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Reconstructed light-harvesting system for photosynthetic reaction centres

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Abstract

A model of a photosynthetic unit was constructed using purple photosynthetic bacterial reaction centres and either bacteriochlorophyll *a* or chlorophyll a molecules embedded together in liposome membranes. For such systems, called reconstituted photoreaction units, we measured the fluorescence, fluorescence excitation and absorption spectra. Our results showed that pigments directly incorporated into the lipid bilayer of the liposomes without the support of polypeptides functioned as light-harvesting antennae for the reaction centres. Light energy absorbed by the donors (pigments) was transferred to the acceptors (reaction centres). We determined the charge transfer in our system by measuring the light-induced absorbance change and photocurrent response. The system with reconstructed antennae exhibited enhanced efficiency in both the photobleaching of reaction centres at 860 nm and photoelectric conversion. We used the fluorescence-quenching method to investigate the donor–acceptor energy transfer between the membrane-bonded pigments and the reaction centres. From the fluorescence measurements we evaluated a critical donor–acceptor transfer distance of 37–40 Å as sufficient for Förster resonance energy transfer. Our results showed that the constructed model system is promising as a starting point for investigations into the construction of biodevices for conversion of solar energy into electrical energy.

Keywords: Bacteriochlorophyll; Chlorophyll; Reaction centre; Liposome; Photocurrent; Light harvesting; Energy transfer; Fluorescence quenching

1. Introduction

In photosynthetic bacteria, primary reactions of photosynthesis, light harvesting and charge separation occur in photoreaction units (PRUs) composed of light-harvesting antenna complexes (LHs) and reaction centres (RCs). One consequence of these reactions is the generation of an electrochemical potential across the membrane [1–3].

For purple photosynthetic bacteria the three-dimensional structures of RCs as well as the main electron transfer chains have already been determined [4–6]. In such organisms the RCs are surrounded by LHs. These LHs enhance the efficiency of the photoreaction by photon capture and the transfer of excitation energy to the RCs. Purple photosynthetic bacterium *Rhodobacter sphaeroides* (*R. sphaeroides*) contains two kinds of light-harvesting antenna complexes: LHI (B875), which closely surround the RC and function as an exciton mediator, and LHII (B800/850), which surround the LHI and function as an absorbing–transferring complex. Both LH complexes contain bacteriochlorophyll *a* (Bchl) and carotenoids non-covalently bound to α- and β-polypeptides. In *R. sphaeroides* about 20 Bchl chromophores located in LHI are close to an RC [7,8].

The properties of whole LH and RC complexes located in liposomes have already been reported by many researchers [9–11]. The excitation energy transfer for such systems or for isolated pigments located in lipid monolayers, black lipid membranes, liposomes and artificial membrane systems has been extensively studied [12–17]. Investigations show that efficient energy transfer also occurs between pigments that are not bound to polypeptides [18,19].

In photosynthetic organisms the conversion process of light energy into charge separation is very efficient [6], so there is great interest in investigating the structure and function of intact bacterial cells, isolated bacterial protein–membrane fragments or, as in the present work, artificially constructed systems. Model systems are usually simpler than natural ones, thus making their features on a molecular level easier to describe. The model used in this work is semiartificial in that it contains native RCs and pigments isolated from organisms, located together in the model membranes. Such a system allows us to conduct various kinds of molecular manipulations. A lipid bilayer liposome (LP) is potentially an excellent model of a biomembrane because it has a composition very similar to native systems.

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In this paper we successfully demonstrate a simple reconstituted PRU composed of purified light-harvesting pigments and native RCs in lipid bilayer membranes. The pigments, chlorophyll *a* (Chl) and Bchl, were directly embedded in liposomes without the support of polypeptides. For our experimental system we used an aqueous suspension of liposomes. The efficiency of excitation energy transfer from such artificial antenna systems to RCs was shown by the enhancement in the photobleaching of the RCs, by the generation of a photocurrent and by the fluorescence quenching of the pigments.

2. Materials and methods

2.1. Preparation of RCs, Bchls and Chls

The RCs of *R. sphaeroides* R-26 were isolated and purified as follows according to the procedure of Clayton and Wang with a slight modification [20]. Bacteria were cultured under illumination of 2 klx by tungsten lamps for 3–5 days with succinate as a carbon source as described previously [21]. The RCs were solubilized from the chromatophores using lauryl-dimethylamine *N*-oxide (LDAO, 2%) in a Tris–HCl buffer (10 mM, pH 8.2) and purified through sucrose solution layers in centrifuge tubes. Further purification was done based on ammonium sulphate fractionation by gel filtration through a Sepharose CL-6B column. The final concentration of the solubilized RCs ranged from 60 to 90 μM.

