ELASTIC INTERACTIONS OF PHOTOSYNTHETIC REACTION CENTER PROTEINS AFFECTING PHASE TRANSITIONS AND PROTEIN DISTRIBUTIONS

J. RIEGLER AND H. MÖHWALD
Technische Universität München, Physik Department E22 Lehrstuhl f. Biophysik, D 8046 Garching, Federal Republic of Germany

ABSTRACT Reaction-center proteins of Rhodopseudomonas Sphaeroides reconstituted into phosphatidylcholine vesicles shift and broaden the fluid-gel transition of the lipid bilayer. The amount of broadening and temperature shift of the transition depend both on protein concentration and on lipid chain length. In particular, the direction of the transition temperature shift is very sensitive to lipid chain length. Electron micrographs show homogeneous protein distribution on the fluid surface whereas the solid phase contains protein aggregates the type depending on chain length. The results can qualitatively be understood in the framework of a mattress model of lipid/protein interactions in membranes.

INTRODUCTION

The study of membranes as the basic structure of biological cells is of great interest for understanding nature. In the fluid mosaic model (Singer and Nicolson, 1972) of a lipid bilayer, integral proteins are embedded in the membrane and membrane adjacent proteins bind to it. Membrane-associated biological activity like ion transport, enzyme activation, photosynthesis, and so on are mediated by lipid/lipid, lipid/protein, and protein/protein interactions. In bacterial photosynthesis for example, there is a cooperation between the membrane-bound reaction center proteins (RC), antenna proteins (LHCP), cytochrome bc_1 complexes, and the water soluble cytochrome *c*. Much work has been done on pure bilayer membranes, less on bilayer/membrane-associated protein interactions, and only few on bilayer/membrane-intrinsic protein interactions (Abney and Owicki, 1985).

Lipid/protein interactions may originate from electrostatic and from elastic forces (Sackmann, 1978). In both cases structure and charge distribution of lipid and protein are essential. As an important contribution, lipid/protein interactions should arise from the elastic energy associated with distortion of the membrane due to protein incorporation (Mouritsen and Bloom, 1984): The rigid hydrophobic α-helical-parts of the membrane-spanning proteins cause a deformation of the hydrophobic lipid tails due to length matching. This leads to stretching or compression of the hydrophobic lipid tails depending on the lengths of the surrounding lipids. Protein aggregation can be generated as consequence of lipid/protein length mismatch. The lipid gel to fluid transition is influenced because lipids change lengths in passing the transition. Thus variation of the lipid chain length and measuring the protein influence on lipid phase transitions or lateral protein distribution give information on elastic membrane forces.

Such a study is performed here using phospholipids with choline headgroups and RC of the photosynthetic bacterium Rhodopseudomonas Sphaeroides. The protein is distinguished by its rather well-known structure, and the influence of elastic forces on phase diagram and on lateral protein distribution can be assessed in accordance with theoretical predictions.

MATERIALS AND METHODS

RC of Rhodopseudomonas Sphaeroides (wild type 241) were grown and isolated in the laboratory of Prof. Scheer (Univ. Munich). The procedure followed standard methods (Jolchine and Reiss-Husson, 1974). Purity was checked by absorption spectroscopy, the ratio of the 280/802 nm-peaks was <2, that of the 865/756 nm-peak ~1 (in dim light). The start solution for all reconstitutions had an RC concentration of 0.15 mM/l in 0.1% Lauryldimethylaminoxide (LDAO), the RC solubilizing detergent. The lipids L-α-Dimyristoyl-phosphatidylcholine (D14PC) and L-α-Dipalmitoyl-phosphatidylcholine (D16PC), purchased from Fluka, Buchs, L-α-Dipentadecanoyl-phosphatidylcholine (D15PC) from Avanti (Birmingham, AL), and L-α-Dilauroyl-phosphatidylcholine (D12PC) from Sigma (Munich, Federal Republic of Germany), were used without further purification. NaCl, EDTA, and Tris-HCl were of PA-standard.

Reconstitution was achieved by mixing the RC solution with PC vesicles at $T > Tc$ and at a proper redox potential (<0 mV). For that unilamellar vesicles of the pure lipid were prepared by the reverse evaporation technique (Rüppel and Sackmann, 1983; Szoka and Papahadjopoulos, 1978). The vesicle buffer contained 12 mM NaCl, 0.2 mM EDTA, and 0.25 mM Tris-HCl at pH 8.4. The corresponding amount of RC-solution (typical: 2 μl/ml suspension) was added to the vesicle suspension (1 mg lipid/ml) on strong stirring under nitrogen atmosphere and $T > Tc$. After 1 h of incubation the RC-lipid-solution was twice centrifuged (2,000 g, 10 min), the vesicle-sediment (<1/100 original volume) was twice diluted to original volume. After this procedure the vesicle suspension contained ~20% of active RC compared to the original RC input (tested by laser-flash studies). Taking into account the lipid

determination (modified phosphate determination: Cooper, 1981) the active protein/lipid ratio amounted to 30% of the inweight. The active protein content is given in the Results section, but the total protein content may be up to a factor of two larger. More information about reconstitution problems is given in the Discussion section of this paper.

