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Protein/lipid interactions in phospholipid monolayers containing the bacterial antenna protein B800–850

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Studies on monomolecular layers of phospholipids containing the antenna protein B800–850 (LHCP) and in some cases additionally the reaction center of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* are reported. Information on monolayer preparation as well as on protein/lipid and protein/protein interaction is obtained by means of fluorescence spectroscopy and microscopy at the air/water interface in combination with film balance experiments. It is shown that a homogeneous distribution of functional proteins can be achieved. This can be transformed into a regular pattern-like distribution by inducing a phospholipid phase transition. Although the LHCP preferentially partitions into the fluid lipid phase, it decreases the lateral pressure necessary to crystallize the lipid. This is probably due to an increase in order of the fluid phase. A pressure-induced conformation change of the LHCP is detected via a drastic change in fluorescence yield. A highly efficient energy transfer from LHCP to the reaction center is observed. This proves the quantitative reconstitution of both types of proteins and indicates protein aggregation also in the monolayer.

Introduction

Protein/lipid interactions are of fundamental importance for the function of biological membranes, and their study therefore is one prime objective of many research efforts. From a biophysical point of view these studies have gained interest because techniques to isolate, characterize and reconstitute membrane proteins have been developed to a high degree. Thus systems with a rather well-defined structure can be designed. One biological system to which the above statements apply especially well is the bacterial photosynthetic apparatus [1–3]. Its function depends on membrane organisation and can be studied by optical techniques in a detailed way. The investigation of intermolecular interactions in monolayers at the air/water interface provides the advantage that this model system and its environmental parameters can be varied to a large extent. This concerns chemical composition, temperature, lateral pressure, phospholipid phase state and the electrostatics of the interfacial area.

Of special importance for the studies reported

Abbreviations: LHCP, bacterial antenna protein B800–850 (light-harvesting chlorophyll-protein); DLPA, DL-α-dilaurylphosphatidic acid; DMPA, DL-α-dimyristoylphosphatidic acid; DMPE, DL-α-dimyristoylphosphatidylethanolamine; DLPC, DL-α-dilaurylphosphatidylcholine; DP-NBD-PE, DL-α-dipalmitoylnitrobenzoaxadiazolephosphatidylethanolamine; BChl, bacteriochlorophyll *a*; LDAO, *N,N*-dimethyldodecylamine-*N*-oxide (lauryldimethylamine-*N*-oxide).

here are achievements of fluorescence microscopy applied to monolayers developed recently by our group [4,14]. This technique enables us to control the growh of large crystalline domains of phospholipids as has also been reported by two other groups [5,6]. In this work fluorescence microscopy is applied to monitor the functional reconstitution of proteins into monolayers, to study protein/lipid and protein/protein interactions. On doing this we specialize in the antenna protein B800–850 (LHCP) and the reaction center of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* R26 reconstituted into monolayers of charged and uncharged phospholipids. The function is characterized by fluorescence properties of the antenna and fluorescence quenching due to energy transfer to the reaction center. Using these model proteins we demonstrate not only the potential of the technique, but additionally provide surprising results on the interdependence between protein function and membrane environment.

Experimental

Materials

Rps. sphaeroides 2.4.1. was grown anaerobically on Hutner's medium [7]. Reaction centers were prepared by a modification of the procedure of Jolchine and Reiss-Housson [8] which involved two subsequent extractions of the chromatophores with 0.25% LDAO (Cogdell, R.J., personal communication, LDAO from Fluka, Buchs, Switzerland). The B800–850 light-harvesting complex was isolated from the reaction-center-depleted pellet according to Ref. 9.

All bacteriochlorophyll protein complexes were dialyzed against Tris-HCl buffer (20 mM; pH 8.0) containing LDAO ($5 \cdot 10^{-4}$ M). The lipids used in this investigation, DLPA, DMPA, DMPE and DLPC, were purchased from Fluka (Buchs, Switzerland) and checked for purity by thin-layer chromatography, and used without further purification. The dye labeled lipid DP-NBD-PE was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL, U.S.A.).

The water used was destilled and filtered (Milli-Q, Millipore Corp., El Paso, TX, U.S.A.). The solvents used were reagent grade.

Protein reconstitution into phospholipid monolayers

To prepare monolayers the phospholipid or phospholipid/dye mixture, respectively, was spread from a chloroform/methanol solution (3 : 1, both Merck, Darmstadt, F.R.G.). After solvent evaporation and recording the pressure-area isotherm the lipid monomolecular film was reexpanded into the fluid state to yield a pressure of 1–5 mN/m.

The antenna protein was kept in an 0.1% LDAO solution at pH 7 as described in the Materials subsection. Just before spreading an aliquot this solution was diluted 1 : 20 into Millipore-filtered water. 50 μl of this solution were then spread in approx. 30 droplets onto the fluid phospholipid monolayer in order to establish a homogenoeus distribution. Each droplet spreads over an area of some 10 cm^2 thus distributing the protein solution on the surface. This rather simple procedure yields a homogeneous LHCP/phospholipid layer at least over dimensions larger than 2 μm over the whole surface as can be judged by fluorescence microscopy.