The Bchl was isolated from *R. sphaeroides* as described by Katoh et al. [22]. Cells were collected by centrifugation and washed with a 10 mM Tris–HCl buffer solution (pH 8.2). Cells were again centrifuged and the resulting pellet (6 g wet weight) was mixed with 6 ml of a 15 mM Tris–HCl buffer solution (pH 7.8), 30 mg of ascorbic acid monosodium salt and 108 ml of methanol. After 10 min extraction the solution was centrifuged at 5000 *g* for 5 min and the supernatant was mixed with dioxane in a volume ratio of 7:1. Then chilled water was added until the Bchl precipitated. The solution was incubated at −20 °C for 20 min and centrifuged at 8000 *g* for 2 min and the resulting pellet was dissolved in approximately 36 ml of ethanol. The obtained solution was then filtrated and dried in a rotary evaporator and the resulting material was applied on a Sepharose CL-6B column with 20:1 hexane/2-propanol to remove carotenoids and pheophytin.

The Chl was extracted from spinach leaves and purified according to the method described by Katoh et al. [22]. Washed leaves (70 g) mixed with 400 ml of acetone were mechanically homogenized, filtrated and then diluted in dioxane (dioxane/extract ratio 1:7). Chilled water was gradually added until a dark green precipitate was formed. After storage for 20 min at −20 °C the mixture was centrifuged at 6000 *g* (1 min) and the resulting pellet was dissolved in 80 ml of ethanol. The solution was then filtrated and dried and the resulting material was applied on a DEAE-Sepharose CL-6B column with acetone to remove carotenoids and pheophytin and with 10:3 acetone/methanol solution to improve Chls. After vacuum drying, the Chl *a* and Chl *b* were dissolved in 100 ml of hexane/2-propanol (20:1 v/v) and applied on a Sepharose Cl-6B column to separate the Chl *a*.

The obtained solutions with Bchl or Chl were then filtrated and dried in a rotary evaporator. The dried pigments were stored in a refrigerator (−20 °C). Prior to use the dried pigments were dissolved in ethyl ether and filtrated again and the concentration was determined spectroscopically [23]. All procedures in the Bchl and Chl preparation were carried out under dark and cold (4 °C) conditions.

2.2. Reconstitution of photoreaction units from RCs and pigments into liposomes

Before use, L-α-phosphatidylcholine (L-α-lecithin) type IV-S from soy beams (hereinafter called SPC; Sigma Chemical Co., USA) was washed twice with acetone. In our reconstitution of RCs and pigments into liposomes we modified the method described by Mimms et al. [24]. An ethyl ether solution containing an appropriate concentration of SPC and pigment (Bchl or Chl) was dried as a thin film on the wall of a glass tube in an argon atmosphere. The dried pigment–SPC mixture was dispersed in buffer solution (10 mM Tris–HCl at pH 8.2) with an appropriate concentration of RCs, vigorously stirred and then sonicated until the solution became transparent by using a low energy bath-type sonicator (28 kHz, 10 min) and a sonda-type sonicator (100 W, twice for 3 s). The RC–pigment–SPC solution was frozen, stored in liquid nitrogen, thawed slowly at room temperature and then sonicated again to give well-fused membranes [25]. All procedures involving pigments or complexes were done in the dark at 4 °C. The basic concentration of RCs was adjusted to 48 μM (RC/LP ratio 1:800). The RC solution was added to the pigment–SPC solution at an RC/pigment molar ratio of 1:20. Using this procedure, we produced small unilamellar vesicles with diameters ranging from 200 to 350 Å [26].

Fig. 1 (see Section 3) shows a schematic diagram of the reconstituted PRUs, hereinafter called pigment–RC–LP or, specifically, Bchl–RC–LP and Chl–RC–LP. As a reference we also prepared liposomes only with pigments (Bchl/Chl–LP) and only with RCs (RC–LP). The concentrations of RCs, pigments and lipids were the same as those for the pigment–RC–LP solutions.

As an electron mediator in the photobleaching and photocurrent measurements we added ubiquinone (UQ), either UQ_{10} or UQ_0 respectively, to the samples. The UQs were dissolved in acetone and added to the reconstituted liposome samples in an amount less than 1% v/v. The optimal ratio of UQ_{10} to RCs was 10:1 and that of UQ_0 to RCs was 200:1.

2.3. Spectroscopic measurements

Absorption spectra of the samples were obtained using a UV-160 Visible Recording Spectrophotometer (Shimadzu

Co. Ltd., Japan). Fluorescence spectra were measured at 20 °C using an F-4500 Fluorescence Spectrophotometer (Hitachi Co. Ltd., Japan).

The flash-induced transmittance changes were measured using a home-constructed system that included a 100 W tungsten halogen lamp as a light source, a monochromatic light (model CT-25GT, Japan Spectroscopic Co. Ltd.) and a photomultiplier (model R632, Hamamatsu Photonics Co. Ltd., Japan) as a detector. The output signal was collected using a digital storage scope (model KDS 103, Graphtec Co. Ltd., Japan). The light flashes were given by a xenon photographic strobe lamp (model Auto 30SR Thyristor, Sunpak Co., Japan) and passed through a 250–650 nm bandpass filter (type CS4-76, Corning Co. Ltd., USA). The measuring beam was cut by a 760 nm sharp cut-off filter (type ITF-50S-76IR, Sigma Koki Co. Ltd., Japan).

