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RECONSTITUTED ENERGY TRANSFER FROM ANTENNA PIGMENT-PROTEIN TO REACTION CENTRES ISOLATED FROM *RHODOPSEUDOMONAS SPHAEROIDES*

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SUMMARY

Efficient energy transfer has been reconstituted between an antenna pigment-protein and reaction centres isolated from the photosynthetic membrane of *Rhodopseudomonas sphaeroides*. The reconstituted system has fluorescence induction kinetics and fluorescence yields similar to those obtained from antenna bacteriochlorophyll in chromatophores. The results indicated that closed reaction centres quench fluorescence from the antenna pigment-protein, although not as strongly as photochemically active reaction centres. The measurement of fluorescence yields from chromatophores of the reaction centreless mutant PM-8 and of the parent strain Ga confirmed these observations.

The fluorescence yield from the reconstituted system was approximately the same whether the reaction centres had been closed by photo-oxidation of the bacteriochlorophyll electron donor or chemical reduction of the primary acceptor, indicating a similar lifetime for the excited singlet state in both states of the reaction centres.

INTRODUCTION

In all photosynthetic organisms, light quanta are absorbed by bulk antenna pigments and transferred to reaction centres where photochemistry takes place. The pigmented membranes of photosynthetic bacteria contain only one type of reaction centre associated with a photosynthetic unit of 30–100 bacteriochlorophylls. The study of these organisms has yielded some insight into the process of energy transfer between antenna and reaction centre pigments.

The close coupling between the fluorescence from the antenna and the photochemical ability of reaction centres indicated that energy migration was at the level of singlet excitation quanta [1, 2]. From measured fluorescence yields of 5–6 % from antenna bacteriochlorophyll in chromatophores and a calculated radiative lifetime of 18 nsec for bacteriochlorophyll in situ [4], Clayton and Wang [3] deduced that the trapping rate or non-radiative lifetime of absorbed quanta was approximately 1 ns. However, Borisov and Godik [5, 6] proposed that this represents a bacteriochloro-

phyll component unconnected with reaction centres, while a second connected component has much shorter lifetimes. Borisov and Godik base their view on their observation that in many species of photosynthetic bacteria the mean fluorescence lifetime (determined by phase fluorimetry at a single modulating frequency) diminishes as the yield increases in response to stronger illumination. They infer that a variable component of fluorescence, from antenna bacteriochlorophyll that communicates with reaction centres, makes a greater contribution to the total as the reaction centres become closed (photochemically inactive) in strong light. The average lifetime decreases as the proportion of this short-lived variable fluorescence becomes greater. This point has not yet been settled definitively.

Theorists have described models of this exciton migration, but uncertainties about the geometrical arrangement of the antenna pigments in vivo have made detailed application and testing of these theories difficult. We undertook to purify antenna pigment-proteins and reaction centres, and to recombine them in vitro so as to restore energy transfer of light quanta absorbed by the antenna pigments to reaction centres. As a result we have developed a model system capable of manipulation, and have gained information about the trapping of energy by reaction centres.

MATERIALS AND METHODS

Reaction centres were prepared by lauryl dimethyl amine oxide treatment of chromatophores from the carotenoidless mutant R-26 of *Rhodopseudomonas sphaeroides*, and purified by ammonium sulfate fractionation as previously described [7]. The antenna pigment-protein was isolated from the wild-type strain ATH 2.4.1 of *Rps. sphaeroides* by a modification of the method of Clayton and Clayton [8]. Purified chromatophores (15 mg/ml) were treated with 1 % lauryl dimethyl amine oxide and diluted 3-fold with 0.01 M Tris · HCl pH 7.6 (henceforth called buffer). After precipitation with ammonium sulfate the pellet was washed twice with a volume of 0.5 % Triton X-100 in buffer equal to the initial volume of the chromatophores. Reaction centres were preferentially solubilised and thus removed by this treatment. The remaining pellet was dissolved in 1 % lauryl dimethyl amine oxide in buffer plus 0.01 M EDTA, and layered onto a discontinuous sucrose gradient (0.6 M and 1.25 M sucrose in buffer plus 0.01 M EDTA). After centrifugation for 105 min at 150 000 $\times g$ the interface was collected, precipitated with ammonium sulfate, washed once with 0.5 % Triton X-100 in buffer and layered onto a second sucrose gradient. The purified pigment-protein ran as a single 9 kdalton band on polyacrylamide gels, with a slight band at 45 kdaltons attributable to a membrane protein. The absorption spectrum of the purified antenna pigment-protein shows a strikingly low peak in the neighbourhood of 260 nm (Fig. 1).

