Biochimica et Biophysica Acta, 548 (1979) 458—470

BBA 47769

THE RECONSTITUTION OF ENERGY TRANSFER IN MEMBRANES FROM A BACTERIOCHLOROPHYLL-LESS MUTANT OF *RHODOPSEUDOMONAS SPHAEROIDES* BY ADDITION OF LIGHT-HARVESTING AND REACTION CENTRE PIGMENT-PROTEIN COMPLEXES

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(Received December 22nd, 1978)

Key words: Reaction center; Energy transfer; Photosynthetic membrane reconstitution; Bacteriochlorophyll; Pigment-protein complex; (Rp. sphaeroides)

Summary

Antenna and reaction centre complexes purified from photosynthetically-grown cells of *Rhodopseudomonas sphaeroides* have been mixed with cytoplasmic membranes prepared from an aerobically-grown bacteriochlorophyll-less mutant of *Rp. sphaeroides* (designated 01) in the presence of 1% sodium cholate. After removal of the cholate by dialysis, the dialysate was subjected to isopycnic centrifugation. Reconstituted cytochrome c_2 photooxidation and cytochrome *b* photoreduction were demonstrated in a pigmented fraction recovered from the sucrose gradient, suggesting that the pigment-proteins were incorporated into the 01 membrane.

The fluorescence properties of the system were examined. The appearance of a variable component after the initial fast fluorescence rise indicated that energy transfer occurred between the antenna and reaction centre proteins in the presence of 01 membrane. The order in which the system was assembled was important. Reconstituted energy transfer with a pre-dialysed reaction centre-antenna complex was more effective than when all the components were

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Abbreviations: P, bacteriochlorophyll dimer acting as primary electron donor; X, 'primary' electron acceptor stable on a μs time scale; Mops, 3-(*N*-morpholino)propranesulphonic acid; F_m, fluorescence yield with all the photochemical traps closed; F_o, fluorescence yield with all the photochemical traps open; F_{var}, slow rise in fluorescence between F_o and F_m.

mixed at once. Energy transfer was also reconstituted between added reaction centre protein and the endogenous antenna present in membranes from the pigmented, but aerobically-grown reaction centre-less mutant PM8dp of *Rp. sphaeroides*.

Preparations of 01 membranes reconstituted with reaction centre exhibited a light intensity dependent cytochrome c_2 photooxidation. At low exciting light intensities, preparations containing reconstituted antenna protein in addition to reaction centres showed greater membrane cytochrome c_2 photooxidation than preparations with the antenna omitted; this improvement was maximal when a pre-dialysed antenna-reaction centre complex was used.

Introduction

The photoheterotrophic bacterium *Rhodopseudomonas sphaeroides* may be grown aerobically in the dark. Under such conditions the synthesis of intracytoplasmic photosynthetic membranes is repressed and only a peripheral cytoplasmic membrane is formed which contains cytochromes of the *b*- and *c*-type [1,2]. This membrane appears to be the site of the incorporation of newly synthesized pigment protein complexes during adaptation to photosynthetic growth [3,4]; during the onset of photosynthetic activity these complexes may orient themselves by association with transport components already present.

The developing photosynthetic membrane contains two spectrally distinguishable forms of light-harvesting pigment-protein complex, *B*-850 and *B*-875 [5]. The function of these antenna bacteriochlorophyll-protein complexes is to capture light energy which then migrates to the reaction centre (designated PX) where it can be converted to chemical energy; the reaction centres thus act as energy traps. The mechanism of the migration is not yet understood (e.g., see Ref. 6).

Emission spectra of whole cells show little fluorescence from *B*-850 [7,8] and it has been suggested that energy from this component is transferred to the reaction centre via *B*-875. These and other considerations have led Monger and Parson to suggest a model for the photosynthetic unit with the reaction centres in interconnected pools of *B*-875 embedded in a matrix of *B*-850 [10]. Such a scheme is compatible with studies on chromatophore induction which suggest that the ratio of *B*-875 to reaction centre is more or less fixed, whereas the amount of *B*-850 can vary widely [5,11].

We have shown that purified reaction centre protein can be incorporated *in vitro* into membranes from an aerobically-grown bacteriochlorophyll-less mutant (01) of *Rp. sphaeroides* and that the resulting reconstituted membranes carry out the light-driven reactions characteristic of cyclic electron flow [12,13] with kinetics approaching those measured in chromatophores [14]. In this communication we describe the fluorescence properties of reconstituted preparations obtained by *in vitro* addition of both purified reaction centre and *B*-850 light-harvesting pigment-protein complexes [15] to membranes from the bacteriochlorophyll-less mutant. The approach of Heathcote and Clayton [16] in which fluorescence induction kinetics were used to monitor

energy transfer between purified antenna pigment-protein complexes and reaction centre traps in suspension has been applied to the reconstituted membrane preparations.