2.4. Photoinduced current response measurements

The photoinduced current responses of the reconstituted liposome systems were measured using a photoelectrochemical cell. The liposome solution was sandwiched between two indium tin oxide (ITO) transparent electrodes that were 100 μm apart. Each electrode had an active area of 10×30 mm^2. The electrochemical cells were kept at 4 °C and were used for the measurements within 10 days after preparation.

The light-induced electrical responses were measured using a current amplifier (model 428, Keithley Co., USA) and a digital storage scope (model KDS 103, Graphtec Co., Japan). As a source of light we used a 300 W tungsten halogen lamp with a 640 nm sharp cut-off filter (type SC-64, Fuji Photo Film Co. Ltd., Japan) and with a water filter 3 cm thick to reduce the IR light energy. Using interference filters, we measured the action spectra between 600 and 1000 nm at 20 nm intervals and then normalized the response by the light intensity. An optical power meter (model TQ8210, Advantest Co. Ltd., Japan) was used to measure the intensity of the monochromatic light. The power meter was calibrated at 1000 nm using a radiometer (YSI-Kettering 65A, Yellow Springs Instrument Co. Inc., USA).

3. Results and discussion

Fig. 1(a) shows a schematic diagram of the proposed model for reconstituted PRUs (pigment–RC–LP) composed of either Bchl or Chl and native RCs in lipid vesicles. Figs. 1(b) and 1(c) show schematic diagrams of the models used for reference measurements, namely pigments in liposomes (Bchl/Chl–LP) and RCs in liposomes (RC–LP) respectively.

In the membranes of photosynthetic bacteria, antenna bacteriochlorophylls are bound to polypeptides, forming LHI 875 nm and LHII 800/850 nm complexes. In our model system, as shown in Fig. 2(a), the lack of polypeptides was reflected in the absorbance spectrum of Bchl–RC–LP, where

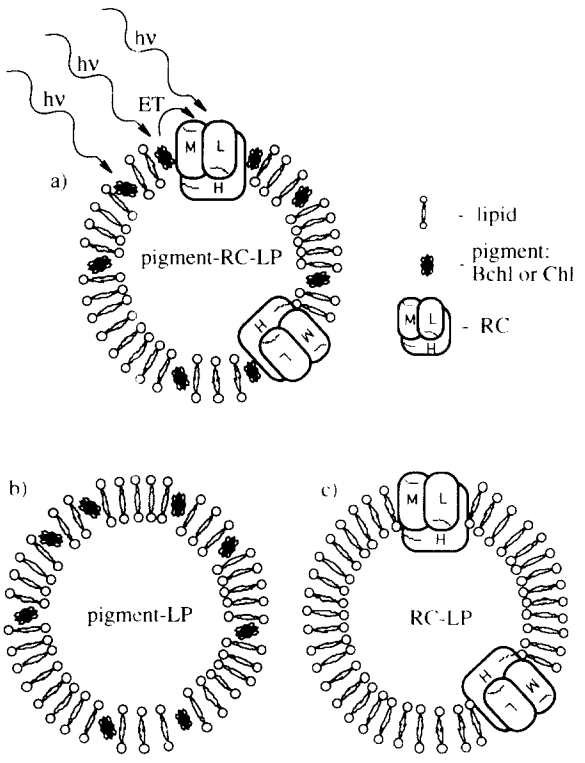

Fig. 1. Schematic illustration of reconstitution of pigments and RCs into liposomes: (a) Bchl/Chl–RC–LP; (b) Bchl/Chl–LP (reference); (c) RC–LP (reference). This is a model of the energy transfer (ET) mechanism within Bchl/Chl–RC–LP showing the light-harvesting ability of the pigment, which transfers its energy to the RCs.

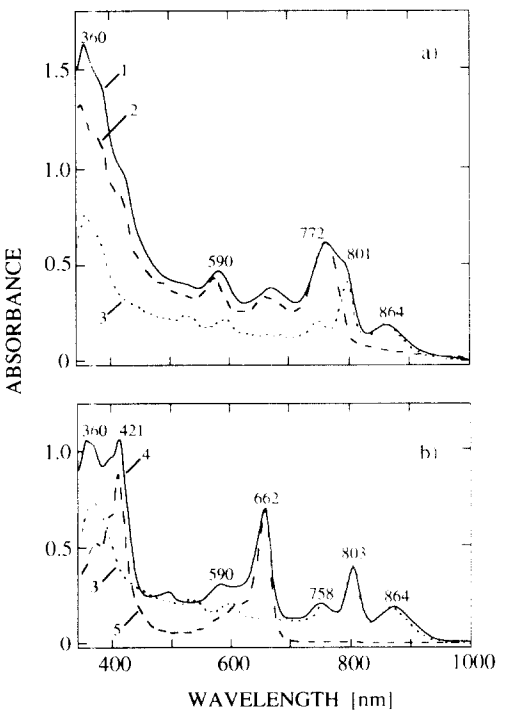

Fig. 2. Absorbance spectra of pigments and proteins reconstituted into liposomes in 10 mM Tris–HCl, pH 8.2 buffer solution: (a) Bchl-pigmented system; (b) Chl-pigmented system. The curves correspond to (1) Bchl–RC–LP, (2) Bchl–LP, (3) RC–LP, (4) Chl–RC–LP and (5) Chl–LP.