Phase transition was measured by transmission changes of the sample due to light scattering. Temperature was changed from high to low values with 30°C/h. Densitometric (Schmidt and Knoll, 1985) and fluorescence depolarization techniques (Lentz et al., 1976) applied to some selected samples gave similar results. Nevertheless, for higher accuracy and for convenience, the light scattering method was preferred. Also other wavelengths and reversed temperature change did not affect measurements of the change in transition temperature due to protein incorporation. A hysteresis of up to 2°C was observed.

RESULTS

Fig. 1 shows transmission changes due to light scattering that reflect phase transition curves of the pure lipid D14PC and of D14PC with two different lipid/protein ratios. The pure lipid transition midpoint is at 24.7°C, the width is as low as 0.5°C, which is in good coincidence with literature data (Silvius et al., 1979). This proves that light scattering is a well working method for phase transition measurements.

Protein incorporation into the lipid matrix changes the transition behavior. For a lipid/protein ratio of 25,000, the transition midpoint lies at 25.1°C and the transition is broadened to 1°C. For the lipid/protein ratio of 25,000 the transition midpoint lies at 25.1°C and the transition is broadened to 1°C. For the lipid/protein ratio of 4,000 corresponding values of 27° and 2.5°C are obtained. This demonstrates that increasing protein contents produce single phase transitions, with increasing shift and broadening compared to the pure lipid.

Phase transition measurements for phospholipid bilayers that have different acyl chains but the same uncharged choline headgroup and that contain the protein in comparable amount, can be compared in Fig. 2. Except for the case of the D12PC, the pure lipid transitions are well observable and only 0.5°C broad. According to literature the D12PC-transition lies around 0° (Mabrey and Sturtevant, 1976; Van Dijck et al., 1976), and is not detectable with the measurement arrangement used. RC reconstituted into the lipid bilayers in any case broaden the transition by up to 4°C. Remarkable is the resulting midpoint temperature shift. There is an upward shift of more than 10°C with D12PC, ~3°C with D14PC, no significant shift with D15PC and a downward shift of ~1°C for the D16PC. One also observes the lower inflection point of D14PC with RC reconstituted never below that of the pure lipid. Likewise the upper inflection point of D16PC containing RC does not exceed that of the pure compound. These results are summarized in Fig. 3 presenting the temperature shift behavior as function of a mean lipid chain length $\bar{d}$ (c.f. Fig. 6 *b* and Discussion section). $\bar{d}$ was derived from x-ray measurements yielding d_f (Lewis and Engelman, 1983). Assuming that d_g is 30% larger than d_f (Janiak et al., 1976), one then calculates $\bar{d} = 0.5\ (d_f + d_g)$: The picture clearly demonstrates that the direction of temperature shift is very sensitive to the chain lengths $\bar{d}$, and that at a mean chain length of 28 Å there is only broadening, no shift of transition.

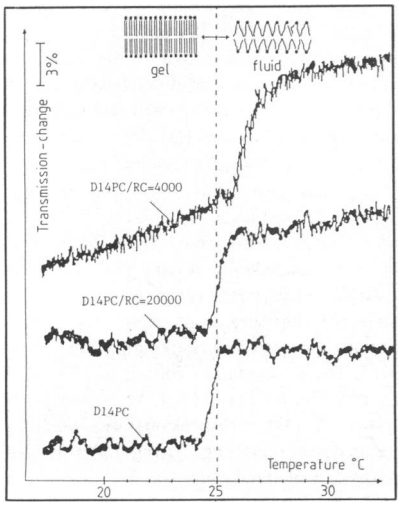

FIGURE 1 Transmission change due to light scattering as a function of temperature for D14PC vesicles containing no RC (*lower trace*) or RC in lipid/protein ratios indicated.

Electron microscopy is a formidable instrument to test the success of RC reconstitution experiments into lipid vesicles and to look for the lateral protein distribution. Whereas the surface of vesicles without protein reconstituted appears flat, RC on the lipid surface are observed as small dots with a diameter of ~100 Å. Freeze-etch preparations (Rüppel and Sackmann, 1983) of artificial lipid/protein bilayer membranes are of striking similarity to natural membrane fragments, for example, erythrozytic surfaces (Fischer and Stoeckenius, 1982).

Electron micrographs of D12PC, D14PC, and D15PC vesicles in the fluid state display the RC being more or less statistically distributed over the whole vesicle surface (Figs. 4 *a*, 4 *b*, 4 *c*). This holds for a simple vesicle. Yet one also realizes that the protein concentrations on different vesicles can be drastically different (see Fig. 4 *c, right*). At most, these vesicles indicate aggregations of 2 or 3 proteins, thus resembling protein reconstitutions into vesicles of natural lipids in the fluid state (Darszon et al., 1980; Riegler et al., 1984).