It is well known that trace amounts (> 1 mol%) of amphiphatic impurities in the phospholipid monolayer seriously affect the pressure-area characteristics in that the transition pressure is increased and, at higher impurity concentrations, the phase transition disappears [10]. Therefore, to check the detergent influence, up to 20-fold the amount of LDAO applied in the protein experiments was added to the phospholipid surface layer. This yielded an area increase of about 7% explaining why in the experiments cited below the detergent does not affect the pressure-area diagram [11]. This additionally proves that the concentration of detergent solubilized in the phospholipid layer is well below 1 mol%, but does not exclude the possibility of boundary detergent at the protein molecules, especially at those protein regions sticking into the gaseous phase. A quantitative study concerning the exchange of detergent between protein/lipid monolayers and subphase is in progress in our laboratory.

Subsequent reconstitution of the reaction center into the antenna/phospholipid monolayer was then achieved by the same procedure described above. The functional integrity of the reaction center was demonstrated after transfer of the

monolayer onto solid support measuring flash-induced absorption changes at 870 nm. This yielded a surface reaction center concentration of 10^{10} cm^{-2} and a decay time of 85 ms, which is characteristic for reaction centers containing one quinone [1].

Fluorescence microscopy and spectroscopy

The fluorescence microscopic device was the one described elsewhere [12]. The excitation light wavelengths were between 440 and 490 nm (Zeiss KP 490 and Schott BG 18), corresponding to the carotenoid absorption. For monitoring fluorescence intensity or recording fluorescence spectra the emission from the field of view was focused onto a light pipe connected to the entrance slit of a 20 cm grating monochromator (Jobin-Yvon H 20 V). The emission was detected using a peltier cooled red sensitive photomultiplier tube (RCA C 31034) and a photon-counting device. The spectral resolution was 8 nm.

Results

Functional reconstitution of B800–850 into phospholipid monolayers

Reconstitution of membrane proteins into monolayers at the air/water interface generally leads to the question to which extent the incorporation is (1) homogeneous, (2) quantitative, and (3) functional.

To contribute to these problems with the technique introduced above we concentrate on the well-known pattern formation of monomolecular layers of pure phospholipids exhibiting crystalline growth in the flat region of the π-A isotherm. With the protein spreading method developed that is described in the second subsection under Experiments we aimed to test (a) whether or not the model peptides show affinity for incorporation into one of two lipid phases coexisting, and (b) how the fluorescence quantum yield reflects different qualities of the protein environment in the phospholipid monolayer.

From both area increase on spreading the protein solution and fluorescence microscopic observation we deduce that a quantitative and homogeneous reconstitution into the monolayer was successful as will be shown in the forthcoming

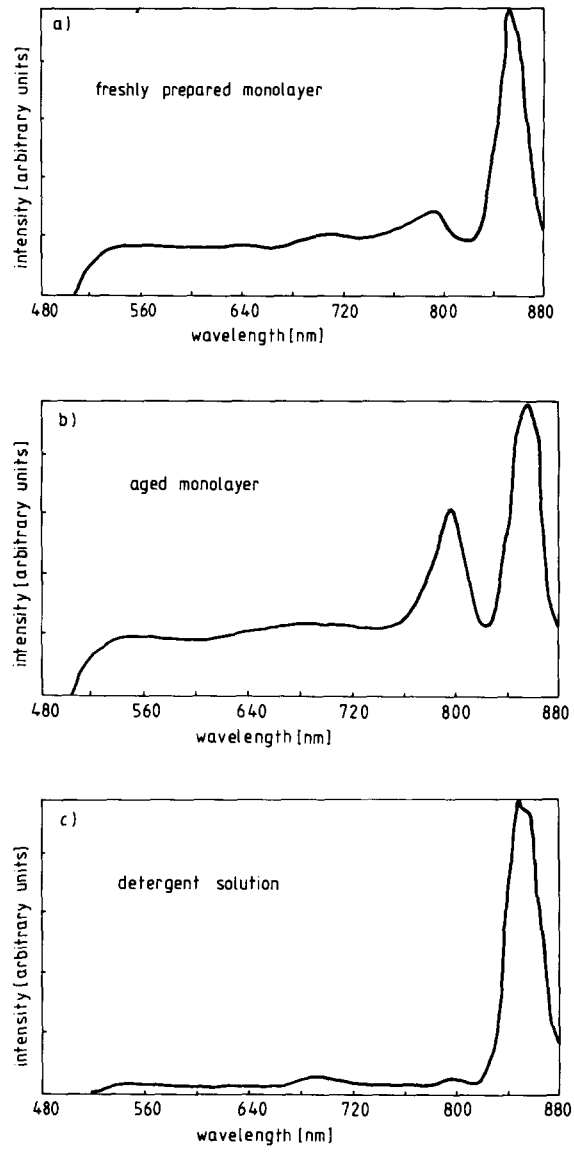

Fig. 1. Fluorescence spectrum of LHCP in DLPA monolayers at the air/water interface. Lipid molecular density is 10/7 nm^{-2}, protein/lipid ratio is 1:100. Spectral resolution, 8 nm. (a) Freshly prepared monolayer; (b) same monolayer after 1 h without inert gas atmosphere; (c) spectrum of LHCP in 0.005% LDAO solution.