the maximum, 772 nm, strongly overlapped the red band maxima of RCs. The absorption maximum of RCs at 360 nm corresponds to the Soret band common to all Bchl forms, that at 590 nm to Q_x Bchl monomer transitions, that at 758 nm ty Q_y of bacteriopheophytin (BPh) and those at 801 and 864 nm to Q_y of Bchl monomer and dimer respectively. The red band maximum (Q_y) of the free Bchl monomer form in Bchl–LP occurred at 772 nm. Fig. 2(a) shows that the absorption of Bchl–RC–LP is a superposition of the spectra for RC–LP and Bchl–LP, resulting in a significant overlap of the three maxima that occurred at 758, 772 and 801 nm.

The pigment absorption maxima for the Chl PRUs (Fig. 2(b)) showed better resolution than those for the Bchl PRUs. Although the spectrum for Chl–RC–LP is a superposition of the RC–LP and Chl–LP absorption spectra, there are also well-visible Chl maxima at 421 nm (Soret band) and 662 nm (Q_y).

The pigment–protein and pigment–pigment interactions in light-harvesting antennae of photosynthetic bacteria create energy levels that are suitable for the efficient transfer of excitation energy to the RCs. Changes in absorption spectra due to pigment aggregation have been observed for Bchls in chlorosomes of green photosynthetic bacteria [27]. Similar changes in the positions of energy levels with respect to the monomeric pigment can be formed in model systems by pigment aggregation [18]. In our model system, dimeric or higher aggregated forms of pigments were not observed; only the Bchl or Chl molecules in monomeric form were embedded in the lipid bilayer of the liposomes. The pigments were not bound to RCs to induce an interaction, because our results show that in liposomes with RCs and pigments the absorption maxima for Bchl and Chl were not shifted with respect to the positions of the maxima for the corresponding systems without RCs.

The kinetics of charge recombination in RCs from the excited states $Bchl_2^+Q_A^-$ and $Bchl_2^+Q_B^-$ (where Q_A and Q_B are quinone A and B respectively) were measured for RCs introduced into liposomes with and without pigments. Fig. 3(a) shows examples of typical flash-induced transient signals for Bchl–RC–LP and RC–LP observed at 860 nm. The kinetics of the transmittance decay after a flash of light in the presence of UQ_{10} were different from those in the absence of UQ_{10}. In the presence of excess UQ_{10} the charge recombination process occurred mostly from the $Bchl_2^+Q_B^-$ energy level and the best curve fit was a single-exponential decay with a typical slow relaxation time of $\tau_{Q_B} = 1.6 \pm 0.2$ s. For the samples without UQ_{10} the kinetics of the decay were double exponential with a fast component with $\tau_{Q_A} = 90 \pm 10$ ms and a slow component with $\tau_{Q_B} = 1.6 \pm 0.2$ s. The exact contribution of the fast component to the decay depended on the sample preparation and usually ranged from 60% to 80%. This means that the electron backscattering from the Q_B position was less efficient because of the depletion of terminal acceptors, which made the charge recombination from the $Bchl_2^+Q_A^-$ energy level more preferable. No differences in decay times were observed between the Bchl/

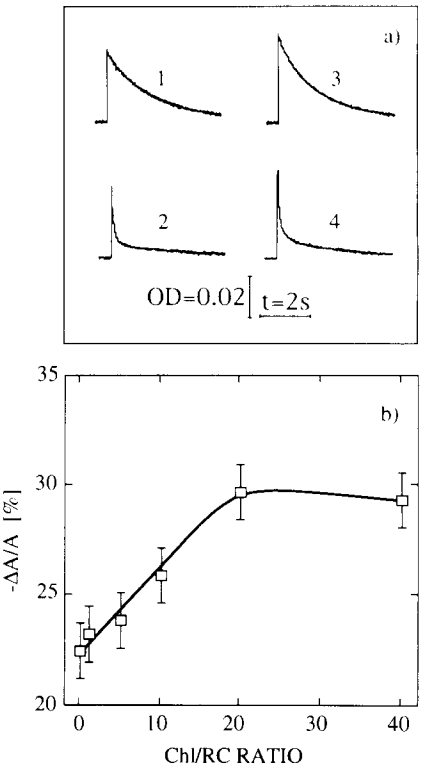

Fig. 3. (a) Examples of flash-induced transient signals for RC–LP (curves 1 and 2) and Bchl–RC–LP (curves 3 and 4). Curves 1 and 3 correspond to samples with UQ_{10} mediator and show a single-exponential decay with $\tau_{Q_B} = 1.6 \pm 0.2$ s. Curves 2 and 4 correspond to samples without UQ_{10} and show a double exponential decay with $\tau_{Q_A} = 90 \pm 10$ ms and $\tau_{Q_B} = 1.6 \pm 0.2$ s. The RC and Bchl concentrations were 1.2 and 24 μM respectively in 10 mM Tris–HCl, pH 8.2 buffer solution. (b) Dependence of flash-induced relative absorbance changes $\Delta A/A$ on Chl/RC ratio for Chl–RC–LP system. The RC concentration was kept constant at 1.2 μM. The transient signal was measured at 860 nm, with the light flash passed through a bandpass filter of 250–650 nm. The accuracy of the measurements was $\pm$ 1.2%.

