Biochimica et Biophysica Acta 904 (1987) 290–300
Elsevier

BBA 73755

Phospholipid and pigment alterations after fusion between *Rhodobacter sphaeroides* chromatophores and acidic liposomes

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(Received 10 April 1987)
(Revised manuscript received 3 August 1987)

Key words. Chromatophore, Fusion, Liposome, Membrane; Phospholipid, Freeze-fracture electron microscopy

Wild-type *Rhodobacter sphaeroides* chromatophores were fused at acidic pH, or by freezing and thawing, with liposomes of soybean phospholipids, phosphatidylserine, phosphatidylglycerol or diphosphatidylglycerol. Equilibrium centrifugation after fusion yielded several fractions. Freeze-fracture electron microscopy showed that fusion resulted in the formation of unilamellar vesicles of diameters larger than that of chromatophores. The lateral density of the intramembrane particles decreased; the asymmetry between the two fracture faces was lost after fusion with soybean phospholipids or phosphatidylserine or phosphatidylglycerol, but gradually disappeared in parallel with diphosphatidylglycerol enrichment. After fusion with phosphatidylserine, when the fractions were frozen from below the lipid transition temperature intramembrane particles aggregated into patches surrounded by smooth lipid zones. A massive incorporation of the fusogen phospholipid was observed in the fractions together with a strong decrease of phosphatidylglycerol and a lower decrease of phosphatidylcholine and aminolipid. The 800 nm absorption band of the B800–850 antenna complex was reduced or suppressed depending on the nature of the lipids while the spectroscopic alteration of B875 chromophore was weaker. The light-induced bandshifts of carotenoid and antenna bacteriochlorophyll were also much weaker or absent; this could result from a desorganization of the B800–850 antenna, or from an impaired capacity to sustain a photoinduced membrane potential. The reaction center was not affected by the fusion, and the polypeptide composition of the various fractions did not show qualitative differences from the chromatophore pattern. Spheroplasts did not show the same capacity of fusion as chromatophores.

Introduction

Photosynthetic membranes offer an excellent system to study the structure-function relationship of their components. The light-driven reactions that characterize these systems are easily identifiable and are closely dependent on a well-defined structure. In facultative photoheterotrophic purple bacteria the structural models of the intracytoplasmic membrane vesicles (chromatophores) and at the same time their chemical composition (essentially lipids and pigment-protein complexes) allow to think that the interaction of lipids with pigments and proteins plays a role in the organization and function of this membrane. Some experi-

Abbreviations PC, phosphatidylcholine, PS, phosphatidylserine, BChl, bacteriochlorophyll, RC, reaction centre, Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid

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mental evidence indicates that lateral diffusion and collisions of interacting membrane components are involved in many biochemical and biophysical processes at molecular level.

Fusion of liposomes with membranes from different sources has been previously used to dilute membrane proteins within the lipid bilayer in a number of vesicle systems. Several techniques for fusion have been developed for inner mitochondrial membranes [1], chloroplasts [2,3], chromatophores [4–10] and bacterial cells [11]. Enrichment of the lipid bilayer portion of biological membranes with exogenous phospholipids provides a valuable tool for studying the role of specific protein–protein and protein–phospholipid interactions. To this purpose, the phospholipid bilayer composition of chromatophores from *Rhodobacter sphaeroides* Y (wild-type strain) was modified by enriching the original membrane with different phospholipids at various phospholipid/protein ratios. We observed several alterations at the level of pigments and phospholipid after these fusions.