subsection DLPA. The functional integrity of the proteins was assessed by means of the fluorescence spectra. These are shown for freshly prepared in Fig. 1a and for aged in Fig. 1b antenna proteins reconstituted into DLPA. For comparison the flu-

orescence spectrum of LHCP in detergent solution (Fig. 1c) is added.

Fig. 1a and c show the pronounced emission band at $\lambda = 858$ nm due to the two BChl chromophores absorbing at 850 nm which are probably associated with the heavier (9 kDa) subunit [13]. The lack of fluorescence from the BChl chromophores absorbing at 800 nm in the lighter subunit demonstrates an efficient energy transfer to the 'dimeric' BChl. We therefore conclude on the functional integrity of the relevant part of the protein containing the chromophores.

Without the protective inert gas atmosphere we observe a second fluorescence band rising at 800 nm in the time course of hours (1b). This indicates disruption of energy transfer to the 'dimeric' energy trap.

By keeping the monolayer under an argon atmosphere and in dim light we are able to preserve the LHCP function over hours as judged by the above criterion (data not shown).

Protein / lipid interaction

DLPA. Fig. 2 presents the π-A isotherms of DLPA at $T = 21°C$ (pH 5.5) without (leftmost trace) and after deposition of $3 \cdot 10^{13}$ molecules and $6 \cdot 10^{13}$ molecules of the antenna protein, respectively. For the subphase pH between 4 and 8, the DLPA head group is known to be singly deprotonated. The concentration c_p of the protein solution added was determined by absorption

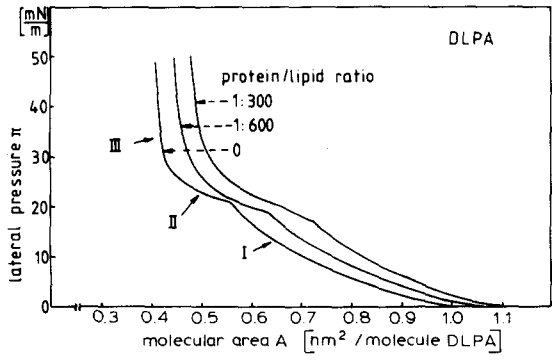

Fig. 2. Pressure-area diagrams of DLPA monolayers containing LHCP in different protein/lipid ratios. The ratios given in the figure are calculated assuming complete incorporation. T, 21°C; pH 5.5. In some diagrams there are indications for two break points in the slope between region I and II.

spectroscopy using the value of $3.19 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ for the extinction coefficient at $\lambda = 850$ nm (Scheer, H. and Cogdell, R.J., unpublished results). The most interesting features of the isotherms after protein deposition are (i) the increase in area and (ii) the decrease of the pressure π_m indicative of the onset of the lipid phase transition.

Calculating the average area A_p per LHCP protomer (α, β-$BChl_3$) in the monolayer at a lateral pressure π of 30 mN/m we obtain $A_p = A_p/N_p \approx 20$ nm^2, where A_p is the area increase due to incorporation of N_p protein molecules or a radius of $r_p \approx 2.5$ nm per LHCP molecule. The decrease of π_m on antenna incorporation is of the order of 10 mN/m per 1 mol% LHCP added.

Control experiments were performed by measuring the isotherms of the protein/lipid-monolayer at different temperatures (data not shown). Hence we deduce the temperature dependence of the pressure π_m of 1.4 $mN \cdot m^{-1} \cdot K^{-1}$. For the pure phospholipid monolayer values of 1 $mN \cdot m^{-1} \cdot K^{-1}$ are reported [15].

Observation of the fluorescence microscopic images leads to the same conclusion. As reported earlier [4–6,10,14] phospholipid monolayers labeled with fluorescent dyes exhibit a characteristic pattern formation on compression from the fluid state to the state of coexisting crystalline and fluid phases. This effect is due to different solubilities of the dye molecules in different phospholipid phases. In analogy with this we may assume that LHCP is squeezed into the fluid lipid phase. An alternative explanation that LHCP remains in solid domains, but is quenched there, can be ruled out from electron microscopic observations [16]. The protein/lipid monolayer shows homogeneous fluorescence in the fluid state (region I in Fig. 2).