Chl–RC–LP and RC–LP samples. The observed decay times agree well with results reported earlier [28,29]. The unchanged kinetics in the light-induced transmittance changes of RCs in the artificial system with pigments suggest that the presence of Bchl or Chl in liposomes did not influence the relaxation time of electron backscattering from quinones to the Bchl special pair located inside the RC. Therefore effects observed as a result of pigment addition should be related to the excitation energy transfer from these pigments to the RCs rather than to a redox-type interaction of the pigments with the RCs. If it exists, the latter type of interaction should be reflected in the kinetics of the absorption change decay.

To compare the photobleaching effect between the RC–LP and pigment–RC–LP samples, which differ in absorbance (Fig. 2), we measured the relative changes in the absorbance (i.e. $\Delta A/A$). This parameter enabled us to determine the absorbance changes divided by the amount of energy absorbed by the molecules. Fig. 3(b) shows the photobleaching $\Delta A/A$ dependence on the Chl/RC ratio at 860 nm. The largest changes in $\Delta A/A$ occurred when the ratio of pigment molecules to RCs was 20:1. This ratio agrees well with the

in vivo situation for *R. sphaeroides* where one RC is surrounded by about 20 pigment molecules in each LH complex [7,8]. A further increase in the pigment concentration did not improve the efficiency of photobleaching, which suggests that most of the energy transfer to the RCs is from pigments that are sufficiently close to the RCs, probably from neighbours located within a distance comparable with the critical radius R_{da} of resonance excitation transfer. These results agree well with those in the literature [15] reporting that the energy transfer efficiency increases as the acceptor concentration increases but is practically independent of the donor concentration above a certain critical level. Because the mutual pigment–protein orientation and distances in our model were certainly less optimal in comparison with natural LH complexes, we expected a maximum of $\Delta A/A$ at a Chl/RC ratio higher than 20:1. Fig. 3(b) shows that the observed maximum is not very sharp, but an additional increase in the number of pigment molecules, which causes a decrease in the LP/pigment ratio, could drastically change the condition of proteins and pigments in LPs. Therefore we considered the pigment/RC ratio of 20:1 to be the optimum for our experiments.

Fig. 4 shows the spectra of $\Delta A/A$ for pigment–RC–LP and RC–LP in the wavelength range from 760 to 1000 nm. The maximum occurred in the region of Bchl dimer absorption, around 860 nm, which agrees well with data reported previously [28,30]. In all cases here (Bchl and Chl as pigments) the absorbance changes were greater for pigment–RC–LP

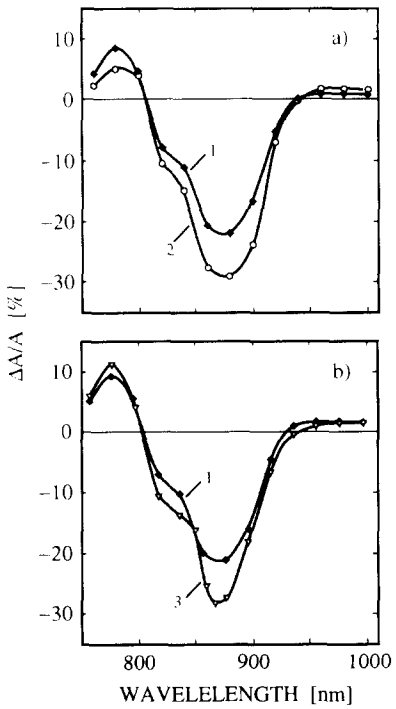

Fig. 4. Spectra of flash-induced relative absorbance change $\Delta A/A$ for reconstituted PRUs suspended in 10 mM Tris–HCl, pH 8.2 buffer solution: (a) Bchl-pigmented system; (b) Chl-pigmented system. The curves correspond to (1) RC–LP, (2) Bchl–RC–LP and (3) Chl–RC–LP. The pigment and RC concentrations were 24 and 1.2 μM respectively.