Materials and Methods

Rhodobacter sphaeroides (wild-type Y strain) were grown anaerobically in the light in a synthetic medium as described previously [12]. Purified chromatophores were isolated from crude French-Press extracts on sucrose density gradients according to Niederman and Gibson [13]. They were dialysed against 20 mM Tes buffer (pH 7.55) and concentrated by centrifugation. Spheroplasts were prepared by incubation of the cells in a buffer containing 0.2 M Tris (pH 8.0), 2 mM EDTA, 0.2 mg lysozyme/ml, 20% sucrose, and Brij 58 at 0.015% final concentration; after 1 h 0.1 M $MgCl_2$ and DNAase were added. Debris were eliminated by centrifugation for 15 min at $800 \times g$. Spheroplasts were pelleted and purified on a sucrose density gradient (30 to 55%) in 10 mM Tris buffer at $150000 \times g$ for 12 h

Soybean phospholipids were purified from commercial grade phosphatidylcholine type II-S (Sigma) by petroleum ether solubilisation and acetone precipitation according to Singleton et al. [14]. Bovine heart and bacterial diphosphatidylglycerol (resp. Sigma and Serdary), bovine spinal cord PS, PC and phosphatidylglycerol both from egg yolk (Lipid Products, Nutfield, U.K.) were used without further purification. Egg yolk PC was also prepared and purified in our laboratory according to Singleton et al. [14].

Unilamellar liposomes were prepared by extensive sonication of phospholipid dispersions. The phospholipid extract (in chloroform/methanol, 2:1, v/v) was taken to dryness under N_2, and suspended to a final concentration of 20 mg/ml in 20 mM Tes (pH 7.5). The suspension was dispersed by shaking and sonication at 2°C under a N_2 stream with the titanium probe of a MSE sonifier vibrating at maximal amplitude (8 μm) for 50 min, with intermittences of 15 s to avoid heating. Sonication of PS (spinal cord) was done at 20°C. Undispersed phospholipids and Ti debris were removed by centrifugation at $100000 \times g$ for 1 h at 4°C.

Fused vesicles were prepared by two different methods. (a) Fusion induced by low pH: 0.5 ml of chromatophores or spheroplasts suspension (0.7 or 1.8 mM BChl) were mixed with 0.5 ml liposomes (20 mg/ml). The fusion was induced at 30°C by lowering the pH to 6.5 by addition of 200 mM HCl, as described by Schneider et al. [1]. Two additional aliquots of liposomes were added after 15 min and 30 min of incubation and the pH maintained at 6.5. After 45 min, the pH was adjusted to 7.5 by addition of 200 mM KOH. The control chromatophores were treated in the same way with addition of buffer solution instead of liposomes. (b) Fusion induced by freezing and thawing: chromatophores (0.5 ml, 0.7 mM BChl) were mixed with liposomes (1.5 ml, 20 mg/ml) at pH 7.5. The mixture was frozen by a modification of the method of Siegel et al. [2] with a vigorous stirring in liquid nitrogen, and allowed to thaw for 20 min at room temperature. Two freeze-thaw cycles were performed. Control experiments were carried out by adding buffer solution instead of liposomes.

The fused membranes were layered on a continuous sucrose density gradient (20–40%) (0.6–1.23 M) in 20 mM Tes (pH 7.5) and centrifuged in a SW 41 rotor at 38000 rpm for 15 h at 5°C. Gradients were fractionated by puncturing the bottom of the centrifuge tube and collecting the drops. Refractive index was measured in all the fractions. To remove sucrose, each fraction

was centrifuged at $90000 \times g$ in a Beckman Airfuge.

Phospholipids were extracted from chromatophores and fusion bands and washed as described by Folch et al. [15]. Separation of phospholipids was carried out on thin-layer chromatography plates pre-coated with silica gel 60 (Merck). Chloroform/methanol/acetic acid (65 : 24 : 4, v/v) was used as solvent system. This system allowed a good separation of diphosphatidylglycerol and phosphatidylglycerol. The spots were visualized by exposing the plates to iodine vapours. Lipid phosphorus was determined by the Bartlett method [16] in the total lipid extracts and in the TLC spots scraped from the plates both previously digested by perchloric acid 70%. On the other hand, lipids were also quantified following chromatography by charring and densitometry according to Macala et al. [17]. Proteins were determined according to Peterson [18] with a bovine serum albumin standard. BChl was measured spectrophotometrically after methanol/acetone (7 : 2, v/v) extraction, using an absorption coefficient of 76 $mM^{-1} \cdot cm^{-1}$ as described by Clayton and Clayton [19].