It is important to note that fluorescence emission leading to an image is collected from the focal plane as can be demonstrated by imaging the entrance diaphragm of the excitation light. On compression into the phase coexistence region (region II in Fig. 2) pattern formation is observed as shown in Fig. 3. The fact that these patterns can be imaged shows that the fluorescence is emitted from molecules within the focal plane. This again proves that LHCP is incorporated into the monolayer, or at least adsorbed to it.

On further compressing the protein/lipid

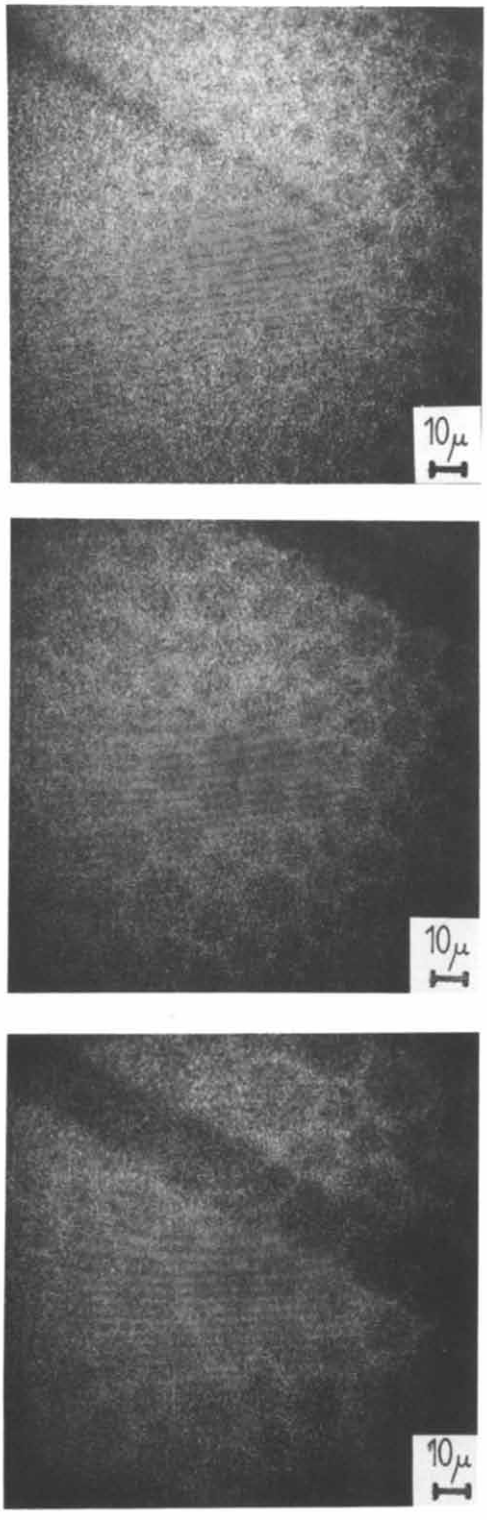

Fig. 3. Fluorescence micrographs of DLPA monolayers containing 1.5 mol% LHCP. The pressure is chosen to maintain the monolayer in the solid/fluid phase coexistence region II and is increased from Fig. 3a to 3c. One observes the fluorescence of LHCP embedded in the fluid phase. Hence the solid regions, the size of which increases with pressure, are dark due to a low protein solubility.

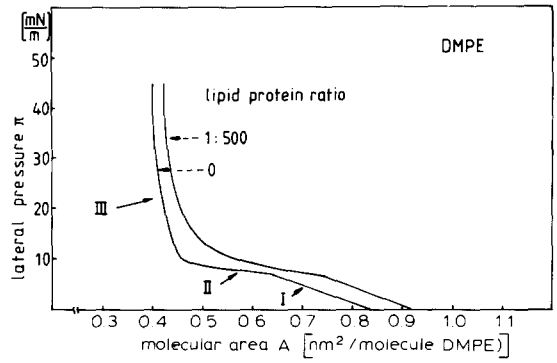

Fig. 4. Pressure-area diagrams of DMPE monolayers without and with LHCP incorporated. T, 22°C (pH 8), using 10 mM Tris buffer.

monolayer to increase the area ratio of the solid lipid (region III, Fig. 2) the fluorescence decreases below the detection limit. These effects are totally reversible, i.e., the fluorescence recovers again on decompression and the patterns dissolve on expanding below π_m, etc.

The observation of decreasing transition pressure π_m on protein incorporation and alteration of size and shape of the patterns formed (compared to the patterns observed with phosphatidic acid monolayers doped lipoid dye probe [10,17]) strongly suggests occurrance of protein/lipid interaction.

DMPE. In order to test how electrostatic interaction between the charged phospholipid head groups and the LHCP is involved in the findings reported in the previous subsection the uncharged phospholipid-matrix DMPE was tested for protein incorporation. Fig. 4 shows the π-A data for comparison with Fig. 2. Obviously, the onset of the phospholipid phase transition is much less decreased compared to the DLPA case. The area increase on protein incorporation yields similar values as the evaluation for DLPA.