Our results are in accord with the view that a photosynthetic membrane may be assembled *in vivo* by insertion of pigment proteins into an existing cytoplasmic membrane.

Methods

Growth of cells

Two mutants of *Rhodopseudomonas sphaeroides*, 01 and PM8dp, were grown aerobically, and R-26, GVP, and wild-type strain 2.4.1. grown anaerobically in the light as described previously [12], using the medium of Sistrom [17]. 01 is a bacteriochlorophyll-less mutant, prepared as described previously [12]. PM8dp is a pigmented mutant containing light-harvesting protein complexes but lacking reaction centre polypeptides; this was confirmed using SDS-polyacrylamide gel electrophoresis (unpublished observations). Strains R-26 and GVP are carotenoid-less (blue-green) and defective in carotenoid synthesis (green), respectively.

Preparation of membranes

Membranes from aerobically and photosynthetically-grown cells were prepared as follows. Cells were washed and resuspended in 20 mM 3-(*N*-morpholino)propane sulfonic acid (Mops), 100 mM KCl buffer, pH 7.0. After two passages through a French pressure cell at 18 000 p.s.i., the suspension was centrifuged at 30 000 $\times g$ for 25 min. The supernatant was removed and centrifuged at 104 000 $\times g$ for 90 min. The transparent membranous pellets were resuspended in the same buffer. Membranes from 01 cells were purified further by centrifugation on a discontinuous sucrose density gradient as indicated in the appropriate figure legends.

Preparation of reaction centres

Reaction centres were prepared from the blue-green mutant R-26 as described previously [13] using the detergent lauryl dimethyl amine oxide [18]. Reaction centres were prepared from the wild-type strain 2.4.1 using the method of Jolchine and Reiss-Husson [19]. In each case before use the final preparation was dialysed for two days against 10 mM Tris-HCl, pH 7.5, containing 2% sodium cholate.

Preparation of antenna pigment-protein

Photosynthetically-grown cells of GVP or 2.4.1. were used to prepare the *B*-850 pigment-protein complex as described by Clayton and Clayton [15]. The final preparations were dialysed for three days against 4 l of 10 mM Tris-HCl, pH 7.5, and dissolved in 10 mM Tris-HCl, pH 7.5, containing 2% sodium cholate.

Spectroscopy

Steady-state light-induced cytochrome absorbance changes were measured

in a dual-wavelength spectrophotometer with the photomultiplier protected by a filter of saturated copper sulphate and the actinic light masked by a Kodak far-red filter (No. 88A). The measuring light beams were chopped at 200 Hz. Spectra of membrane and protein preparations were measured on a Cary 14 R spectrophotometer.

Fluorimetric measurements were made either in the laboratory of Professor L.N.M. Duysens at the State University, Leiden, The Netherlands, using the apparatus previously described [20], or in this laboratory using a fluorimeter constructed within this Department (Figs. 3 and 5). In the latter instrument, exciting light over the range 360—620 nm was supplied by a 55 Watt quartz halogen lamp filtered through a Corning blue glass filter (No. 9782) and transmitted to the cuvette surface along a fibre optic (supplied by Rank Optics, Leeds, U.K.). The light emitted from the front face of the cuvette was detected by an EMI 9684B photomultiplier with an S1 photocathode. The photomultiplier was protected from scattered actinic light by an RG830 filter (Oriel Scientific, Ltd., Kingston-upon-Thames, U.K.), and a suitable Balzers interference filter (see figure legends for details). The signal from the photomultiplier, after amplification, was stored on a Datalab DL 905 transient recorder connected to a Bryans 27000 chart recorder. 'False light' due to exciting light reaching the photomultiplier was less than 10% of the measured emission.

Reconstitution of pigment-proteins into membranes from aerobically grown cells

Membranes prepared from 01 cells and purified by gradient centrifugation (see appropriate legends) were mixed with reaction centres and/or antenna proteins in the presence of 1% sodium cholate and sonicated soya bean phospholipids (2:1, w/w, with 01 membrane protein). The sodium cholate was removed by dialysis against two changes of 4 l of 20 mM Mops, 100 mM KCl buffer, pH 7.0, at 4°C for 42 h. Membranes from the aerobically-grown mutant PM8dp were mixed with reaction centres and treated in the same way.

Estimation of bacteriochlorophyll

Samples were extracted with acetone-methanol (7 : 2, v/v) as described by Cohen-Bazire et al. [21] and bacteriochlorophyll estimated at 770 nm using the extinction coefficient $E_{mM} = 75$ [22].