In the solid phase the distribution of proteins depends considerably on lipid chain length. Vesicles were quenched 2–11°C below the transition temperature. For D12PC the vesicles appear shrunken, the surface is rough and furrowed (Fig. 5 *a*). Therefore proteins are difficult to detect. Yet they are present in the expected amount, as is proved from electron micrographs of the same preparation after rewarming (c.f. Fig. 4 *a, left* picture). Nevertheless the electron micrographs allow the conclusion that the RC are statistically distributed and not patched. The situation is different with D14PC, D15PC, and D16PC (Figs. 5 *b, c,*

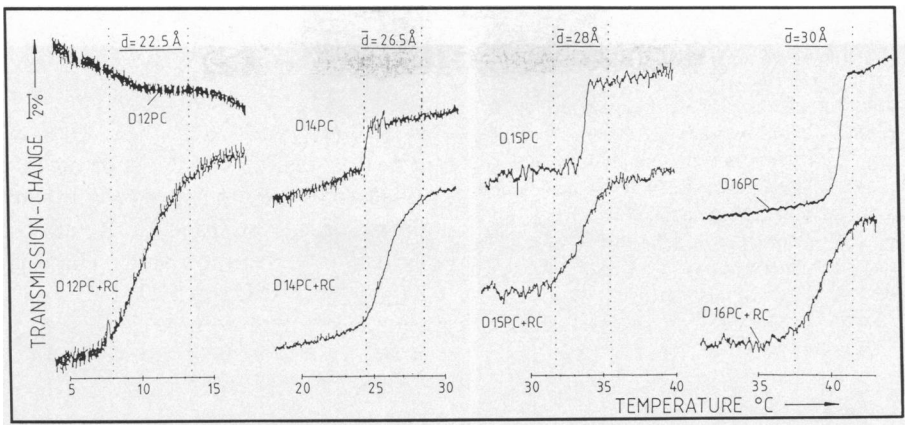

FIGURE 2 Phase transition behavior of diacylphosphatidylcholine vesicles containing no RC and RC in a lipid/protein ratio of 10,000. The parameter $\bar{d}$ characterizing the membrane thickness is defined in Fig. 6.

and *d*). All micrographs reveal that the P'_{β}-ripple phase of the lipids remains undistorted. In the case of D14PC, where electron micrographs taken at temperatures 2° and 8°C below *Tc* look identical, the proteins seem to concentrate at the place of intersection of planes with parallel ripples (Fig. 5 *b*). Yet a considerable amount of RC sits in the ripple phase, but always between the rooftops. This sometimes leads to remarkable pearl string structures with inter-protein distances at ~50–100 Å, but no intimate protein contact. In many other cases with lower protein surface concentration, single proteins are positioned at crystal defect points (Fig. 5 *b*). The longer D15PC lipid chain has a stronger effect on protein aggregation. Most of the RC patch at defect planes (Fig. 5 *c*), only a few single RC can be found in the ripple phase between the ripple rooftops (Fig. 5 *c*). The distance between the patched proteins is lowered compared to D14PC. Patching is very strong in the D16PC case (Fig. 5 *d*). Typically pronounced single or double RC strings are found at places where ripple crystal sheets meet. The RC appear pushed into the area between different crystalline domains. Sometimes the inter-protein distance is so low that the single protein is not discernible.

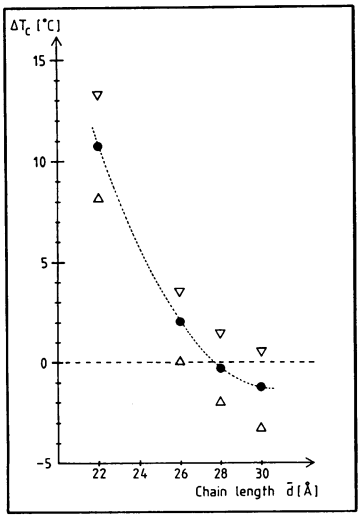

FIGURE 3 Shift of phase transition ΔT_c as a function of mean membrane thickness $\bar{d}$ for comparable protein content (lipid/protein ratio 10,000). (●) midpoint shift (▽), (△) upper and lower inflection point shift, respectively.

DISCUSSION

We have demonstrated that RC proteins reconstituted into uncharged phosphatidylcholine vesicles shift and broaden the fluid-gel transition of the lipid bilayer. To exclude any trivial effects due to the reconstitution process and residual detergent, control experiments substituting the detergent solubilized RC by pure detergent, otherwise performing the same procedure, were accomplished. Even the addition of up to 400 μl of 0.3% LDAO to 5 ml vesicle suspension (i.e., detergent volume concentration 0.02%), a 20 times higher concentration than the comparable one used for our highest protein/lipid ratio did not affect transition behavior. (For detergent volume concentration exceeding 0.02% our dialysis procedure does not work because the vesicles are destroyed by detergent and not centrifugable).

This proves that our preparation procedure is effective concerning detergent removal and that the observed lipid transition changes are caused by incorporated proteins. Nevertheless we cannot exclude tightly bound detergent molecules around the RC. In any case we would not expect the observed very specific chain-length dependence of transition change and protein aggregation to be caused only by some residual detergent molecules.