SDS-Polyacrylamide gel electrophoresis was made according to Laemmli and Favre [20], after delipidation of the samples by isopropanol/hexane (2 : 3, v/v) [21].

Optical spectra were measured at 25 °C and 77 K with an Aminco DW 2A spectrophotometer equipped with a side illumination accessory. Reaction center was monitored at 865 nm in light minus dark difference spectra. Cytochrome *c* content was measured from reduced-minus-oxidized difference spectra in the wavelength region 540–580 nm; solid ascorbate and μl amounts of ferricyanide (100 mM) were added to the sample and reference cuvettes until the maximal absorbance was obtained. Formation of a membrane potential was followed during continuous illumination both by the carotenoid shift measured in the wavelength region 510–480 nm, and by the electrochromic shift of BChl at 845 nm; we checked that these effects (when observed) were suppressed by 2 μM gramicidin.

For freeze-fracture electron microscopy the samples were cryoprotected with glycerol (30% (w/w)) and rapidly frozen in liquid propane using conventional Balzer's gold planchets. The fracturing was performed at −150 °C, under a vacuum better than 10^{-6} Torr, with a nitrogen cooled knife. The replication of fractured surfaces was performed using Pt-C. The replicas were cleaned in chromic acid, washed with distilled water and observed in a Philips 301 electron microscope.

The main transition temperature of PS was determined from wide-angle X-ray diffraction diagrams taken with a Guinier camera at temperatures varying between 30 °C and 5 ± 0.1 °C.

Results

Fusion with various phospholipids

After equilibrium centrifugation in a sucrose gradient, chromatophores formed a single band at a buoyant density of 1.151 $g \cdot cm^{-3}$. Liposomes under the same conditions floated at the top of the gradient. After treatment of the chromatophores with acidic phospholipids several pigmented bands were found; their number, their relative abundance and their buoyant density depended on the fusogenic liposome composition [4] and on the fusion lipid/BChl ratio employed in the experiment (Table I). In the soybean phospholipids fusion, most of the pigment and proteins were associated with the densest band and the lightest one contained only liposomes. Fusion with PS liposomes yielded pigments and proteins more evenly distributed from band 2 to band 4, and band 1 contained some protein (see Fig. 1D). Similarly with the other acidic phospholipids protein was found even in the lightest band of the gradient (Table I). Freeze fracture data showed in these lightest fractions the presence of smooth vesicles (like liposomes) and some particulated vesicles (Fig. 1D). Except for soybean phospholipids band 1, all the other bands contained fused chromatophore-liposome products; their phospholipid/protein ratio was always higher than in chromatophores, even in the most dense band 4 (Table I).

These results indicate that chromatophores display different affinity for different phospholipids [4]. In fusion experiments with neutral phospholipids, (i.e. pure PC) contradictory results have been obtained (see Discussion).

To check if some proteins were lost during the

TABLE I

BUOYANT DENSITIES AND COMPOSITION OF FUSION BANDS

Bands 1, 2, 3 and 4 were obtained after fusion at low pH with mixtures of liposomes (0 5 ml, 10 mg lipid) and chromatophores (0 5 ml, 1 8 ml BChl) Additional bands 1^b and 3^b were observed by the freezing and thawing method with mixtures of diphosphatidylglycerol or phosphatidylserine liposomes (0 5 ml, 10 mg lipid) and chromatophores (0 5 ml, 0 7 mM BChl) Abbreviations PL, phospholipid, RC, reaction center, n d , not determined, cyt, cytochrome