Results

Fig. 1 shows spectra of the various preparations used for fluorimetry measurements. The antenna proteins isolated from strain 2.4.1. and mutant GVP of *Rp. sphaeroides* show very similar spectra, each with the characteristic 800 and 850 nm peaks described in earlier work on purified antenna [15] and also seen in the chromatophore spectra. The pigmented membranes isolated from the aerobically-grown reaction centre-less mutant PM8dp are noteworthy as they appear to contain bacteriochlorophyll and carotenoid molecules bound to proteins in the same way as in chromatophore membranes, as indicated by the absorption spectrum. The asymmetry of the absorbance peak in the near infrared region indicates that the *B*-875 component usually associated

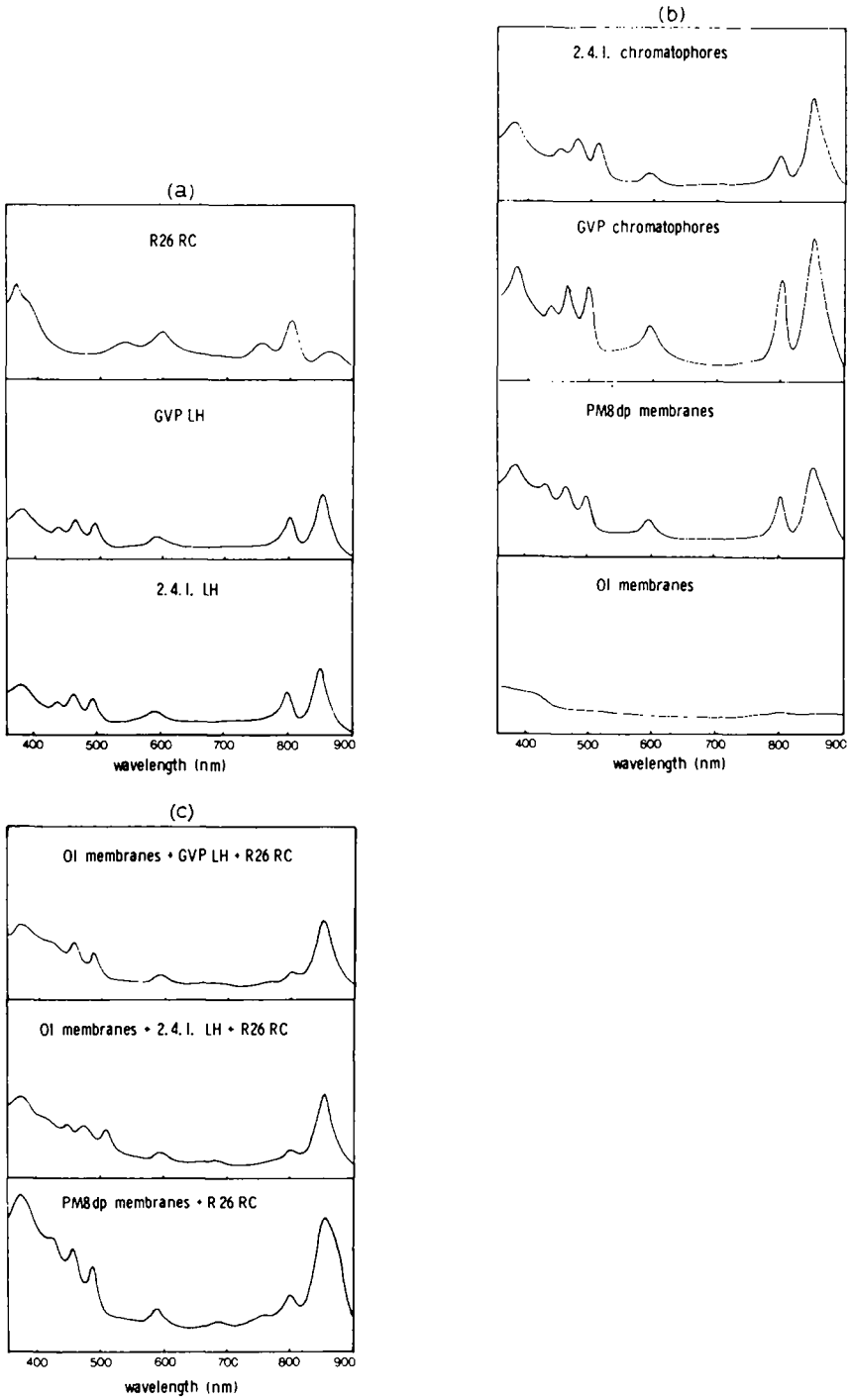

Fig. 1. Absorption spectra of fractions from *Rp. sphaeroides*; (a) isolated pigment-proteins, (b) membranes isolated from aerobically and photosynthetically-grown cells, and (c) membranes reconstituted with reaction centres and/or antenna. Each spectrum is labeled according to the sample examined. Isolation of membranes, pigment-proteins and reconstitution procedure all as described in Methods. LH, light-harvesting (antenna) pigment protein; RC, reaction centre.

with reaction centres is also present in these membranes in a larger proportion to the bulk *B*-850 antenna bacteriochlorophyll than is found in chromatophores.