Our result can be understood by an elastic force mattress model (Mouritsen and Bloom, 1984) of the protein/lipid bilayer (Fig. 6 *a, b*). Its basic structural variables are the length of the hydrophobic region of the protein and the temperature-dependent thickness of the lipid bilayer. For minimizing energy, the hydrophobic parts of the membrane spanning protein and the bilayer should match. Proteins may be rather rigid, whereas the bilayer thickness changes by 20–30% as consequence of the phase transition

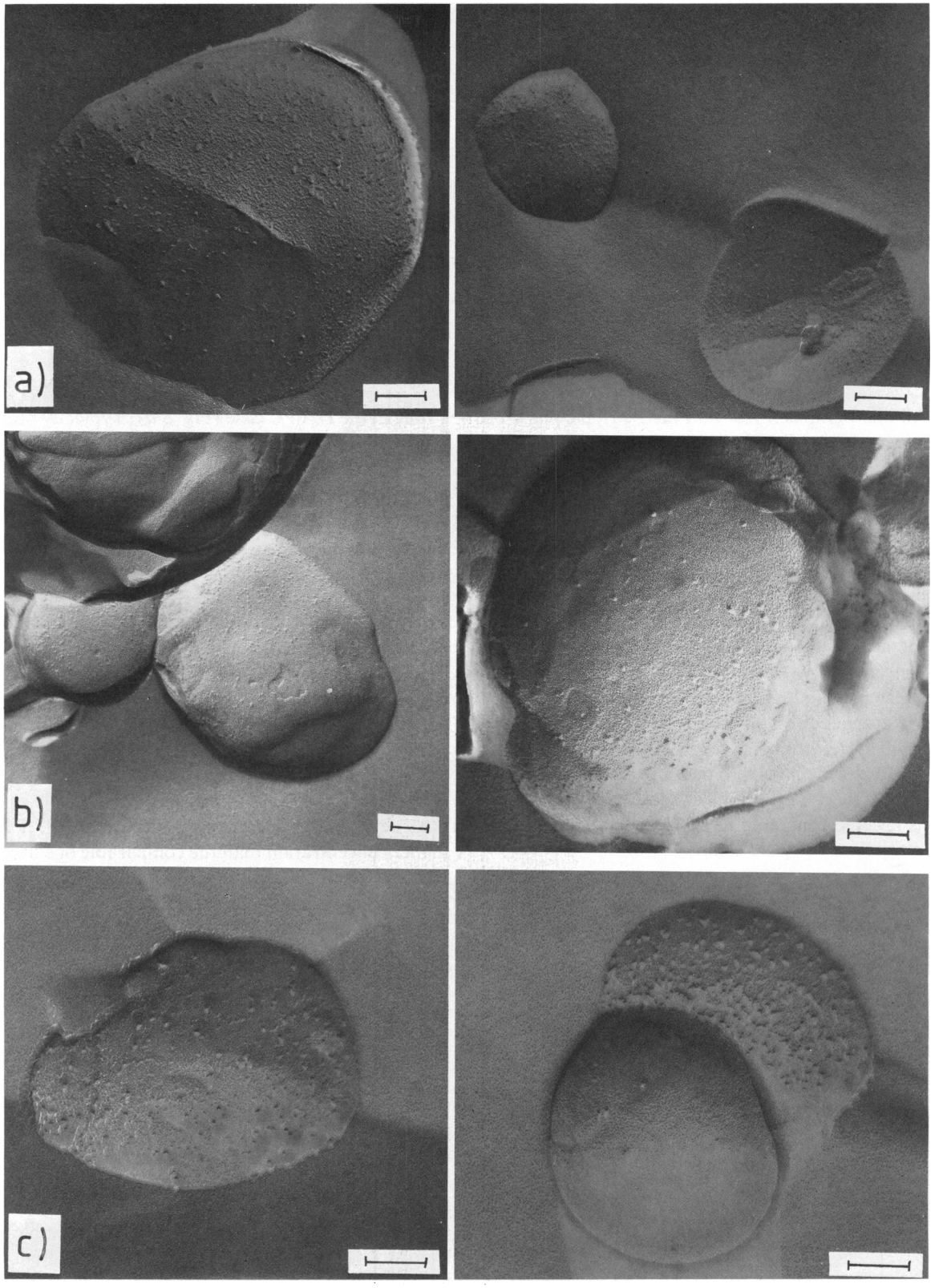

FIGURE 4 Freeze-etch electron micrographs of RC containing diacylphosphatidylcholine vesicles kept in the fluid phase before freezing. (*a*) D12PC, freezing temperature 23°C. (*b*) D14PC, freezing temperature 35°C. (*c*) D15PC, freezing temperature 38°C. The bar indicates 100 nm.