In the fluid phase fluorescence microscopy again shows a homogeneous distribution. Fig. 5 shows the fluorescence images in the phase coexistence

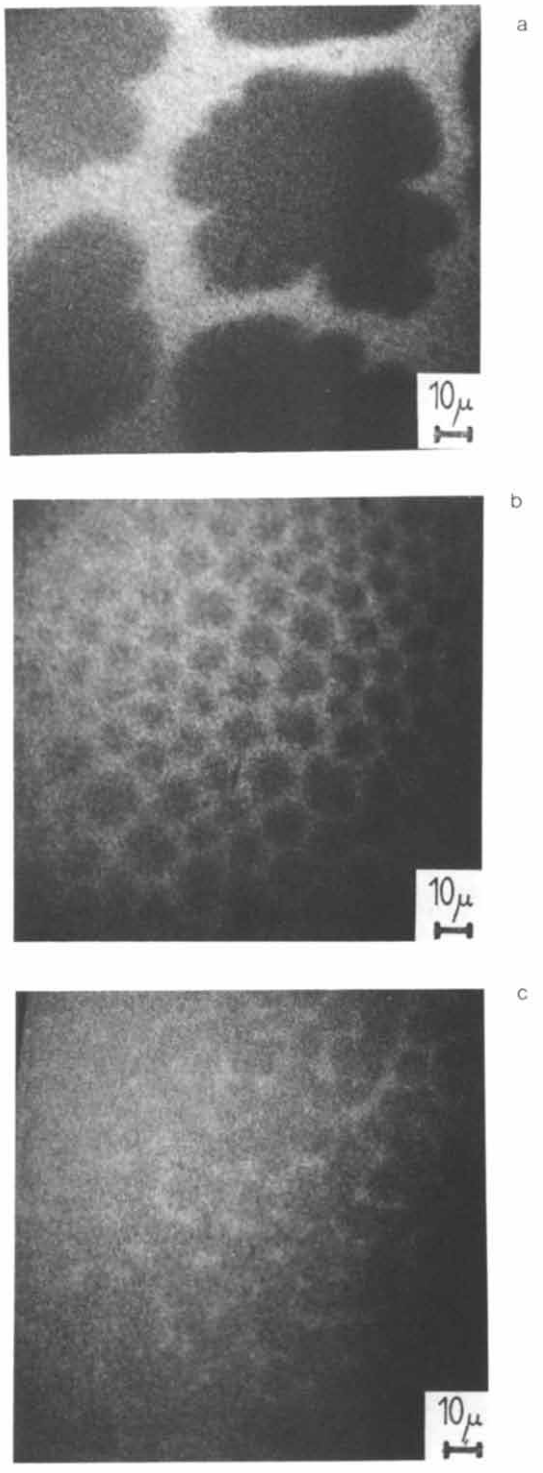

Fig. 5. Fluorescence micrographs of DMPE monolayers in the phase coexistence region II (T, 21°C, pH 8). (a) with 1 mol% DP-NBD-PE; no LHCP; (b) with 1 mol% DP-NBD-PE; 0.2 mol% LHCP; (c) no DP-NBD-PE; 0.2 mol% LHCP.

region visualized by incorporation of 0.5 mol% of the dye DP-NBD-PE, without (Fig. 5a) and with (Fig. 5b) LHCP incorporated, respectively. For comparison Fig. 5c shows the solid domains visualized by means of the LHCP intrinsic fluorescence. The following features have to be noted.

(a) The patterns emerge on passing π_m in the case of pure phospholipid, whereas in the protein/lipid monolayer the dark domains can only be visualized on compressing to higher pressures, where the biphasic region should be terminated and the compressibility $\kappa = -1/A\ \partial A/\partial\pi$ is decreasing.

(b) The solid domains in the latter case are very small compared to the monolayer without protein incorporated.

DLPC. In a third set of experiments DLPC was tested as a matrix for protein incorporation. Like DMPE it is uncharged, but due to the short hydrophobic chains does not exhibit any fluid/solid phase transition on compression at room temperature. Therefore, it provides a suitable system to rule out the influence of the phospholipid phase transition on the protein fluorescence behaviour.

As expected, no inhomogeneous fluorophore distribution could be detected throughout passing the entire isotherm. Like in the former cases fluorescence is emitted out of the focal plane. The fluorescence intensity, however, is not a linear function of fluorophore density. Fig. 6 shows a

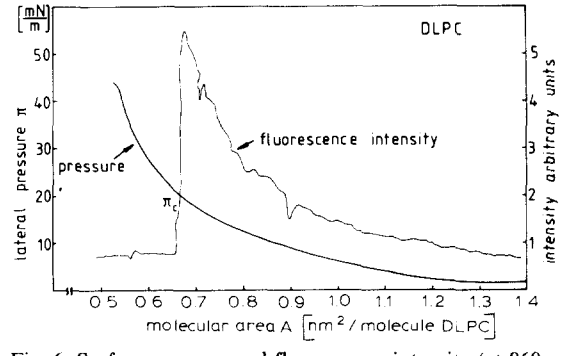

Fig. 6. Surface-pressure and fluorescence intensity (at 860 nm) as a function of molecular area for DLPC monolayers containing 0.6 mol% LHCP. T, 21°C (pH 8), using 10 mM Tris buffer. The increase in intensity on decreasing the area from 1.40 to 0.68 nm^2/molecule is not always as smooth as indicated in the figure.

characteristic trace of the intensity at $\lambda_{em} = 860$ nm as a function of monolayer area. Additionally the corresponding π-A isotherm is shown.