A preparation of antenna and reaction centre proteins reconstituted into 01 membranes by the cholate dialysis procedure was layered onto a 10—60% (w/w) linear sucrose gradient in an attempt to demonstrate the association of these pigment proteins with the 01 membrane fraction. As also demonstrated previously [13,23] the membrane and pigmented components appeared as a single band in the gradient (figure not shown). This pigmented fraction exhib-

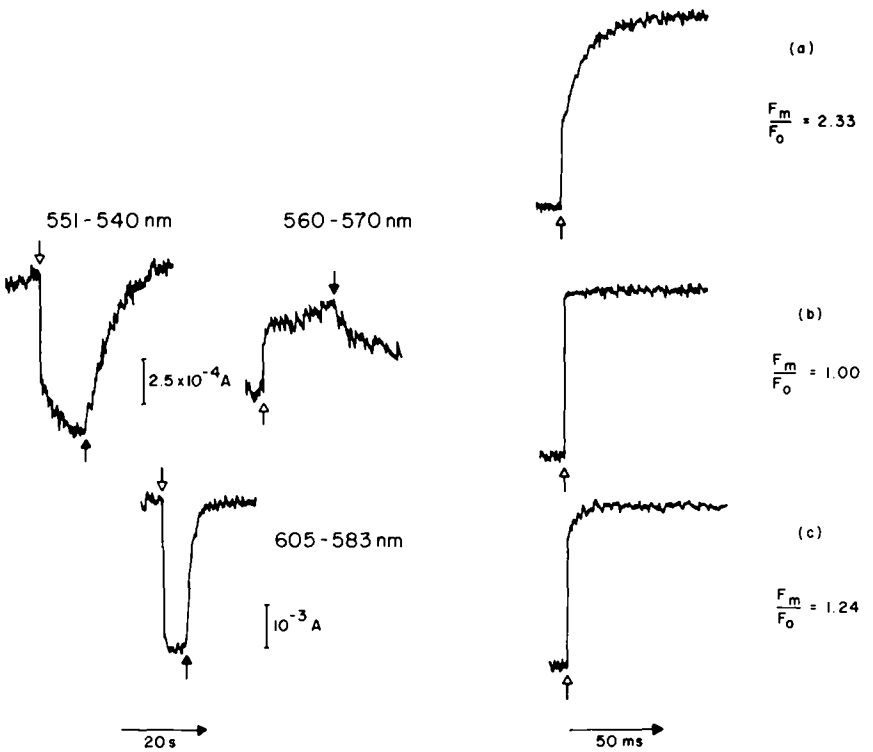

Fig. 2. Steady-state cytochrome absorbance changes in the pigmented fraction obtained from isopycnic centrifugation of reconstituted membranes. 01 membranes were prepared as described in Methods but resuspended material was layered on a 0.6 M/1.2 M sucrose step; after centrifugation at 75 000 × *g* for 4 h a clear brown fraction was collected from the interface between the sucrose layers, having been separated from a translucent pellet consisting of cell wall material. Reaction centre (1.4 nmol/mg of 01 protein) and antenna (9 nmol Bchl/nmol reaction centre) isolated from photosynthetically-grown R-26 and 2.4.1. wild-type, respectively, were reconstituted as in Methods. Three hundred and fifty microliters of the reconstituted membrane preparation was layered onto a 12 ml, 10—60%, w/w, sucrose gradient made up in 1 mM Tris/1 mM EDTA, pH 7.5. After centrifugation at 100 000 × *g* for 11 h the pigmented fraction was recovered and examined for reconstituted light-induced cytochrome absorbance changes in a dual wavelength spectrophotometer (see Methods), in the presence of 10 mM sodium fumarate, 0.1 mM sodium succinate and 10 μg antimycin A.

Fig. 3. Fluorescence induction traces from (a) GVP chromatophores, (b) GVP antenna, and (c) 01 membranes reconstituted with R-26 reaction centres and 2.4.1. antenna, followed by isopycnic centrifugation of the reconstituted membranes. All preparations had an absorbance $A^{1cm}_{850nm} = 5.0$. The measuring wavelength was 859 nm for (b) and (c), 906 nm for (a). The scale of the ordinate is different for each trace. Isopycnic centrifugation of reconstituted membranes was as for Fig. 2.

ited the reconstituted light-induced steady-state cytochrome absorbance changes which result from an interaction between the pigment proteins and the 01 membrane cytochromes [13,14]. Fig. 2 shows that cytochrome c_2 and a cytochrome b (probably b_{+50} (see Ref. 14)) participate in reconstituted electron flow in these membranes.