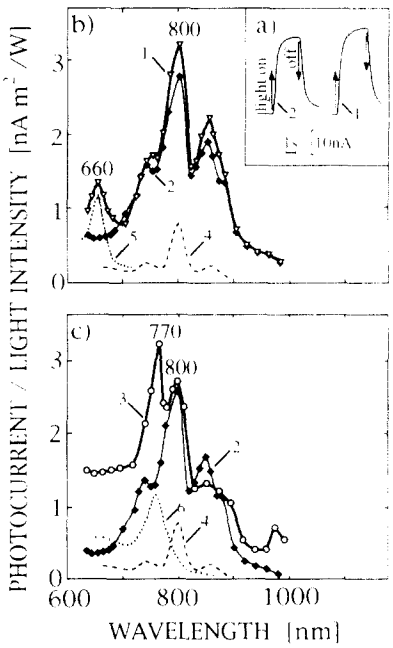

Fig. 5. (a) Examples of photocurrent response in sandwiched photocells with Chl–RC–LP (curve 1) and RC–LP (curve 2) at excitation wavelength $\lambda = 800$ nm. (b, c) Photocurrent action spectra for (b) Chl- and (c) Bchl-pigmented sandwiched samples. The full curves correspond to (1) Chl–RC–LP, (2) RC–LP and (3) Bchl–RC–LP. Responses were normalized by the light intensity (average light intensity $P = 12.9$ W m^{-2} at $\Delta\lambda_{1/2} = 9 \pm 1$ nm). The pigment and RC concentrations were 960 and 48 μM respectively. The broken curves show the absorbance red band maxima for (4) RC–LP, (5) Chl–LP and (6) Bchl–LP.

than for RC–LP. At the maximum (860 nm) the net enhancement of photobleaching in the presence of pigments was more than 20%. These results suggest that pigments reconstituted into liposomes in positions close to RCs are able to transfer energy to RCs and increase the efficiency of energy conversion in artificial systems.

Measurements of the photocurrent generation provided information about the charge distribution in our reconstituted PRUs. For this purpose we introduced reconstituted systems between two transparent ITO electrodes of a photoelectrochemical cell. Since it is well known that sandwiched cells do not exhibit identical electrical properties, for the analysis we only used data averaged over several samples.

The inset in Fig. 5(a) shows an example of the photocurrent response for the sandwiched cells of Chl–RC–LP and RC–LP (after irradiation at 800 nm) when a light is switched on and off. The higher value of the photocurrent for Chl/Bchl–RC–LP than for RC–LP can be explained by the efficient excitation energy transfer from the pigments to the RCs. The irradiated electrode of the cell with either Bchl/Chl–RC–LP or RC–LP always produced a positive charge (cathode), meaning that the electrons were always transferred to the dark (not illuminated) electrode of the symmetrical photocell. It is known that isolated pigments in polymer or liquid crystal matrices can also generate a photocurrent [19,31]. However, this effect can be neglected in our system model, because the current generated in pigment–LP was much lower

than that in RC–LP. To eliminate the charge mediator effect of the pigments, we used an excess of additional efficient electron mediator (i.e. UQ_0).

Figs. 5(b) and 5(c) show the photocurrent action spectra normalized by the power of the incident light for the Bchl/Chl–RC–LP and RC–LP sandwiched photocells. These figures show that the presence of pigments always enhanced the photocurrent, especially in the shorter excitation wavelength region. The positions of the action spectra maxima agree well with the absorbance maxima for RCs, but additional maxima occurred that were related to the pigment absorbance, namely at 770 nm for Bchl and at 660 nm for Chl.

The photocurrent responses, i.e. the net differences between light and dark current levels averaged over all measured samples, were higher for pigment–RC–LP than for RC–LP. Thus the photocurrent enhancement and maxima positions of pigmented systems cannot be explained simply by charge transfer but rather by energy transfer from the pigment molecules to the RCs.

Our photocurrent response measurements confirmed that in our model the pigments function as quasi-antennae, because pigment–RC–LP showed a stronger photoresponse than RC–LP. Comparison of the photocurrent action spectra with the absorbance spectra suggests that energy transfer from the pigment molecules to the RCs occurs rather than direct charge transfer from the pigments to the electrodes. The relative increase in the photocurrent response caused by the presence of pigments in liposomes containing RCs was greater when the light intensity was low (less than 13 W m^{-2}). The photoresponse as a function of light intensity was almost linear for RC–LP, whereas it became non-linear for pigment–RC–LP (data not shown). For a light intensity higher than 13 W m^{-2} the photocurrent response for the pigmented systems reached saturation. These results indicate that our model system is more effective at lower light intensities that are of the order of sun illumination in the natural environment.

To support our hypothesis that excitation energy transfer occurs from pigment molecules to RCs, we carried out donor fluorescence-quenching measurements. When in vivo, the RCs can serve as an energy trap, because the Bchl in an RC has the lowest, or almost lowest, energy level of all Bchl molecules in the photosynthetic apparatus [6]. Therefore we expect that the functional incorporation of RCs into lipid membranes in which each RC is closely surrounded by Chl/Bchl molecules can lead to the 680/790 nm fluorescence quenching owing to energy transfer from the pigments to an RC that is in a lower energy state. The Chl and Bchl molecules should work as energy donors for Förster resonance energy transfer to the RC acceptor. Our use of the donor fluorescence-quenching method was valid because at room temperature only weak, sensitized fluorescence of the acceptor occurred. In an equilibrium state, in single unilamellar vesicles, an increase in the energy transfer from donor to acceptor should be confirmed by a decrease in the yields of other paths of energy deactivation. Quenching of the donor fluorescence should also be observed. Then an increase in the number of acceptors surrounding each donor causes a decrease in the donor fluorescence. Experiments in which the number of pigment molecules is increased are not sensitive enough for energy transfer investigation, because the energy transfer efficiency is almost independent of the donor concentration [15]. This fact can be explained by the active sphere effect around each donor molecule, meaning that energy transfer occurs when an acceptor is within a certain distance of the donor. For resonance excitation energy transfer the radius of this effective sphere should be comparable with the critical distance R_{da} in the Förster theory. The increase in acceptor concentration causes an increase in the yield of quenching until saturation is reached, i.e. when statistically all donors have an acceptor within their active sphere. In our model, instead of an active sphere, we introduced a two-dimensional circular portion of the liposome membrane which consists of one RC and appropriate numbers of pigments.