Both reaction centre and antenna pigment-protein preparations were stored at −20 °C in 0.3 % lauryl dimethyl amine oxide in buffer. To reconstitute energy transfer between them, they were mixed together and diluted 4-fold with buffer. The resulting solution, of about 10 absorbance at 850 nm, was either stored at 4 °C or dialysed for 3 times 24 h against buffer. These preparations were further diluted in buffer, to give an absorbance at 850 nm of 0.3 (1 cm path), just before measurements. At this dilution artifacts due to self-absorption were negligible.

To monitor the efficiency of energy transfer from the antenna pigments to

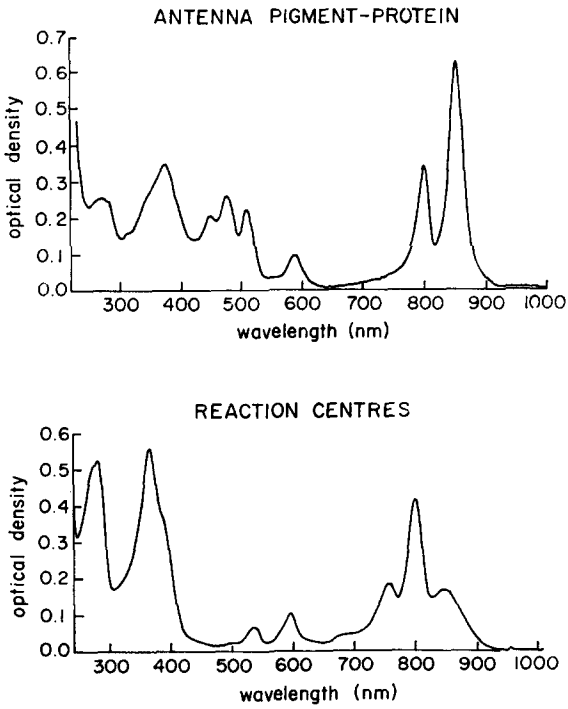

Fig. 1. The absorption spectra of an isolated antenna pigment-protein and of isolated reaction centres from the photosynthetic membranes of wild type and R-26 strains of *Rps. sphaeroides*, respectively.

reaction centres, the quantum efficiency of bacteriochlorophyll photooxidation in reaction centres was determined. The initial slope of bleaching of reaction centre bacteriochlorophyll at 863 nm was measured, and the quantum efficiency ϕ_p was determined from these measurements, as described by Wraight and Clayton [9]. We did not take the special optical precautions needed [9] for maximum absolute accuracy. Consequently, the relative values of ϕ_p were reliable within 10 %, but absolute values could have been in error by as much as 25 %.

Fluorescence spectra were recorded on the apparatus previously described by Wang and Clayton [3]. The yield and kinetic variations of the fluorescence were measured with a simple fluorimeter employing interference filters to separate the actinic and measuring wavelengths, as described by Clayton et al. [10]. 800 nm actinic light of the same intensity was used for all fluorescence yield and kinetic measurements. The signal from a constant reference light source was recorded after each measurement, to compensate for changes in the photomultiplier high voltage between measurements, and any change of photomultiplier sensitivity with time. The yield of fluorescence measured in this way was compared with the known fluorescence yield of open (photochemically active) reaction centres at 902 nm ($3.5 \cdot 10^{-4}$, according to Zankel et al. [4]).