Fusion lipid		Soybean phospholipids		Phosphatidylglycerol			Diphosphatidylglycerol			Phosphatidylserine			
Band	Buoyant density (g/cm^3)	PL/protein		PL/protein		BChl/RC	PL/protein		BChl/RC	PL/protein		BChl/RC	cyt c/RC
		w/w	-fold increase	w/w	-fold increase	(molar ratio)	w/w	-fold increase	(molar ratio)	w/w	-fold increase	(molar ratio)	(molar ratio)
1	1 078 ± 0 007	∞	∞	18 58	68 8	100	n d	n d	n.d.	33 64	124 6	n d	0 51
$1b^+$	1 090						2 59	9 6	n.d				
2	1 103 ± 0 007	5 39	19 9	4 21	15 6	134	2 71	10 0	160	9 86	36 5	78	0.45
3	1 113 ± 0 009	absent		absent		–	2 28	8 4	140	3.61	13 4	98	0.50
$3b^+$	1 117									2 21	8 2	n d	
4	1 130 ± 0 009	1.19	4 4	3.66	13 5	113	1 85	6 8	181	1 76	6 5	71	0 49
Control chromatophores													
	1 151	0.27	1 0			133			196			131	0 78

fusion process the polypeptide composition of the different bands after fusion between chromatophores and soybean phospholipids, PS or diphosphatidylglycerol liposomes was analyzed; it was similar to that of chromatophores, at least qualitatively (not shown). Nevertheless, the molar ratio cytochrome c/reaction center that was constant in PS fusion bands 1 and 4 (Table I), was 36% lower than in the native chromatophores, which might indicate a small loss of cytochrome c_2 during the fusion process.

Ultrastructure of the fusion products

Freeze-fracture images of control chromatophores showed PF faces with densely-packed intramembrane particles and EF faces smooth or with only few intramembrane particles (Fig. 1A) Spheroplasts presented the opposite morphology (Fig. 1B).

The electron microphotographs of all gradient bands showed unilamellar vesicles with a very heterogeneous distribution of size. No indication of multiple concentric membrane structures was

TABLE II

MEAN VESICLE DIAMETER AND MAXIMAL RELATIVE ENLARGEMENT OF FUSED VESICLES

Fusion lipid		Soybean phospholipids		Phosphatidylserine		Diphosphatidylglycerol	
Band	Buoyant density (g/cm^3)	mean vesicle diameter [a] (nm)	max relative enlargement [b]	mean vesicle diameter [a] (nm)	max relative enlargement [b]	mean vesicle diameter [a] (nm)	max relative enlargement [b]
1	1 078	30 ± 5		40 ± 37		28 ± 9	
$1b^+$	1 090					534 ± 277	25 7
2	1 103	108 ± 67	7 5	103 ± 52	4 0	960 ± 300	63 5
3	1 113			356 ± 228	12 6	122 ± 117	10 9
$3b^+$	1 117			660 ± 210	43 2		
4	1 130	54 ± 16		54 ± 16		68 ± 19	

Chromatophores diameter (nm) native 55 ± 16, low-pH control 54 ± 18, freeze/thaw control 55 ± 19 Spheroplasts 140 ± 70

[a] Mean values ± S D Only the 'fused vesicles' within the heterogeneous mixture were counted, in each fraction and in each case their number was variable

[b] Expressed as ratio of diameters of the biggest vesicles and of the chromatophores