Fig. 3 illustrates the fluorescence kinetics observed at the onset of illumination of the pigmented fraction examined in Fig. 2. Chromatophores from photosynthetically-grown cells and the antenna protein isolated from such chromatophores were also examined fluorimetrically as a comparison. The F_o part of the fluorescence rise represents light absorbed by the antenna system but emitted before reaching an open trap (a reaction centre in state PX) because of inherent inefficiencies in the system. In a reconstituted system such as that described here, a part of this light may arise because some antenna molecules are not coupled to the open reaction centres.

Excitation energy that does reach the open traps can be used for photochemistry, and when electrons accumulate on the reducing side of the reaction centre, or the supply of electron donors to P^+ runs out, the states PX^- and P^+X, respectively, are reached. Quanta incident on the antenna proteins are no longer translated into photochemical energy by the reaction centres and more energy is emitted as fluorescence (F_m) over and above the basal level F_o. In the time during which this saturation level is reached that portion of the total fluorescence rise, F_{var}, occurs. The time taken to achieve F_m depends on the rate of input of quanta to the antenna (light intensity), the rate of energy migration within the antenna, and the rate of electron transport to and from the reaction centre. The fluorescence rise of the isolated antenna bacteriochlorophyll (Fig. 2b) demonstrates that when no energy transfer to a reaction centre is possible, the maximal fluorescence is composed entirely of an 'F_o' ($F_m/F_o = 1$). Thus, traces 2a and 2b represent for our purposes two extremes of energy transfer, the chromatophores showing a relatively high efficiency of energy transfer ($F_m/F_o = 2.33$). The pigmented fraction from the sucrose gradient containing antenna and reaction centre proteins bound to 01 membranes also shows some reconstituted energy transfer ($F_m/F_o = 1.24$). This suggests that after purified antenna and reaction centre proteins are mixed with bacteriochlorophyll-less 01 membranes in the presence of 1% sodium cholate, dialysis results firstly in the binding of reaction centres to antenna protein in a manner that permits energy transfer between these proteins. Secondly, this photoactive complex associated with 01 membranes in the sucrose gradient can photoreduce cytochrome b and photooxidise cytochrome c_2 in the membrane (Fig. 2).

This phenomenon of reconstituted energy transfer was examined in more detail using different antenna and reaction centre preparations, and different ratios of the two proteins to each other and the amount of membrane present (Table I). In each case the ratio F_m/F_o was used as the criterion for reconstituted energy transfer. As the amount of reaction centre per mg 01 membrane protein increased, and the amount of antenna bacteriochlorophyll per reaction centre decreased, the ratio F_m/F_o increased in all preparations to a maximum of 1.20—1.30 (Fig. 3). Dialysis of antenna with reaction centres in the presence of 1% sodium cholate and without 01 membranes appeared to increase the amount of reaction centres which interact with the antenna proteins in a

TABLE I

THE RECONSTITUTION OF VARIOUS ANTENNA AND REACTION CENTRE PREPARATIONS INTO 01 AND PM8dp MEMBRANES AND THE EFFECT ON FLUORESCENCE INDUCTION KINETICS

All preparations except chromatophore were dialysed as described in the Methods section. The absorption maximum of each sample was 850 nm: all samples used were balanced at $A^{1cm}_{850nm} = 1.4$. All measurements at 875 nm except where indicated. Bchl, bacteriochlorophyll; LH, light-harvesting (antenna) pigment protein; RC, reaction centre.

Preparation	F_m/F_o	nmol Bchl/ nmol RC	nmol RC/mg membrane protein	F_{max} / F_{max} measured in absence of RC
01 membranes + GVPLH + R26 RC	1.03	309	0.07	0.87
	1.14	190	0.7	0.90
	1.18	39	1.4	0.79
	1.24	9	1.4	0.95
01 membranes + 2.4.1 LH + R26 RC	1.10	190	0.7	1.47
	1.14	38	1.4	1.00
	1.16	10	1.4	1.00
01 membranes + GVP LH + 2.4 1. RC	1.31	9	1.4	0.90
01 membranes + 2.4.1 LH + 2.4.1. RC	1.20	10	1.4	0.78
PM8 dp membranes + R26 RC *	1.02	259	0.07	0.87
	1.13	26	0.7	0.88
	1.25	13	1.4	0.78
R26 RC + GVPLH	1.41	9	—	0.86
R26 + 2.4.1. LH	1.38	10	—	1.11
GVP chromatophores *,**	2.37	—	—	0.66
2.4.1. chromatophores *,**	2.00	—	—	0.64

* Measurements made at 907 nm.

** Denominator used was F_{max} from the appropriate isolated antenna protein.

manner that permitted energy transfer. The ratio F_m/F_o increased to about 1.4, but did not approach the ratio obtained from chromatophores of 2.0—2.4. No significant differences were observed with the different antenna and reaction centre preparations.