Fig. 6 shows fluorescence spectra of Bchl/Chl–RC–LP for constant pigment and liposome concentrations and various RC concentrations. The fluorescence emission with a maximum at 790 nm (F790) is related to the Bchl molecules with the excitation mostly from the maximum at 360 nm. For the Chl molecules, excitation at 440 nm leads to fluorescence F680. The uptake of RCs into liposomes is limited, and when the saturation level is exceeded, the amount of free acceptors

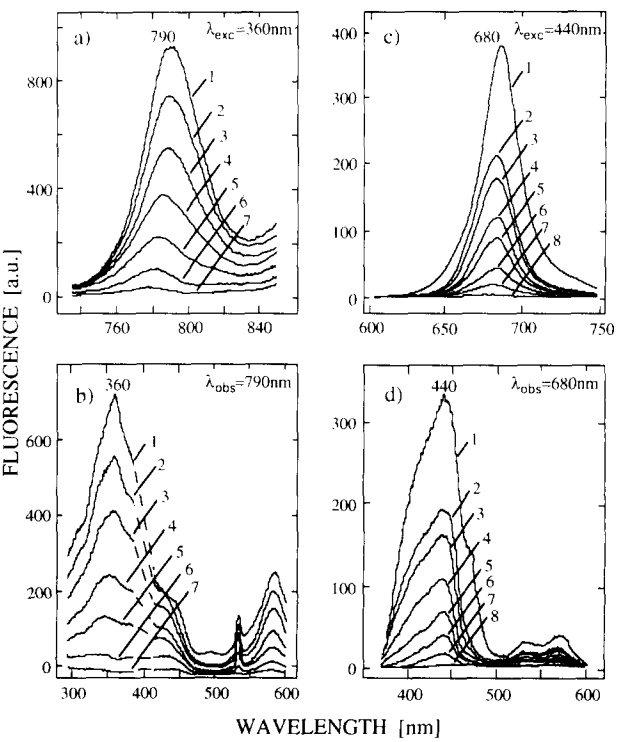

Fig. 6. Fluorescence spectra of reconstituted PRUs in buffer solution for (a, b) Bchl–RC–LP and (c, d) Chl–RC–LP: (a, c) emission spectra; (b, d) excitation spectra. The pigment and lipid concentrations were kept constant at 10 and 450 μM respectively. The curves represent increasing pigment/RC ratios of (1) Bchl/Chl–LP (no RC), (2) 40:1, (3) 20:1, (4) 10:1, (5) 5:1, (6) 2:1, (7) 1:1 and (8) 1:2.

in the aqueous phase could be easily increased [12]. Furthermore, a high surface density of RCs perturbs the lipid environment and may eventually cause disruption of the bilayer structure [12,32]. We therefore restricted the amount of added acceptor to a maximum RC/LP ratio of 1:45, with constant pigment and phospholipid concentrations of 10 and 450 μM respectively. As expected, an increase in acceptor concentration led to a decay in the fluorescence, and when there was less than one acceptor molecule per donor molecule, the donor fluorescence was completely quenched (Fig. 6).

The energy transferred from the donor is proportional to the fractional loss of donor fluorescence given by the formula [15]

$$\Phi_d = 1 - \frac{\Phi_{fd}}{\Phi_{fd0}} \quad (1)$$

where Φ_{fd} and Φ_{fd0} are the donor fluorescence quantum yields in the presence and absence of an acceptor respectively. Assuming that all fluorescence and absorbance measurements can be done under the same energy conditions, the fractional loss of donor fluorescence can be calculated as

$$\Phi_d = 1 - \frac{F_d/A_d}{F_{d0}/A_{d0}} \quad (2)$$

where F_d and F_{d0} are the donor fluorescences in the presence and absence of an acceptor respectively and A_d and A_{d0} are the donor absorbances in the presence and absence of an acceptor respectively.