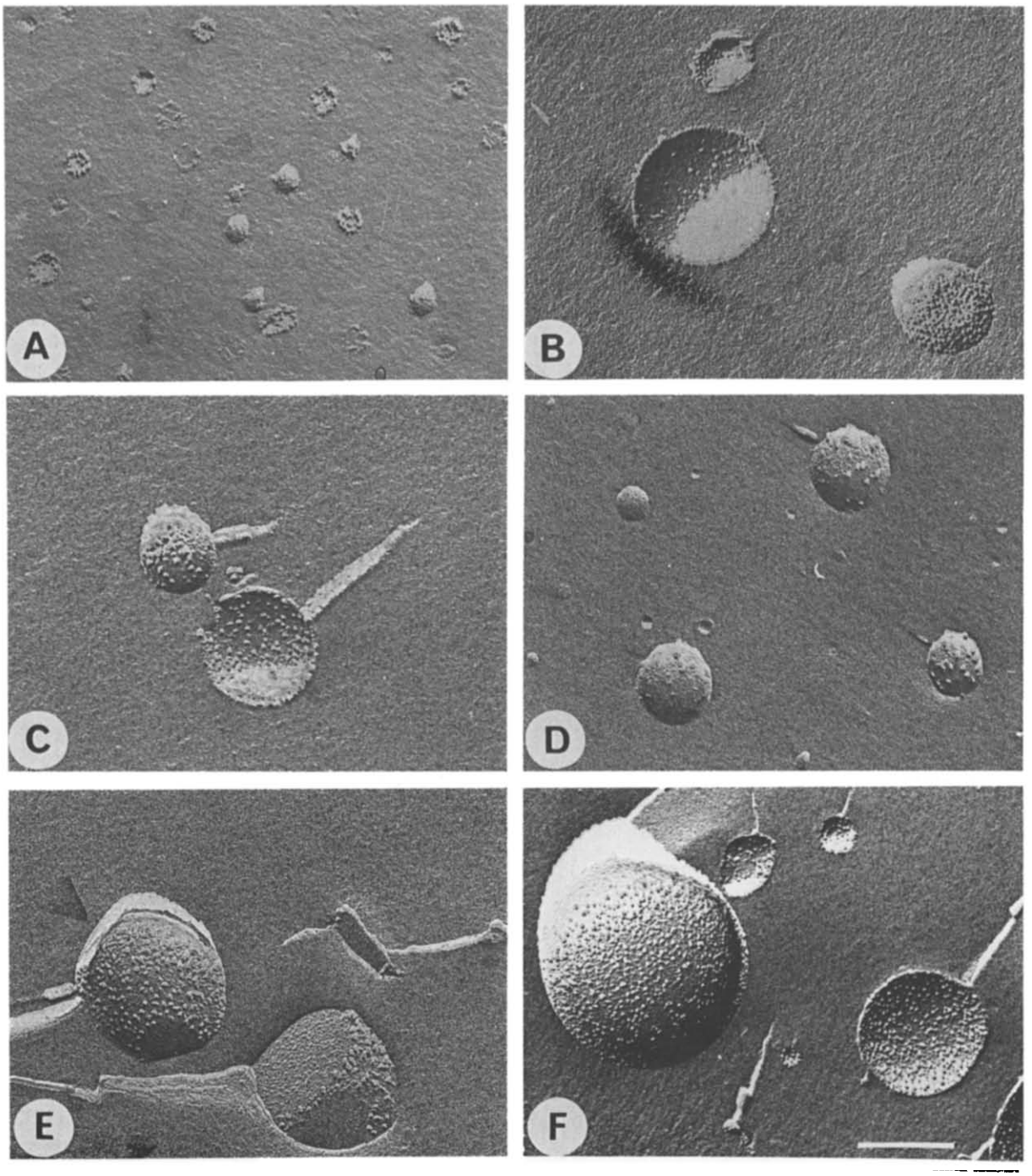

Fig. 1 Freeze-fracture electron micrographs of some fractions analysed in this study (A) Native chromatophores, (B) native spheroplasts, (C) soybean phospholipids fusion band 2, (D) PS fusion band 1, (E) PS fusion band 3, frozen from below 17 °C, (F) diphosphatidylglycerol fusion band 2 Magnification is the same for all micrographs (×75000) The bar in F represents 200 nm Shadowing is from bottom to top

detected in the different populations inspected; nor any evidence of liposomes adhering to the membrane surface following the low pH or the freezing and thawing procedures.

The soybean phospholipids band 1 was formed exclusively of liposomes (smooth vesicles) (not shown). In band 4, the vesicle diameter was rather similar to that of native chromatophores; in the other lighter band, it was significantly larger (Table II). In all enriched vesicles, the intramembranous particles were remarkably less densely packed than in chromatophores, and their lateral distribution was uniform and equal in two halves of the bilayer (Fig. 1).