Whereas at values below a critical pressure π_c fluorescence increases stronger than linearly with molecular density, at π_c a sudden drop in emission intensity is observed. This decrease is completely reversible and can, in this case, not be correlated with the phospholipid phase behaviour. The drastic decrease in emission should then reflect the fluorescence quantum yield response to the variation of the lateral pressure mediated by the phospholipid environment.

Protein / protein interaction

As a last test of functional integrity of the antenna protein (as well as a promising direction to future work) reaction centers were incorporated into the LHCP/phospholipid monolayer. In vivo the reaction center serves as the lowest level energy trap, and therefore it is expected that after functional incorporation of reaction centers into functional arrays of LHCP in phospholipid monolayers the 858 nm-fluorescence of the BChl 'dimer' should be quenched due to energy transfer to the lower state reaction center.

Such a quenching can indeed be observed in the ternary monolayer system (Fig. 7).

The fluorescence of the pure LHCP/phospholipid system (upper trace) is drastically decreased after addition of $6 \cdot 10^{10}$ molecules/cm^2 of reaction center and has vanished after deposition of $12 \cdot 10^{10}$ molecules/cm^2 (corresponding to average reaction center: LHCP ratios of 1 : 200 and 1 : 100, respectively). Again, this is an additional proof of the incorporation of the antenna protein into the monomolecular layer and, above that, of course shows that the reaction center also is incorporated into the 2-dimensional system. It also shows that the natural function, energy transfer from B800–850 to reaction center, can be reconstituted in the monolayer system.

Discussion

Functional reconstitution into monolayers

The new technique introduced is able to clarify some general questions connected with protein reconstitution into monolayers which are discussed subsequently in more detail.

Homogeneous protein distribution. Applying the more conventional technique of dropping or rinsing the protein solution onto a certain surface area one observes only clusters of proteins unevenly distributed over the surface. This is understandable as molecular diffusion over distances of 1 cm requires times longer than 10^6 s. Convective flow was measured below 10 μm/s. Hence distribution over distances of 1 cm still requires more than 10^3 s. Our simple technique of uniformly distributing small droplets was superior probably for the following reasons.

(i) The droplets were small enough to lie on the hydrophobic surface and not to penetrate immediately into the subphase. In the latter case the membrane protein would have returned to the surface, basically at the point of immersion and not been distributed.

(ii) The reduced surface tension of the detergent containing water droplets allow for spreading on the hydrophobic surface.

Incorporation into the monolayer. The LHCPs added are embedded into the phospholipid monolayer or, at least, adsorbed very close to it. We deduce this statement from the following observations.

(a) By means of fluorescence microscopy we are able to obtain images of the protein distribution. As the observation is made by focusing on the water surface with a depth of field of about 1 μm the proteins are at least as close to the surface.

(b) Reaction center reconstituted additionally in a rather low surface concentration are very efficient quenchers. This requires that energy donor (LHCP) and acceptor (reaction center) are contained within a thin layer of thickness below 10 nm. A crude estimate indicates that the proteins otherwise would be diluted too much to allow for energy transfer. If the amount of reaction center sufficient for quantitative energy transfer would have been homogeneously distributed in the subphase the volume concentration of RC would be below 10^{-10} M. Hence we conclude that the RC is incorporated into or adsorbed onto the monolayer as a distance closer than 10 nm to it.

(c) The increase in fluorescence intensity with molecular density for pressures below π_c indicates

that the protein is coupled to the surface layer. This again implies an incorporation into or at least a tight binding to the surface.

(d) Indications that the LHCP and reaction center are contained in the surface layer at last also result from electron micrographs of shadowed monolayers after transfer on a solid support. These additionally show linear aggregates of LHCP that will be reported in another publication [16].

Quantitative reconstitution. Data on the amount of reconstituted LHCP (or reaction center) result from the area increase on protein addition. The protein radius calculated from this (≈ 2.5 nm) is large in view of the fact that the monomeric protein consists of merely two transmembrane α-helices. This may be due to a tilt of the α-helices with respect to the surface normal and/or protein bound detergent molecules. An indication against the former suggestion results from the fact that the absorption dichroism of transferred LHCP lipid matrix on solid support was measured as expected for α-helices (pependicular to the membrane) [16]. Yet this is no proof as the protein orientation on water surface and on solid support may be different. The facts that the protein influences the pressure–area diagram and that the LHCP fluorescence can be imaged at the water surface (Fig. 5c) show that we succeded in quantitative reconstitution.