The fluorescence of reaction centres measured at 902 nm was contaminated by scattered actinic light plus the 'tail' of fluorescence bands from bacteriopheophytin and other chromophores emitting at shorter wave lengths. This background of 'false'

light could be discounted by comparing the decline, during constant illumination, of both the fluorescence at 902 nm and the absorbance at 863 nm, as the bacteriochlorophyll became photo-oxidized. As expected, these light-induced decreases had identical kinetics. The initial 'true' fluorescence f could therefore be computed from the relation

$$f/\Delta f = A(863\text{ nm})/\Delta A(863\text{ nm})$$

These absorbance measurements were made with a Cary 14R spectrophotometer, using the same intensity of 800 nm actinic illumination as was used in the fluorescence measurements.

The fluorescence yield of reaction centres was, however, being measured at 902 nm, whilst the fluorescence of the antenna pigment-protein or chromatophores was monitored at 863 and 890 nm respectively. To compensate for differences in the bandwidth of the different measuring interference filters, differences in their transmission, and changes in photomultiplier sensitivity with wavelength, the filters were all calibrated with reference to a 910 nm interference filter. The extent of a signal produced by a pin-point black body source (a tungsten lamp at 2860 K) was recorded after the measurements of the fluorescence yield and the reference light source. The fluorescence signal X_λ measured at any wavelength is defined by

$$X_\lambda = S_\lambda I_\lambda$$

where I_λ = the fluorescence intensity at that wavelength and S_λ = sensitivity of the photo-multiplier and filters at that wavelength. S_λ for the 910 nm filter was assumed to be 1. The ratio of black body emission at 910 nm to that at the measuring wavelength λ could be determined from the Planck formula

$$\frac{B_{910}}{B_\lambda} = \left(\frac{\lambda}{910}\right)^4 e^{5.53\left(\frac{910}{\lambda} - 1\right)} \quad \text{at 2860 K}$$

The actual signals from the black body at that wavelength and 910 nm had been recorded, giving $(X_\lambda/X_{910})_{BB}$, so

$$S_\lambda = (X_\lambda/X_{910})_{BB}\,(B_{910}/B_\lambda)$$

To compensate for variations in photomultiplier sensitivity and high voltage, the constant reference signals had been recorded at 910 nm and the measuring wavelength. The final factor relating fluorescence yields at the two wavelengths, a_λ could then be calculated from

$$a_\lambda = (1/S_\lambda)\,(X_\lambda/X_{910})_{\text{Ref}}$$

The observed relative fluorescence yield at wavelength λ (measured yield/measured reference signal) could thus be multiplied by a_λ to standardise it in relation to 910 nm. Finally these relative yields were converted to absolute yields by comparison with the known absolute yield of reaction centre fluorescence [4].

RESULTS

One unit of reaction centres was mixed with three units of the purified antenna pigment-protein as based upon their peak absorbances at 800 and 850 nm, respec-

tively. After dialysis or storage, the quantum efficiency (ϕ_p) for bacteriochlorophyll photo-oxidation in reaction centres, monitored by 863 nm bleaching [9], was measured with several different interference filters in the actinic light path (Table I). Undialysed samples showed quantum efficiencies approximately equivalent to the percentage of absorption, at any actinic wavelength, which could be attributed to reaction centres. The quantum efficiency of 863 nm bleaching in the dialysed sample approached unity, indicating complete reconstitution of energy transfer between the antenna pigment-protein and reaction centres.

Measurement of fluorescence induction kinetics in the two samples also suggested a close degree of coupling between the antenna pigment-protein and reaction centres after dialysis. The initial fast phase of fluorescence was followed by a slow rise in fluorescence (Δf) as the reaction centres became photochemically closed (Fig. 2a). The fluorescence from the antenna pigment-protein alone was constant (Fig. 2b). In these measurements, the actinic light intensity was adjusted to maximize the ratio of Δf to the total fluorescence yield f, i.e., to saturate the photochemistry of the reaction centres.