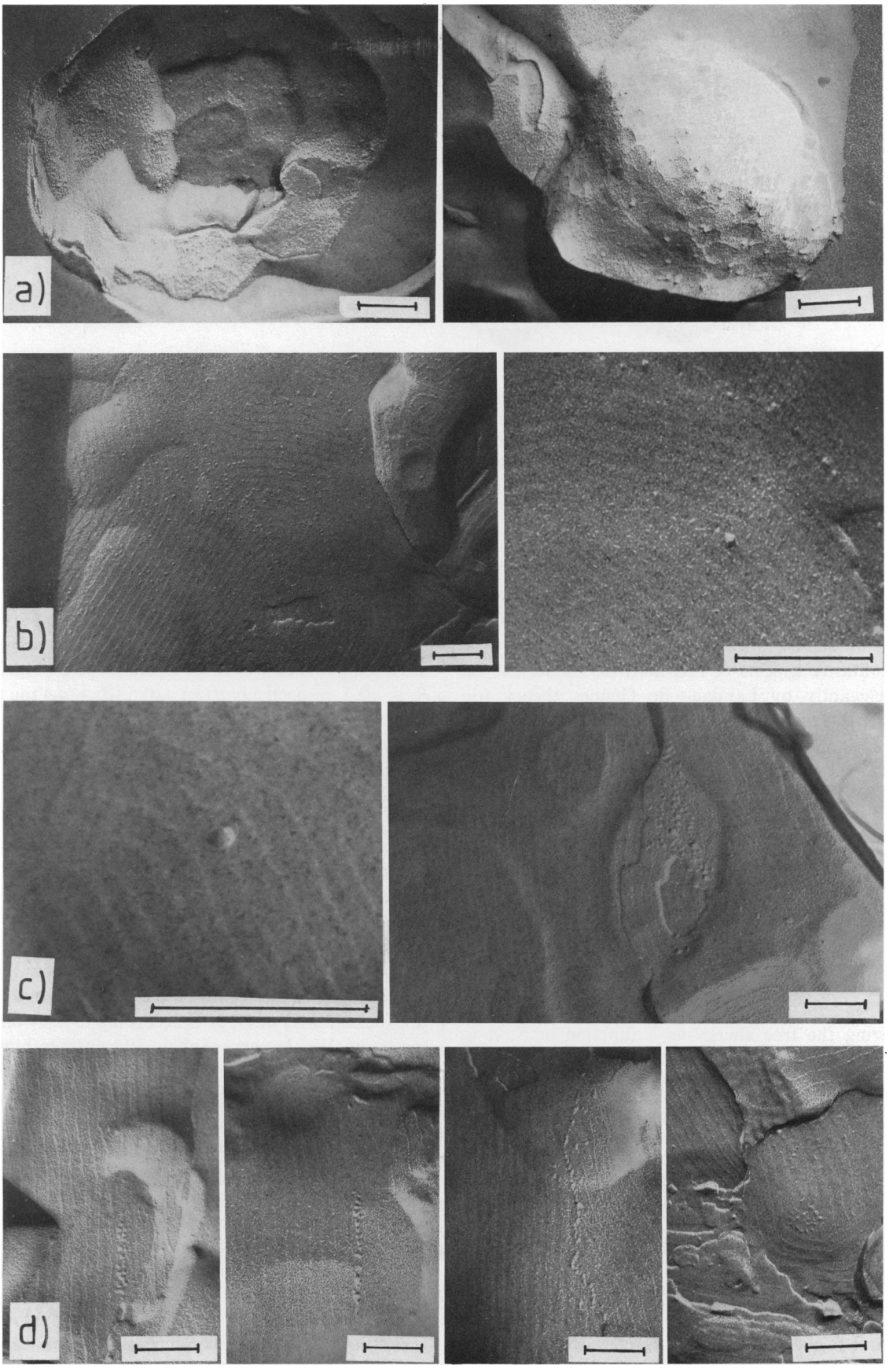

FIGURE 5 Freeze-etch electron micrographs of RC containing diacylphosphatidylcholine vesicles kept in the gel state before freezing. (*a*) D12PC, freezing temperature 4°C; (*b*) D14PC, freezing temperature 23°C; (*c*) D15PC, freezing temperature 23°C; (*d*) D16PC, freezing temperature 35°C. The bar indicates 100 nm.

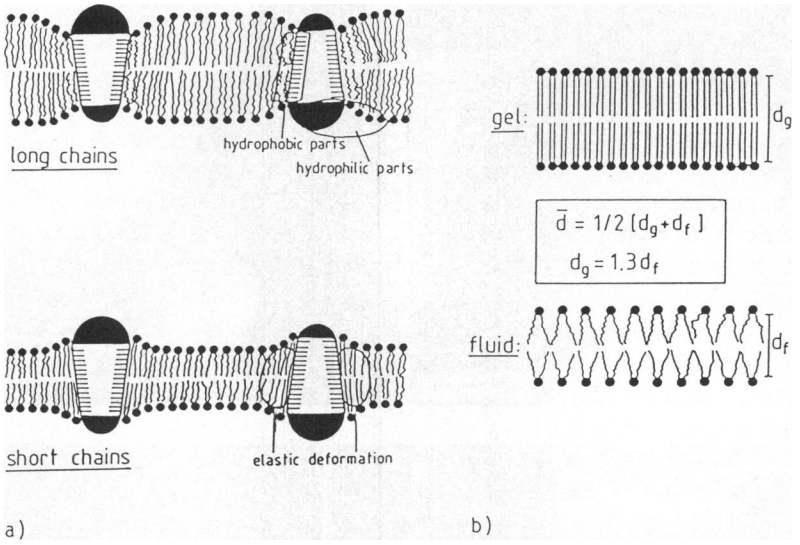

FIGURE 6 Sketch of elastic deformations caused by protein incorporation and definition of parameters characterizing the membrane thickness.

