHC II present in the membranes. It can also be seen in Fig. 3 that incorporation of higher amounts of pigment-protein complexes leads to a decrease in the signal. Separate measurements of K^+-diffusion potentials with a cyanine dye have shown that at these high protein-to-lipid ratios the liposomal membrane has a higher ion permeability. Reconstitution of protonmotive force generation with RC LHC I-LHC II complexes from *Rhodopseudomonas palustris* shows an optimum at a similar protein-to-lipid ratio [10].

Both RC LHC I-LHC II and RC LHC II liposomes show typical band-shift difference spectra (Fig. 2B and C), similar to the one obtained for chromatophores (Fig. 2A, see also Ref. 1). The most consistent interpretation of the shape of these spectra assumes that solubilisation and/or reconstitution of the pigmented proteins causes a slight blue-shift (approx. 4–5 nm) of the field-sensitive carotenoids (i.e., those associated with B800 in LHC II; see Refs. 6 and 7). When this blue-shift is taken into account, it is clear that the carotenoids in chromatophores and in RC LHC I-LHC II complexes give a mirror response when exposed to a $\Delta\psi$, inside negative. This would be consistent with an opposite orientation of the carotenoid associated with B800 in these two samples. Indeed it was found that RC LHC I-LHC II complexes, reconstituted in *E. coli* lipids, do have their cytochrome *c* binding site exposed to the external aqueous phase for more than 95% (Crielaard, W. and Hellingwerf, K.J., unpublished results). This is the same orientation as observed for RC LHC I-LHC II complexes from *Rps. palustris* after reconstitution in *E. coli* phospholipids [10] and contrasts their orientation in chromatophores. Along similar lines it is predicted that LHC II will have opposite orientations when reconstituted either as a single complex or in combination with RC LHC I (compare Fig. 2B with Fig. 2C). A small blue-shift (4 nm) has recently been observed in the absorption spectrum of carotenoids in the LHC II complex from *Rps. acidophila,* upon incubation with a low concentration of an ionic detergent (lithiumdodecylsulphate) [20]. However, since no differences in the absolute spectra of the different preparations were observed, the actual shift is probably obscured by the carotenoids associated with B850, which do not necessarily have to show an identical shift as the field-sensitive carotenoids. The results described in Fig. 2 make it unlikely that either of the two complexes has been reconstituted in a random orientation. In that case a

doubling of the number of minima and maxima in the $\Delta\psi$-induced difference spectrum would be expected.

The size of the protein particles found in the fracture faces in the electron micrographs is comparable to those reported by Varga and Staehelin [11] for *Rps. palustris*. For RC LHC I-LHC II complexes an average of 15 nm was observed (compared to 12.5–15 nm in Ref. 11). Particles caused by LHC II complexes have an average diameter of 11 nm. This is slightly larger than the corresponding complexes from *Rps. palustris*.

The most attractive feature of the carotenoid absorbance change, the linear relationship with the $\Delta\psi$ (cf. Ref. 1), has been preserved in the liposomes. Fig. 4 shows that both potentials negative and positive inside provoke a linear response with a proportionality constant of $6.6 \cdot 10^{-6}$ $(mV)^{-1}$ per μM BChl. This constant is probably different from the proportionality constant in chromatophores, due to the shifts in maxima and minima. The linear response implies that the postulated local field in the carotenoid region [6] has not been abolished by the isolation and reconstitution.

Liposomes containing electrochromically active pigment protein complexes can be used for several purposes. By fusing bacterial membrane vesicles with liposomes containing pigment-protein complexes [21] the carotenoid absorbance change can be used as a membrane potential probe in non-photosynthetic membranes. Such a system is well suited to investigate further the discrepancies between the membrane potential deduced from distribution measurements of lipophilic ions and from the carotenoid absorbance change [2,3,22]. However, measurements of the band-shift in RC LHC I-LHC II liposomes with a $\Delta\psi$, generated by illumination (cf. Ref. 10), have not yet been possible. These measurements are complicated by the spectral overlap between the band shift and absorbance changes due to the light-dependent changes in the redox state of cytochrome *c* [10].

Acknowledgements

This study was supported by the Netherlands Foundation for Chemical Research (SON) which is supported by the Netherlands Organization for the Advancement of Pure Research (NWO). The electron microscopy was kindly performed by J. Zagers and Dr. M. Veenhuis. We thank D. Molenaar for valuable discussions.

References

1 Jackson, J.B. and Crofts, A.R. (1969) FEBS Lett. 4, 185–189.
2 Clark, A.J. and Jackson, J.B. (1981) Biochem. J. 200, 389–397.
3 Crielaard, W., Cotton, N.P.J., Jackson, J.B., Hellingwerf, K.J. and Konings, W.N. (1988) Biochim. Biophys. Acta 932, 17–25.
4 Witt, H.T. and Zickler, A. (1973) FEBS Lett. 37, 307–310.
5 Witt, H.T. (1979) Biochim. Biophys. Acta 505, 613–621.
6 Holmes, N.G., Hunter, C.N., Niederman, R.A. and Crofts, A.R. (1980) FEBS Lett. 115, 43–48.
7 Webster, G.D., Cogdell, R.J. and Lindsay, J.G. (1980) Biochim. Biophys. Acta 591, 321–330.
8 Matsuura, K. and Nishimura, M. (1977) Biochim. Biophys. Acta 462, 700–705.
9 Carcia, A.F., Gad'on, N. and Drews, G. (1985) Arch. Microbiol. 141, 239–243.
10 Molenaar, D., Crielaard, W. and Hellingwerf, K.J. (1988) Biochemistry 27, 2014–2023.
11 Varga, A.R. and Staehelin, L.A. (1985) J. Bacteriol. 161, 921–927.
12 Sistrom, W.R. (1960) J. Gen. Microbiol. 22, 778–785.
13 Moor, H. (1964) Z. Zellforsch. 62, 545–580.
14 Clayton, R.K. (1960) Biochim. Biophys. Acta 75, 312–323.
15 Clark, A.J., Cotton, N.P.J. and Jackson, J.B. (1983) Biochim. Biophys. Acta 723, 440–453.
16 De Grooth, B.G. and Amesz, J. (1977) Biochim. Biophys. Acta 462, 237–246.
17 De Grooth, B.G. and Amesz, J. (1977) Biochim. Biophys. Acta 462, 247–258.
18 Wraight, C.A., Cogdell, R.J. and Chance (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 471–511, Plenum Press, New York.
19 Van Walraven, H.S., Krab, K., Hagendoorn, M.J.M. and Kraayenhof, R. (1985) FEBS Lett. 184, 96–99.
20 Robert, B. and Frank, H.A. (1988) Biochim. Biophys. Acta 934, 401–405.
21 Crielaard, W., Driessen, A.J.M., Molenaar, D., Hellingwerf, K.J. and Konings, W.N. (1988) J. Bacteriol. 170, 1820–1824.
22 Ferguson, S.J., Jones, O.T.G., Kell, D.B. and Sorgato, M.L. (1979) Biochem. J. 180, 75–85.