Using either broad bandwidth blue light or an 800 nm interference filter in the actinic light path the fluorescence spectra from all systems containing the antenna pigment-protein peaked at 865 nm. The fluorescence yields (ϕ_f) from the antenna pigment-protein plus reaction centre mixtures and from the antenna pigment-protein alone are shown in Table II. The undialysed antenna pigment-protein alone had a very high ϕ_f of 63 %. Simply adding reaction centres in a 1 : 3 ratio reduced ψ_f by about one third, and dilution of the mixture four-fold with buffer followed by storage for three days reduced the ϕ_f further to 23 %. In the latter case some photochemical coupling was evidenced by a small Δf in the fluorescence induction kinetics (Fig. 2c). Dialysis of this antenna pigment-protein plus reaction centre mixture reduced ϕ_f to a

TABLE I

THE QUANTUM EFFICIENCY OF BACTERIOCHLOROPHYLL PHOTO-OXIDATION IN REACTION CENTRES MIXED WITH AN ANTENNA PIGMENT-PROTEIN, BEFORE AND AFTER DIALYSING AWAY THE DETERGENT PRESENT

The ratio of antenna pigment-protein to reaction centres was 3 : 1, meaning three units of antenna pigment-protein based on absorbance at 850 nm and one unit of reaction centres based on absorbance at 800 nm. Samples were diluted 4-fold with buffer and then dialysed or stored for 3 days. They were brought to an absorbance of 0.3 at 850 nm just before measurement.

Actinic light wavelength (nm)	ϕ_p*		Percent of absorption at that wavelength attributable to reaction centres alone
	Undialysed antenna pigment-protein plus reaction centres	Dialysed antenna pigment-protein plus reaction centres	
510	0.03	0.98	3
585	0.14	0.91	14
600	0.26	0.97	28
800	0.37	0.94	24
850	0.16	0.91	7
900	0.54	0.98	59

* The average of measurements taken at two different actinic light intensities.

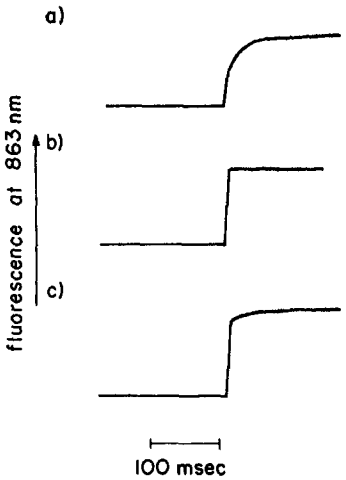

Fig. 2. Fluorescence induction kinetics from: (a) dialysed antenna pigment-protein plus reaction centres; (b) dialysed antenna pigment-protein in the absence of reaction centres; (c) undialysed antenna pigment-protein plus reaction centres. Measuring wavelength was 863 nm, actinic wavelength was 800 nm. The scale of the ordinate is different for each trace; for absolute values see Table II.

TABLE II

Fluorescence yields from the antenna pigment-protein in the presence or absence of reaction centres and detergent, and for chromatophores from a reaction centreless mutant of *Rps. sphaeroides* (PM-8) and a wild strain (Ga). Undiluted samples (first two rows) or samples diluted fourfold with buffer (rows 3–5) were either dialysed or stored for 3 days. All samples were brought to an absorbance of 0.3 at 850 nm just before measurement. The ratio of antenna pigment-protein to reaction centres (when both were present) was 3 : 1, as defined in the legend of Table I.

Sample	Measuring wavelength (nm)	ϕ_f(final)*	$\frac{\phi_f(\text{final})}{\phi_f(\text{initial})}$
Undialysed, undiluted antenna pigment-protein	863	0.63	1.0
Undialysed, undiluted antenna pigment-protein plus reaction centres	863	0.43	1.0
Undialysed, diluted antenna pigment-protein plus reaction centres	863	0.23	1.1
Diluted and dialysed antenna pigment-protein	863	0.093	1.0
Diluted and dialysed antenna pigment-protein plus reaction centres	863	0.028	2.2
Chromatophores from strain PM-8	890	0.089	1.0
Chromatophores from strain Ga	890	0.039	2.0

* The maximum value attained during constant saturating illumination.

value corresponding to those in vivo [4], and gave efficient excitation transfer between the two components (Table I and Fig. 2a). However, dilution followed by dialysis of the antenna pigment-protein alone reduced ϕ_f by a factor of 7 (from 63 to 9 %), suggesting that only part of the observed fluorescence quenching in the dialysed antenna plus reaction centre mixture was due to trapping by the reaction centres. These data indicate that reaction centres quench antenna fluorescence (from 9 to 3 %; rows 4 and 5 in Table II) even when the reaction centres have been photochemically closed by saturating light*. This could be expected from the low yield of fluorescence from purified reaction centres (0.1 % when photochemically closed) [4].