Functional reconstitution. In the experiments we were able to preserve the fluorescence spectrum as given above for the intact protein over our measurement times of hours. This was achieved by working under argon atmosphere and in dim light. The pH was not a very sensitive parameter because even experiments without using any buffer gave similar results *. The possible photodestruction by the intense excitation for fluorescence measurements does not occur due to surface convection: molecules in the field of view of the microscope are moved out of this after a time of less than 1 min. During this period no decrease of the fluorescence is detected.

A second indication that the proteins incorporated are still in function results from the fact that also the energy transfer to the reaction center can be reconstituted. This, however, does not rule out that other parameters like aggregation are influenced by varying the protein environment.

Protein / lipid interaction

Influence of lipid environment on protein structure. One surprising result is the finding that the fluorescence yield drastically depends on surface pressure and lipid phase. To substantiate that this change is due to a structural deformation of the protein within the membrane we first have to rule out some more trivial mechanisms.

(a) On increasing the pressure the antenna might be squeezed into the subphase and thus not contribute to the monolayer fluorescence. This, however, would be observable in the pressure/area diagrams as a decrease in slope on squeezing out the protein. These slopes do not change at all at the pressure corresponding to the abrupt fluorescence drop in DLPC monolayers. For the other two lipid systems the fluorescence decrease occurs at pressures corresponding to the termination of the phase transition where the slope even increases.

(b) At a distinct pressure protein aggregates might be formed that increase the efficiency of singlet-singlet annihilation. This quenching process can, however, be ruled out as we observe a linear dependence of fluorescence intensity on excitation intensity. This result is intelligible from an estimate of the concentration of excited molecules in our experiment, where the excitation intensity is below 10^{20} photons per cm^2 per s. Thus less than 10^{17} molecules per cm^2 per s of the monolayer are excited, as its optical density is below 10^{-3}. Assuming an excited state life time of 10^{-9} s, an upper limit of the density of excited states of 10^8 molecules per cm^2 is obtained. This is much smaller than the protein density of 10^{12} per cm^2 and thus even focusing the energy into aggregates of 10^3 molecules size cannot produce a sufficiently high excitation intensity to allow singlet–singlet annihilation.

(c) We can also rule out that a change in transition dipole orientation is responsible for the steep fluorescence decrease, as due to the high objective aperture we basically observe unpolarized light.

The conclusion that the fluorescence yield of

* B800–850 is quite stable down to pH 1.5 (Steiner, R. and Scheer, H., unpublished results).

the B800–850 protein depends on environment is supported from the fact that its value also varies by more than a factor of 3 for the protein in different detergent micelles [18]. Hence, even in cases where the pressure inside different micelles is not expected to vary too much the fluorescence properties are affected.

To be able to conclude on the pigment environment inside the protein it is important to ask for the molecular mechanisms leading to a change in fluorescence yield. As the transition is electronically allowed, its transition dipole moment is not sensitively dependent on the environment of the fluorophore group [19]. This also holds for the dimer if the transition originates from the lower of the two energy levels that result from the electronic interaction of the monomer excited states [20,21]. As this argument is valid in the present situation the transition dipole moment quite probably remains unaffected by a pressure change. Hence we suppose either the energy transfer within LHCP or the rate of radiationless transitions is varied. The latter can be caused by freezing in or allowing a vibration to serve as a decay channel. This possibility was discussed by Pearlstein [22].

Alternatively, a radiationless transition can be induced by a change in the energetic position of the lowest excited singlet state due to a variation of porphyrin environment within the protein. The chlorophyll microenvironment has already been discussed as contributing significantly to the excited state energy [13], and could thus also open more efficient decay paths by creating a degeneracy between the excited state and the proper vibrational level of the electronic ground state. Without detailed structural data, no distinction between the two mechanisms is possible.

The possibility of variation in energy transfer from carotenoid to the BChl 'dimeric' trap, proposed by Cogdell [23], will be investigated after modification of the apparatus. If energy transfer is involved fluorescence should not be affected by pressure variation if the excitation occured into the BChl bands instead of the carotenoid band.

Besides this biophysical aspect that a change in the membrane environment causes a change in protein structure and thus in a functional property, one may also speculate if this is a biologically important regulation mechanism. The pressure near 20 mN/m is not entirely improbable for the inside of a membrane. A local pressure change, e.g., as a consequence of a change in the ionic environment of the membrane after electron transfer within the reaction center could open a radiationless decay channel and thus prohibit too frequent energy transfer to the reaction center. This idea is intriguing, but also highly speculative, and deserves further consideration in dedicated experiments with vesicle systems.