Membranes from the aerobically-grown, pigmented but reaction centreless mutant PM8dp appear to contain both antenna chlorophyll proteins, *B*-850 and *B*-875, providing an opportunity to study reconstituted energy transfer from both proteins to the reaction centre. From Table I it can be seen that the addition of reaction centres to a membrane structure containing native antenna proteins, but 'loosened' by the addition of 1% sodium cholate, permits some of the reaction centres to interact with the light-harvesting proteins in such a way that energy transfer is reconstituted, after the cholate is removed by dialysis. As found in the other reconstitution experiments in Table I, decreasing the ratio of antenna bacteriochlorophyll to reaction centre increases F_m/F_o.

Numbers in the extreme right column of Table 1 indicate the extent to which antenna bacteriochlorophyll fluorescence (F_m) was quenched by the added reaction centres when they had reached their photochemically closed state; on average, maximal fluorescence was lowered by only 10%.

All the measurements of fluorescence emission in Table I were made at

three wavelengths, 863, 875, and 907 nm to give results over the width of the emission spectrum. The F_m/F_o values selected for inclusion in Table I were mostly measured at 875 nm, since the emission maximum of isolated antenna preparations used in these reconstitution studies was found to be at 875 nm. Chromatophores show an emission maximum of a higher wavelength due to fluorescence emission from the *B*-875 antenna present. Since the emission maximum was shifted to higher wavelengths the pigmented PM8dp membranes reconstituted with reaction centres bore more resemblance to the 'natural' energy transfer system than that reconstituted from the addition of isolated *B*-850 antenna reaction centre proteins to bacteriochlorophyll-less 01 membranes.

The effect of adding a pre-dialysed antenna-reaction centre preparation to 01 membranes

The highest ratio F_m/F_o in Table I was observed when antenna and reaction centre proteins were mixed in 1% sodium cholate in the absence of 01 membrane, and then dialysed to remove the cholate. This step was incorporated into the reconstitution procedure as a pre-dialysis step; in the second step antenna/reaction centre complex was mixed with 01 membranes and 1% cholate and dialysed as usual. Experiments were carried out in order to investigate if this additional pre-dialysis step results in a membranous preparation more able to harvest and utilize incident light energy than a system assembled from the same components but in a one-step process.

Light quanta incident on the antenna in chromatophore membranes are ultimately converted to photochemical energy with cytochrome b_{+50} as the acceptor and cytochrome c_2 the electron donor for P [24]. Thus the steady-state level of cytochrome c_2 photooxidation was used as an indication of the photochemical activity for this study. In a preparation better able to harvest the incident quanta and pass the energy to the cyclic electron transport system in the membrane, it would be expected that very low exciting light intensities would result in more cytochrome c_2 photooxidation; this is borne out in the results shown in Fig. 4. When GVP antenna protein is reconstituted into the 01 membrane, the reaction centres present utilize the incident light more efficiently for cyclic electron flow in the membrane when compared with a preparation that contains reaction centres alone bound to 01 membranes. The most efficient system for light utilization results from the addition of a pre-dialysed antenna-reaction centre complex to 01 membranes; this increased efficiency is also manifested in Fig. 5 as an increase in the ratio F_m/F_o from 1.33 to 1.57 for 01 membranes reconstituted with reaction centres and antenna in one- and two-step processes, respectively. The absolute fluorescence of undialysed GVP antenna appears to have been quenched by 50% by the presence of closed reaction centres (Fig. 5b), and a further 8% by the presence of closed reaction centres in the pre-dialysed complex after cholate dialysis with 01 membranes (Fig. 5c). A separate experiment showed that the effect of cholate dialysis treatment on GVP antenna was to lower its fluorescence yield by 34% (results not shown), so the real quenching effect of photochemically closed reaction centres in the presence of antenna in the reconstituted preparations was calculated to be a lowering of F_{max} by 24%. This quenching effect was