From this, it would be predicted that the fluorescence yield from 'closed' chromatophores of the photochemically competent Ga strain of *Rps. sphaeroides* should be lower than that from chromatophores of the reaction centreless mutant strain PM-8 derived from Ga. To test this, chromatophores were prepared from aerobically grown cultures, and cultures of PM-8 and Ga were chosen that matched well with regard to the shapes of absorption and fluorescence spectra. The fluorescence yields are shown in Table II; the ϕ_f for PM-8 (9 %) and the light-saturated Ga chromatophores (4 %) agree closely with those for antenna dialysed in the absence (9 %) or presence (3 %) of reaction centres.

Mayne [11] reported that intense, prolonged illumination of chromatophores with blue light caused a gradual decline in ϕ_f and ϕ_p. This effect, which was observed

TABLE III

The effect of varying the ratio of antenna pigment-protein to reaction centres on fluorescence yield and induction kinetics from dialysed and undialysed mixtures of both components. All samples were diluted 4-fold with buffer before 3 days' storage or dialysis, and were brought to an absorbance of 0.3 at 850 nm just before measurement. Ratios of antenna pigment-protein to reaction centres are as defined in the legend of Table I.

Sample	$\frac{\phi_f(\text{final})}{\phi_f(\text{initial})}$	ϕ_f(final)
Undialysed 5 : 1 antenna pigment-protein : reaction centres	1.0	0.24
Dialysed 5 : 1 antenna pigment-protein : reaction centres	2.2	0.042
Undialysed 10 : 1 antenna pigment-protein : reaction centres	1.0	0.34
Dialysed 10 : 1 antenna pigment-protein : reaction centres	1.7	0.065
Undialysed 20 : 1 antenna pigment-protein : reaction centres	1.0	0.32
Dialysed 20 : 1 antenna pigment-protein : reaction centres	1.4	0.13
Undialysed 50 : 1 antenna pigment-protein : reaction centres	1.0	0.74
Dialysed 50 : 1 antenna pigment-protein: reaction centres	1.2	0.15

* Unless stated otherwise, the values of ϕ_f pertain to final values measured with saturating illumination, such that the reaction centres have become closed.

in the reaction centreless strain PM-8, as well as in chromatophores from the wild-type strains of *Rps. sphaeroides*, was attributed to the photo-oxidative formation of quenching sites in the antenna pigments. This process was reversed or prevented by dithionite or anaerobic conditions. We observed the same phenomenon in the antenna pigment-protein, and therefore tested all the experimental samples by adding dithionite. Dithionite caused a slight increase in ϕ_f when added to dialysed antenna and PM-8 chromatophores (9–10 %). When added to Ga chromatophores or dialysed antenna plus reaction centres, the major effect was to eliminate Δf, although there was a decrease in the total ϕ_f from the systems when dithionite was added (20 %). The latter phenomenon was not further investigated, and was not always observed in chromatophores.

Finally, the ratio of reaction centres to antenna pigment-protein was decreased in an attempt to reveal any specific binding interactions (Table III). As the proportion of reaction centres decreased the ϕ_f(final)/ϕ_f(initial) decreased and ϕ_f (final) increased.

DISCUSSION

The absorption spectrum of chromatophores of *Rps. sphaeroides* has peaks at 800, 850 and 870 nm (B 800, 850 and 870), attributed to antenna bacteriochlorophyll molecules in differing physical environments. The isolated and purified antenna pigment-protein contains B800 and B850 plus carotenoids but lacks B870 (Fig. 1). Dissociated by boiling in sodium dodecyl sulfate into 9 kdalton units, it probably exists in vivo as aggregates with molecular weights in excess of 100 kdaltons [8].