Influence of LHCP on the lipid matrix. Concerning the influence on the lipid matrix we obtained as most interesting results (a) that although the protein preferentially partitions into the liquid phase it reduces the pressure necessary for solidification, and (b) that it removes the liquid inclusions within the dendrites. Result (a) is surprising as one usually expects that the incorporation of the 'impurity' protein in the monolayer creates disorder in the system which therefore is rendered more difficult to crystallize [24]. This, however, is not necessarily true in the present case. The protein may form a boundary of ordered lipids, thus reducing the entropy of the liquid state. Crystallization therefore needs a smaller entropy change. This reduces the free-energy change of the transition and thus also the transition pressure. Similarly a change in transition temperature of phospholipid vesicles due to protein incorporation is discussed by means of Landau–de Gennes theory (Helm, C., Lösche, M. and Möhwald, H., unpublished results).

The fact that this reduction of π_m is very pronounced for the charged lipid, but less for the uncharged one points to the importance of electrostatic forces: the protein tends to attract negatively charged lipids, but not uncharged ones. This finding is understood regarding the amino acid sequence of B800–850 [12]. Positively charged lysins are positioned at the end of long hydrophobic regions capable of forming α-helices. As the negatively charged amino acids may protrude into the water phase an attractive Coulomb interaction may occur in the polar headgroup region.

The second effect, the removal of the liquid inclusions in the dendrites has been observed after addition of divalent ions and may thus be caused by the same mechanism [26]. These inclusions may be stabilized by electrostatic forces: as the molecu-

lar density between liquid and solid phase differs, the charge density and thus the electric potential in the two phases differ [27]. Besides that the electrostatic potential depends on domain size and is maximal in the center of domain. This means that a higher force is needed to condense a negatively charged phospholipid in such an inclusion close to the center of a dendrite. A positive, especially a polyvalent, ion is, however, attracted to such a defect, thereby locally reducing the surface potential. This facilitates crystal growth at this position. Hence, containing excess positive charges the protein may be edge active like a divalent ion.

These latter conclusions do not only apply for the case of acidic phospholipids, but also for neutral phospholipids like DMPE. Solids formed from these exhibit an excess dipole moment, and therefore tend to counteract the condensation of other dipolar ions. The dipolar field again can be screened by polyvalent ions, and thus the observations for DLPA and DMPE do not differ qualitatively.

Protein–protein interaction

The protein–protein interaction can in the present case be studied via energy transfer between antenna and additionally reconstituted reaction center. The result of the energy transfer experiment, given in Fig. 7, is a decrease in fluorescence with reaction center concentration. As in this experiment, keeping the surface concentration of the fluorophore B800–850 constant, the pressure is increased by adding reaction center and this again

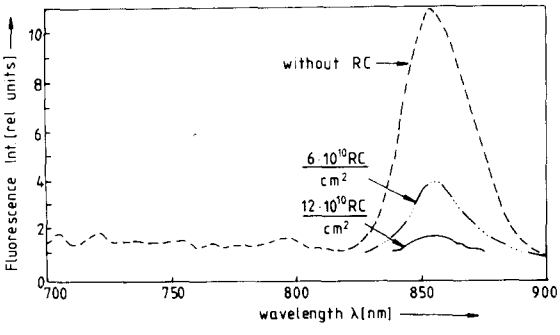

Fig. 7. Fluorescence spectrum of 1 mol% LHCP incorporated into a DMPA monolayer; T, 21°C; pH 5.5. The reaction center densities given in the figure were calculated assuming complete reconstitution.

influences the fluorescence quantum yield, the data are hard to quantify.

Nevertheless, the fluorescence decrease in this case is not dominated by the pressure increase as this is expected to cause, as shown for the RC-free experiments, either a slight fluorescence increase or an abrupt decrease. Both are not observed. Assuming that the fluorescence decrease is solely due to energy transfer one can estimate the energy transfer radius R_c from the concentration $c = 1.2 \cdot 10^{11}\,\mathrm{cm}^{-2}$ necessary for quenching according to:

$$R_c^2 c = 1$$

$2R_c$ corresponds to a mean distance of interacting molecules at a given concentration c under assumption of a statistical distribution. Hence

$$R_c = \sqrt{\pi^{-1}\,12 \cdot 10^{10}}\ [\mathrm{cm}] = 16\ \mathrm{nm}$$

This value is much larger than to be expected according to Förster's theory [28] for a statistical distribution of antenna and reaction center, where values near 5 nm would be reasonable, and indicates that LHCP aggregates are formed favouring energy transfer to the reaction center. LHCP aggregates are also discussed for other in vivo and in vitro systems [25].

We indeed observed aggregates of antenna in electron microscopic experiments with monolayers transferred on a solid support (unpublished results).

Although these experiments will have to be elaborated more quantitatively they clearly show that reaction center can be additionally reconstituted into the surface layer. If dissolved in the subphase they would be diluted too much to effect energy transfer. A quantitative analysis of energy transfer is additionally promising as it also measures other intermolecular interactions like aggregation.

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