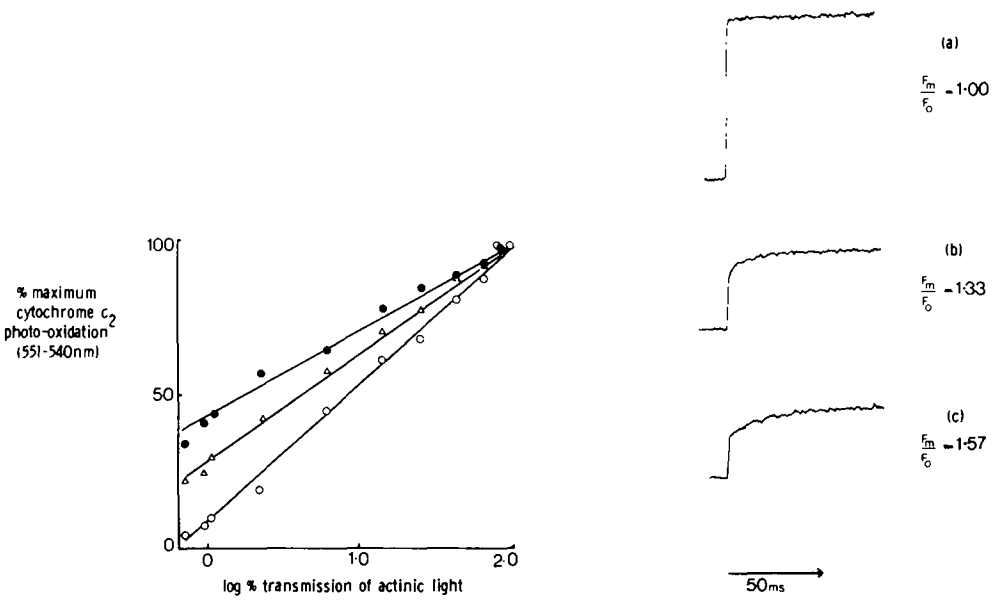

Fig. 4. The variation of the light-induced photooxidation of cytochrome c_2 measured at 551—540 nm (expressed as a percentage of the change at 100% transmission of exciting light), with the logarithm of the percentage transmission of exciting light. The absorption changes were monitored using a dual wavelength spectrophotometer as described in Methods. The intensity of the exciting light was varied using transmission filters calibrated for far red light using a thermopile connected to a millivoltmeter. The samples all contained purified 01 membranes reconstituted with 2.4.1. reaction centres (○), 2.4.1. reaction centres and GVP antenna (△), or a pre-dialysed complex of 2.4.1. reaction centres and GVP antenna (●). 01 membranes were prepared as in Fig. 2. The reconstituted membranes were assayed in the presence of 10 μM diaminodurol and 50 μM ascorbate.

Fig. 5. Fluorescence induction traces from (a) undialysed GVP antenna, (b) 01 membranes reconstituted with GVP antenna and 2.4.1. reaction centres, and (c) 01 membranes reconstituted with a pre-dialysed GVP antenna/2.4.1. reaction centre complex. The measuring wavelength was 859 nm for all samples. The ordinate scale is the same for all three samples. The 01 membranes were prepared as for Fig. 2.

larger than the average 10% (see earlier), although figures as high as 22% are reported in Table I.

Discussion

Several of the results reported here provide strong evidence for insertion of antenna and reaction centre complexes into membranes from the bacteriochlorophyll-less mutant 01 when the isolated components are combined in the presence of cholate and the detergent is removed by dialysis. These include the demonstration that added reaction centre and antenna complexes remain associated with 01 membranes after sucrose gradient centrifugation, the antimycin-sensitive cytochrome *b* photoreduction, cytochrome c_2 photooxidation, and reconstituted energy transfer exhibited by the pigmented fraction recovered from the gradients. When reaction centres and antenna are added in known amounts to the reconstitution mixture they quantitatively associate with the purified 01 membranes in the sucrose gradient [13] which allows an estimate of the relative amounts of each of the complexes incorporated into

the membrane. Similar reconstitution studies [13,14] with membranes from mutants 01 and PM8bg demonstrated that approximately 20% of the reaction centres must penetrate the membrane bilayer well enough to interact with the *b*- and *c*-type cytochromes present and enable them to participate in reconstituted light-driven electron flow upon flash or continuous illumination.

The results presented in Table I clearly show that energy transfer can be reconstituted with combinations of antenna, reaction centre and membranes isolated from various mutants of *Rp. sphaeroides*. This is shown by the appearance of the slow component F_{var} in the kinetics of the fluorescence rise; this is diagnostic of some coupling to open reaction centre traps, with the F_m approached as the photochemistry becomes saturated while energy is transferred from the antenna to the reaction centre. The effect of interaction of the reaction centre and antenna complexes is to quench antenna fluorescence by about 10% even when reaction centres are present in the closed state (P^+X). These results contrast with those obtained by Heathcote and Clayton [16] with dialysed antenna/reaction centre preparations who reported a three-fold quenching of the antenna fluorescence yield by closed reaction centres. In our experiments it is probable that the traps are closed as P^+X, and whole cell studies have yielded no evidence for a quenching effect on fluorescence yield by P^+ [20]. The small amount of quenching observed could be a non-specific effect induced by the proximity of another hydrophobic protein (the reaction centre) to the antenna; the effect of the cholate dialysis procedure on isolated antenna was to quench its own fluorescence by approximately 50%. A similar effect was noticed by Heathcote and Clayton (Ref. 16, see below) and may be due to the aggregation of the protein following removal of cholate by dialysis. In the spectra of reconstituted preparations that contained antenna, reaction centre and 01 membranes after exposure to sodium cholate and dialysis, the ratio of absorbance at 800 nm to that at 850 nm is diminished which suggests that the former component is labile during the cholate dialysis procedure. This effect is not seen during the isolation of the antenna protein from chromatophore, and may not occur until the stabilizing detergent layer is removed from this hydrophobic complex during dialysis. 800-nm excitation of antenna preparations dialysed alone in the absence of reaction centres, and balanced according to 850 nm absorbance [16] would lead to much less absorption of energy, so the further seven-fold decrease in fluorescence yield [16] could be explained partly by the lability of the 800 nm-absorbing species.