(Janiak et al., 1976). Thus the hydrophobic protein and lipid parts mismatch in the gel and/or fluid state of the lipid. The protein tends to match the length of neighboring lipid chains, thus undulating the bilayer thickness and causing elastic stress.

The qualitative aspects of this mattress model can be handled elegantly by Landau—de Gennes theory (de Gennes, 1974), expanding the Gibbs free energy density as a power series of an order parameter S. As in our experiments the membrane thickness is varied, the order parameter S may be defined according to

$$S = (d_f - d)/(d_f - d_g), \tag{1}$$

where d is the membrane thickness and d_f and d_g refer to the (chain-length dependent) thickness in the fluid and gel states at the transition temperature in the unperturbed bilayer. This is equivalent to the order parameter depending on lipid molecule surface area used by Owicki et al. (1978, 1979).

Minimizing the free energy as a function of S, it is possible to make statements on direct physical observables, in special on phase diagrams of lipid/protein mixtures. These predictions strongly depend on S_o, the order parameter at the lipid/protein interface, which is, of course, related to physical bilayer properties as

$$S(r_o) = S_o = (d_f - d(r_o))/(d_f - d_g). \tag{2}$$

In our experiments we always used the same RC-protein, thus the length of the hydrophobic protein part, namely $d(r_o)$, is not changed. We varied lipid chain length, that means d_f and d_g and as an important consequence, S_o. Our experimental results qualitatively agree with the theory: (*a*) transition temperature shift is dependent on protein concentration (see Fig. 1); (*b*) the shift additionally depends on S_o, respectively d_f and d_g, which is related to the unperturbed bilayer thickness. We measure a four times larger transition temperature shift for D12PC compared to D14PC (Fig. 3); (*c*) if S_o is >0.5, respectively $d(r_o) > 0.5\,(d_f + d_g)$ the transition temperature is increased. This is observed in the case of D12PC and D14PC (Fig. 3); (*d*) if S_o is <0.5, respectively $d(r_o) < 0.5\,(d_f + d_g)$ the temperature is decreased. This is the case for RC in D16PC (Fig. 3). (*e*) if $S_o = 0.5$, respectively $d(r_o) = 0.5\,(d_f + d_g) =$ (average bilayer thickness), no temperature shift is predicted. This relation seems to hold for RC in D15PC (Fig. 3). (*f*) The phase transition is broadened with increasing protein content due to a reduced order parameter change at the transition point (Jähnig, 1981; Owicki, 1978, 1979), caused by the spatial averaging of S. All our curves undoubtedly depict this transition broadening.

As a first result of the combination of theory and experiment we would expect the hydrophobic transmembrane length of the RC to be the same as average bilayer thickness (28 Å) of D15PC according to $S_o \approx 0.5$, hereby measured. We thus expect about 19 transmembrane amino acid residues for the RC. Incidentally, this number has also been used by other authors to define the α-helix positions along the amino acid sequences of the L and M subunits of the RC (Williams et al., 1983, 1984). The length of the hydrophobic protein parts is also quite similar to those of another transmembrane protein, the structurally very well known bacteriorhodopsin that has 20 aminoacid residues (Lewis and Engelman, 1983).

Furthermore, Landau theory predicts a critical protein concentration, where the sharp phase transition should vanish, yet the required very high protein/lipid molar ratio (>1/100) was beyond the scope of our preparation possibilities. In addition, theoretical results on the spatial distribution of the order parameter were not checked by

direct measurement, but this raises the question on the microscopic picture of the transition change.

Landau theory, being a mean field theory, does of course not directly determine physical properties on the microscopic level. Also, its applicability may be questioned as the RC/lipid membrane is heterogeneous and as intermolecular interactions may be of short range. This latter statement may not hold that strictly for elastic forces where an inverse square and not an exponential distance dependence is expected (Sackmann et al., 1984).

In addition protein/protein interactions (possibly lipid-mediated) may be so strong, that part of the vesicle is coated with a net-like protein structure, i.e., a macroscopic heterogeneity on the scale of the lipid surface. However, we have never found a more regular macroscopic protein superstructure in the fluid lipid phase by electron microscopy.