For known concentration and mean cross-sectional area S per molecule of RC ($S_a = 3850$ Å^2 [4,33]), Chl/Bchl ($S_d = 92$ Å^2 [17,34]) and lipid ($S_{LP} = 65$ Å^2 [26]) we calculated the average donor–acceptor distance (active circle radius of one acceptor) for a uniform distribution according to the formula

$$R_{da} = \left(\frac{S_{tot}}{\pi c_a}\right)^{1/2} \quad (3)$$

Here S_{tot} is the combined active circle surface of the pigment–RC–lipid double layer and is defined as $S_a c_a + S_d c_d + 0.5 S_{LP} c_{LP}$, where c_a, c_d and c_{LP} are the concentrations of acceptor, donor and lipid respectively. This formula corresponds to the radius used by Heckl et al. [16] in explaining the energy transfer between bacterial antenna protein B800-850 and RCs in a monolayer at the air–water interface. Eq. (3) is valid when the difference between the cross-sectional areas of the donor and acceptor is large, thus making the distance between RCs predominant.

Fig. 7 shows the fractional loss of donor fluorescence (calculated from Eq. (2) using data of fluorescence maxima) as a function of the donor–acceptor distance (calculated from Eq. (3)). Total loss of the donor fluorescence ($\Phi_d = 1$) occurred for a donor–acceptor distance of about 40 Å for Bchl–RC–LP and 37 Å for Chl–RC–LP. The calculated distance depended on the orientation of the donor and acceptor,

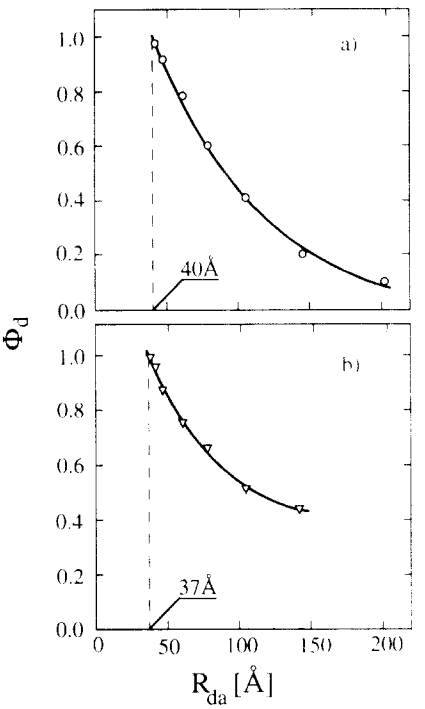

Fig. 7. Dependence of fractional loss of donor fluorescence (Φ_d) on donor–acceptor distance (R_{da}) for (a) Bchl–RC–LP and (b) Chl–RC–LP. Φ_d was calculated from the emission fluorescence maxima F790 (for Bchl) and F680 (for Chl). The values 40 and 37 Å correspond to the effective loss of donor fluorescence ($\Phi_d = 1$) for systems with Bchl and Chl respectively.

and because exact mutual orientations of RC complex and pigment molecule transition moments in liposome membranes are not established, we should regard the obtained values as estimates. The obtained donor–acceptor distance of about 40 Å is low enough for effective Förster resonance energy transfer, as shown for other model systems [15,16]. This value is approximately equal to the radius of an RC (35 Å [4,33]), suggesting that energy transfer occurs only from pigments that are very close to the RCs.

It is well known that photosynthetic pigments (especially Bchl) are very unstable in vitro. Our artificial Bchl/Chl–RC–LP system reconstituted into liposomes has good stability with respect to time as well as to temperature. After 10 days at room temperature and in natural light we observed no significant changes in the basic properties of the samples. Unfortunately, the system was sensitive to high irradiation intensity, reflected in the absorbance spectra as the formation of a denaturation maximum at 670 nm for Bchl and around 620 nm for Chl. A decrease in the efficiency of energy transfer was also seen after frequent, strong photobleaching. Our reconstituted PRU has no natural protection system and we believe that the addition of β-carotene could reduce the triplet state formation and as a result inhibit or at least reduce the observed denaturation process.

4. Conclusions

We constructed a simple reconstituted PRU to simulate the function of natural PRU in photosynthetic bacteria. The data

presented here show that even in such a simple model, photosynthetic pigments (Chl and Bchl) directly incorporated into lipid bilayers in which the pigments are very close to the RCs can function as light-harvesting antennae. This means that apart from light energy absorbed directly by RCs, energy absorbed by pigments can be transferred to RCs and cause an increase in the efficiency of energy conversion.

The effective quenching of donor fluorescence occurred at donor–acceptor distances of about 40 and 37 Å for the systems with Bchl and Chl respectively. These values seem to be reasonable for effective Förster resonance energy transfer from pigments to RCs. Our artificial model system could enhance the photoinduced charge separation across lipid membranes and the photocurrent generation in sandwiched photoelectrochemical cells. The best result of energy conversion was obtained for a pigment/protein ratio of 20:1. The system was more efficient at a lower range of intensity of irradiation and showed properties similar to in vivo systems, i.e. highly stable with respect to time and temperature.

No significant differences in the energy transfer and charge transfer between systems with Bchl and Chl pigments were observed. This was rather unexpected because of various overlaps of the donor fluorescence and acceptor absorbance spectra in these two cases.

The proposed model system is promising as a starting point for investigations into the construction of biodevices for conversion of solar energy into electrical energy.

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