The reconstitution of reaction centres into an array of light-harvesting antenna was studied with membranes from the pigmented but reaction centre-less mutant PM8dp. The 800-nm absorbing component of the mutant membrane appears also to be sensitive to cholate treatment and dialysis, but the PM8dp membranes contain *B*-875 as well as *B*-850, presumably surrounded by the natural lipid environment. We have shown that it is possible to bind reaction centres to these membranes so that energy transfer from the antenna pigments to the reaction centre occurs. Membranes from reaction centre-less mutants 01 and PM8dp show light-induced cytochrome absorbance changes when reconstituted with reaction centres using the cholate dialysis procedure [13]. Such functional assays have established that added reaction centres are able to penetrate the membrane bilayer.

The fluorescence emission maximum of the PM8dp reconstituted system is at a higher wavelength than preparations containing purified *B*-850 antenna due to emission from the *B*-875 antenna species. This suggests that as in chromatophores, energy is transferred within these reconstituted membranes from the *B*-850 to *B*-875 antenna and then to reaction centres.

The absence of a stoichiometric interaction of antenna with reaction centre is in agreement with Heathcote and Clayton [16]. The concentration of the *B*-850 component in the photosynthetic unit is capable of wide variation [11] and perhaps the requirement for a fixed stoichiometry does not arise. The need in our experiments to have a low ratio of antenna bacteriochlorophyll to reaction centre and a high ratio of reaction centre to membrane in order to obtain satisfactory F_m/F_o ratios can be interpreted most simply on the basis of the proteins interacting correctly by chance. The ratio of antenna bacteriochlorophyll to reaction centre in the living cell can vary between 30 and 300 [11], but more reaction centre per antenna is necessary in our reconstitution experiments presumably because only a proportion of those added are capable of trapping quanta harvested by the antenna. The presence of membranes reduced further the chances of this interaction occurring if the hydrophobic proteins are distributed throughout the membrane upon removal of cholate by dialysis. It is possible that during the transition from high to low oxygen tension (<2.5% [25]) such limitations are avoided by synthesising an antenna/reaction centre complex. Evidence for this has been obtained from pulse chase studies [26] which suggest that newly synthesised polypeptides of the antenna and photochemical reaction centre complexes may be incorporated into the photosynthetic membrane at specialized sites as discrete photosynthetic units [27,28]. This would remove the need for individually integrated proteins to find each other in the growing membrane and then achieve the correct orientation for effective energy transfer.

Recent studies in which fluorescence techniques were used to monitor energy transfer within and between antenna/reaction centre units in developing chromatophore membranes suggest that the formation of functional photosynthetic units proceeds from discrete predetermined membrane sites [28,29]. Energy transfer between antenna/reaction centre units does not occur until a later stage in the pigmentation process. This was inconsistent with a statistical building process for the photosynthetic apparatus by random integration of pigments within the membrane [29]. Our work in which antenna and reaction centre complexes are first combined before reconstitution into the membrane also suggests that the inefficiency of energy transfer due to such a statistical building process is substantially reduced by formation of photopigment complex as an antenna/reaction centre unit.

Our results show that insertion of a preformed antenna-reaction centre complex results in more energy transfer to the reaction centre in the reconstituted membrane since the ratio F_m/F_o increased substantially. The antenna and reaction centre proteins bound together during the cholate dialysis procedure may remain associated upon insertion into the hydrophobic environment in the 01 membrane, and such a preparation clearly shows an increased ability to utilize low intensity incident light for photooxidizing 01 cytochrome c_2 when compared to an identical preparation assembled in a one-step

process. This confirms early observations on a similar reconstituted system in which the sodium cholate was removed by dilution [30]. The light intensity dependence of cytochrome c_2 photooxidation in this system suggests that energy transfer can be reconstituted in which light harvested by the antenna enables the closely associated reaction centre both to utilize low intensity light effectively and to pass this advantage on to the cyclic electron transport system in the membrane.

Acknowledgements

C.N.H. is grateful to Professor L.N.M. Duysens for providing laboratory facilities for some of this work, and to the Science Research Council for financial support. The authors are grateful to Drs. Henk van der Wal for assisting in some experiments, and acknowledge the skilled technical assistance of Mrs. E. Burd and Mrs. J. Fielding.

N.G.H. and R.v.G. have fellowships from the E.E.C. Solar Energy Programme D. This work was supported by grants from the Science Research Council and the E.E.C. Solar Energy Programme D.

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