Concerning the equilibrium the situation is similar for $S_0 > 0.5$ (short chains) and $S_0 < 0.5$ (long chains) regarding temperature shift (up for $S_0 > 0.5$, down for $S_0 < 0.5$) and the binary desintegration of the fluid and gel phase. Nevertheless theory predicts maximum segregation for large length mismatch and large values of the lipid chain elastic constant (Mouritsen and Bloom, 1984). The latter condition implies that segregation will occur more likely in the gel than in the fluid state. This explains the strong aggregation in solid D16PC (Fig. 5 *d*) and the absence of protein aggregation in fluid D12PC (Fig. 4 *a*), despite the pronounced length mismatch, which is comparable to the solid D16PC.

As a prerequisite of the above discussion one should mention both, that the length mismatch in the gel state increases from 2 Å for D14PC to 6 Å for D16PC, and that the RC do not aggregate without any lipid mediated force. This is demonstrated by the statistical distribution of RC in fluid membranes of D12PC, D14PC, and D15PC in spite of a bilayer/protein thickness mismatch.

Our results concerning the protein segregation are also in coincidence with the observation on bacteriorhodopsin (Lewis and Engelman, 1983), which only segregates for fluid bilayers that are 10 Å thinner than the bacteriorhodopsin hydrophobic length. Judged from this, as the RC is shorter than the bacteriorhodopsin, we would expect RC aggregation for membranes with thickness shorter than that of D10PC.

One basic structural principle of the RC is that it consists of the three subunits *L* ($M_r \sim 31{,}000$), *M* ($M_r \sim 34{,}000$) and *H* ($M_r \sim 36{,}000$?), exhibiting 5, 5 and 1 hydrophobic α-chains, respectively (Williams et al., 1983, 1984; Deisenhofer, 1984). The *H*-unit is anchored to the *L-M*-group at one side of the membrane, giving the protein an asymmetrical shape. The RC is more strongly embedded into the membrane by its 11 α-helices than other membrane bound proteins. It is stable over long time periods (approximately months) in detergent solution and the internal protein function, easily measurable by absorption changes, seems to be independent of the surrounding lipid phase. This presumes that the protein is rather rigid.

The functional test of RC is a convenient way to measure intact protein concentration. For more quantitative statements on protein/lipid interactions in the framework of the mattress model exact data on protein/lipid ratios of course are necessary. Yet we have demonstrated that there are always different protein/lipid ratios on different vesicles (see Fig 4 *b, c*). This only weakly affects the midpoint of the transition, but strongly influences the shape of the transition curve. For D14PC, for example, in one case we observe broadened transitions with an upward shifted lower inflection point (e.g. Fig. 1). On the other hand, the transition can be much stronger broadened with a lower inflection point at 24.3° and an upper one near or even above 30°C (e.g. Fig. 2).

Here we could use the great advantage of the RC that protein incorporation and surface partition can be controlled directly by electron microscopy, a feature that other membrane-bound proteins (Sackmann et al., 1984) miss. A more detailed analysis of the direction of the shadows in Figs. 4 and 5 reveals that all RC's are visible as pickles, never as holes. This indicates that the fracture does not separate the monolayers of the bilayer, or that initially created holes anneal.

In summary the RC-protein is quite suitable for membrane/protein interaction studies. In addition to quantitative aspects, the given experimental procedure will now be extended to lipid mixture/RC, lipid/RC/LHCP, and lipid mixture/RC/LHCP systems. The specific RC/lipid chain interaction may affect lipid phase separations, observable by electron microscopy or other methods. The RC/LHCP energy transfer is distance dependent, hence lipid mediated protein aggregation quenches the LHCP fluorescence. This fact can be used to measure the lipid influence on protein/protein interaction and furthermore to test the overall structure-function relationship.

Finally, we would like to stress that we apparently observe elastic contributions to protein/lipid interactions depending on mismatch of hydrophobic thicknesses. This explains our data irrespective of possible membrane disorder created since the surface of protein is rough and may not fit in any regular structure. In this context it is remarkable that indications of an immobile protein surface imply no contributions to dynamic disorder. We also realize that in the case of D14PC the RC is always embedded between the rooftops. This could be of relevance, considering a model of the P'_β-ripple phase as coexistence of fluid and solid phases (Marder et al., 1984).

This study was confined on one protein and it is hoped that future studies can show if this is a peculiar feature of this rather stiff RC-protein or if it is a more general protein characteristic.

We appreciate helpful discussions with E. Sackmann and many technical advises by H. Scheer and by J. Peschke. The skillful technical assistance

of C. Fahn in preparing the electron micrographs is gratefully acknowledged.

This work was supported by the Deutsche Forschungsgemeinschaft through SFB 143.

Received for publication 19 June 1985 and in final form 18 December 1985.

REFERENCES

Abney, J. R., and J. C. Owicki. 1985. Theory of Protein-Lipid Interactions. *In* Prog. Protein-Lipid Interactions. A. Watts and J. J. H. H. M. De Pont, editors. Elsevier, North Holland, vol. 1.

Cooper, T. G. 1981. Biochemische Arbeitsmethoden. Walter de Gruyter, Berlin.