The results show that efficient transfer of excitation energy from antenna pigments to reaction centre pigments can be restored in a reconstituted mixture of these components. This was evidenced both by antenna-sensitization of reaction centre photochemistry, and by the fact that reaction centres quench antenna fluorescence, with open reaction centres quenching more strongly than closed ones.

The yields of fluorescence, the fluorescence induction transients, and the photochemical efficiencies in the reconstituted system corresponded closely to those in the intact photosynthetic membrane. This correspondence is partly fortuitous since the ratio of antenna pigments to reaction centre pigments was 3 : 1 (based on long wavelength absorption maxima) in these measurements, whereas a ratio of 10 : 1 would correspond more closely to the living cell.

Our measurements with different ratios of antenna pigment-protein to reaction centres did not reveal a specific interaction between the two components; the parameters of fluorescence varied smoothly as the ratios were varied. However, specific binding interactions, with stoichiometric equivalence falling outside the ratios tested, remains a remote possibility.

In chromatophores from strain Ga and in the dialysed antenna plus reaction centre system, the rise in antenna fluorescence as photochemistry was light saturated was 2 to 2.2 fold. This is in crude agreement with the observation that the fluorescence of purified reaction centres increases four-fold when the reactions centres are closed by reduction of the primary acceptor [4, 12].

These values could be expected if the efficiency of energy transfer were about 55 %; they are not consistent with 100 % efficiency, unless the quenching properties of

the reaction centres are modified by association with the antenna pigment-protein. Alternatively, the transfer is efficient for most of the antenna pigment-protein, but a small proportion of unconnected antenna contributes a large component of constant fluorescence.

The results also show that closed reaction centres do quench excitation energy in the reconstituted system, as might be expected from the short lifetime of the singlet excited state in reaction centres. The resulting expectation that fluorescence from chromatophores without reaction centres should be greater than in chromatophores with closed reaction centres was confirmed, comparing a reaction-centreless mutant of *Rps. sphaeroides* with the wild type.

In the illuminated dialysed 'antenna plus reaction centre' system, the reaction centres become photochemically closed due to the oxidation of the electron-donor bacteriochlorophyll. When dithionite was added to the same system, the reaction centres were closed because the electron acceptor(s) were reduced. The rises in antenna fluorescence in either of these cases were of approximately the same magnitude, indicating that the two forms of closed reaction centres have approximately equal quenching action. If such quenching by closed reaction centres results from the capture of singlet excitation quanta by the reaction centres, it follows that the excited singlet state lifetime is the same in both forms of closed reaction centre. The same implication can be drawn from earlier observations of variable fluorescence in chromatophores, comparing the rises in fluorescence associated with the closing of reaction centres with and without oxidation (bleaching) of the reaction-centre donor bacteriochlorophyll (B870) [12]. The excited state lifetime in purified reaction centres closed by chemical reduction of the primary acceptor has been estimated at 20 ps, using the yield of 900 nm fluorescence and the intrinsic lifetime estimated from the long wavelength absorption band area [4]. From the above results, we can now estimate approximately the same lifetime for reaction centres closed by oxidation of the bacteriochlorophyll electron donor, where there is no 900 nm fluorescence available as an indicator of excited state lifetime.

The antenna pigment-protein has exceptionally high fluorescence yields compared to other systems which also have bacteriochlorophyll in a highly condensed state. The absence of strong excimer quenching may be attributable to specific bacteriochlorophyll-protein linkages that prevent the pigment molecules from approaching each other too closely. It is unlikely that lipids are responsible for this separation, as the preparation of the pigment-protein with lauryl dimethyl amine oxide has probably removed most of the native lipids as it does in reaction centre preparations. The lack of strong quenching in this antenna pigment-protein makes it of potential value in constructing model systems for solar energy conversion.

Finally, it is hoped that this combination of antenna pigment-protein and reaction centres may prove to be a useful model for the testing of detailed hypotheses of excitation migration.

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