Darszon, A., C. A. Vandenberg, M. Schönfeld, M. H. Ellisman, N. C. Spitzer, and M. Montal. 1980. Reassembly of protein-lipid complexes into large bilayer vesicles: perspectives for membrane reconstitution. *Proc. Natl. Acad. Sci. USA.* 77:239–243.

Deisenhofer, J. 1984. X-ray structure analysis of a membrane protein complex. *J. Mol. Biol.* 180:385–398.

Fischer, K. A., and W. Stoeckenius. 1982. Membran-Modelle. *In* Biophysik. W. Hoppe, W. Lohmann, H. Markl, and H. Ziegler editors. Springer-Verlag, Berlin.

Jähnig, F. 1981*a*. Critical effects from lipid-protein interaction in membranes. I. Theoretical description. *Biophys. J.* 36:329–345.

Jähnig, F. 1981*b*. Critical effects from lipid-protein interaction in membranes. II. Interpretation of experimental results. *Biophys. J.* 36:347–357.

Janiak, M. J., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyllecithin. *Biochemistry.* 15:4575–4580.

Jolchine, G., and F. Reiss-Husson. 1974. Comparative studies on two reaction center preparations from Rhodopseudomonas sphaeroides Y. *FEBS. (Fed. Eur. Biochem. Soc.) Lett.* 40:5–8.

Lentz, B. R., Y. Barenholz, and T. E. Thompson. 1976. Fluorescence depolarisation studies of phase transition and fluidity in phospholipid bilayers. *Biochemistry.* 15:4521–4528.

Lewis, B. A., and D. M. Engelman. 1983. Bacteriorhodopsin remains dispersed in fluid phospholipid bilayers over a wide range of bilayer thicknesses. *J. Mol. Biol.* 166:203–210.

Lewis, B. A., and D. M. Engelman. 1983. Lipid bilayer thickness varies linearly with acyl chain length in fluid phosphatidylcholine vesicles. *J. Mol. Biol.* 166:211–217.

Mabrey, S., J. M. Sturtevant. 1976. Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. Natl. Acad. Sci. USA.* 73:3862–3866.

Marder, M., H. L. Frisch, J. S. Langer, and H. M. McConnell. 1984. Theory of the intermediate rippled phase of phospholipid bilayers. *Proc. Natl. Acad. Sci. USA.* 81:6559–6561.

Mouritsen, O. G., and M. Bloom. 1984. Mattress model of lipid-protein interactions in membranes. *Biophys. J.* 46:141–153.

Owicki, J. C., and H. M. McConnel. 1978. Theoretical study of protein-lipid interactions in bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 75:1616–1619.

Owicki, J. C., and H. M. McConnel. 1979. Theory of protein-lipid and protein-protein interactions in bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 76:4750–4754.

Riegler, J., J. Peschke, and H. Möhwald. 1984. Two-dimensional electron transfer from cytochrome c to photosynthetic reaction centers. *Biochem. Biophys. Res. Commun.* 125:592–599.

Rüppel D., and E. Sackmann. 1983. On defects in different phases of two-dimensional lipid bilayers. *J. Physique.* 44:1025–1034.

Sackmann, E. 1978. Dynamic molecular organisation in vesicles and membranes. *Ber. Bunsen-Ges. Phys. Chem.* 82:891–909.

Sackmann, E., R. Kotulla, and F. J. Heiszler. 1984. On the role of lipid-bilayer elasticity for the lipid-protein interaction and the indirect protein-protein coupling. *Can. J. Biochem. Cell. Biol.* 62:778–788.

Schmidt, G., and W. Knoll. 1985. Densitometric characterization of aqueous lipid dispersions. *Ber. Bunsen-Ges. Phys. Chem.* 89:36–43.

Silvius, J. R., B. D. Read, and R. N. McElhaney. 1979. Thermotropic phase transitions of phosphatidylcholines with odd-numbered *n*-acyl chains. *Biochim. Biophys. Acta.* 555:175–178.

Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash. DC).* 173:720–731.

Szoka, F., and D. Papahadjopoulos. 1978. Procedure for preparation of liposomes with large internal aqueous space and capture by reverse-phase evaporation. *Proc. Natl. Acad. Sci. USA.* 75:4194–4198.

Van Dijck, P. W. M., B. de Kruijff, L. L. M. van Deenen, J. de Gier, and R. A. Demel. 1976. The preference of cholesterol for phosphatidylcholine in mixed phosphatidylcholine-phosphatidylethanolamine bilayers. *Biochim. Biophys. Acta.* 455:576–587.

Williams, J. C., L. A. Steiner, R. C. Ogden, M. I. Simon, and G. Feher. 1983. Primary structure of the M subunit of the reaction center from rhodopseudomonas sphaeroides. *Proc. Natl. Acad. Sci. USA.* 80:6505–6509.

Williams, J. C., L. A. Steiner, G. Feher, and M. I. Simon. 1984. Primary structure of the L subunit of the reaction center from rhodopseudomonas sphaeroides. *Proc. Natl. Acad. Sci. USA.* 81:7303–7307.