For PS-enriched vesicles the electron microscope images showed a stronger increase of vesicle diameters; some vesicles with an increase up to 43-fold in diameter were obtained by the freezing and thawing fusion method when the initial experimental phospholipid/ BChl ratio was increased (Table II). When frozen from a temperature below the main transition temperature of PS, the intramembrane particles seggregated at the surface of vesicles, out of smooth lipid regions (Fig. 1E). When freezing was done from above this temperature, the lateral distribution of particles was uniform. We have found by X-ray diffraction experiments that the main transition temperature of bovine spinal cord PS in the presence of water was about 17° C.

After fusion with diphosphatidylglycerol liposomes, the diameter of the fused vesicles was again increased. The largest fused vesicle detected by freeze-fracture showed an increase up to 63-fold as compared to chromatophore diameter (Table II) and the intramembrane particles were uniformly distributed and more densely packed than in the vesicles formed by other fusogenic phospholipids (Fig. 1F). For vesicles which were smaller than 150 nm in diameter some residual asymmetry was observed in the particle distribution between the two fracture faces. Convex fracture faces were in this case less particulate than concave ones, a feature reminiscent of chromatophores (not shown).

Apparent diameters of the intramembrane particles were, in all cases, 11.9 ± 2.6 nm.

Phospholipid composition of fused vesicles

The phospholipid composition of the various

TABLE III

PHOSPHOLIPID COMPOSITION OF CHROMATOPHORES AND FUSED FRACTIONS

Phospholipids were extracted from each sample, and lipid phosphorus was determined in the TLC spots after scraping the plates Data were normalized to the PE value of each sample.

Fraction	Relative contents			
	PE	phosphatidyl-glycerol	PC	diphosphatidyl-glycerol
Chromatophores				
native	1	0 90	0 56	0 12
pH control	1	0 64	0 48	0 26
freeze-thaw control	1	0 77	0 52	0 12
pH fusion with PS				
band 2	1	0 12	0 30	0 10
band 3	1	0 26	0 24	0 12
band 4	1	0 52	0 34	0 14
Freeze-thaw fusion with PS				
band 2	1	0.24	0 12	0 02
band 3	1	0 24	0 24	0.06
band 4	1	0.46	0 34	0 10
pH fusion with diphosphatidylglycerol				
band 2	1	0.44	0 44	$\gg 1$
band 3	1	0.48	0 50	$\gg 1$
band 4	1	0.32	0 48	$\gg 1$

bands was modified by the fusion process and was not merely the sum of the chromatophores and exogenous components (Table III). Lipid phosphorus analysis of the chromatophores of this strain of *Rhodobacter sphaeroides* indicated that their phospholipids are composed of 38.7% PE, 34.7% phosphatidylglycerol, 22.0% PC and 4.6% diphosphatidylglycerol. In addition, an ornithine containing lipid (which did not contain phosphorus) was also present (not shown in Table III). In the lipid extract from the fusion bands, quantitative phosphorus and densitometric determinations on the main species resolved by thin-layer chromatography plates indicated that after PS fusion, the phosphatidylglycerol and PC contents relative to that of PE were severely decreased in all the bands. These results were similar whatever the fusion method employed. After diphosphatidylglycerol fusion, the content of phosphatidylglycerol showed an equivalent decrease but a lower decrease was seen in the PC content (Table III). Fusion bands of soybean phospholipids also indicated a decrease of phosphatidylglycerol content relative to that of PE (not shown). Qualitatively, a strong decrease was observed in the ornithine-containing lipid after fusion of the chromatophores with all the fusogen liposomes assayed (not shown). It is noteworthy that in control experiments made at low pH with chromatophores (without liposomes) a relative decrease of phosphatidylglycerol and PC was observed. In control experiments on freezing and thawing of chromatophores, these relative decreases were much smaller than those observed after fusion (Table III).

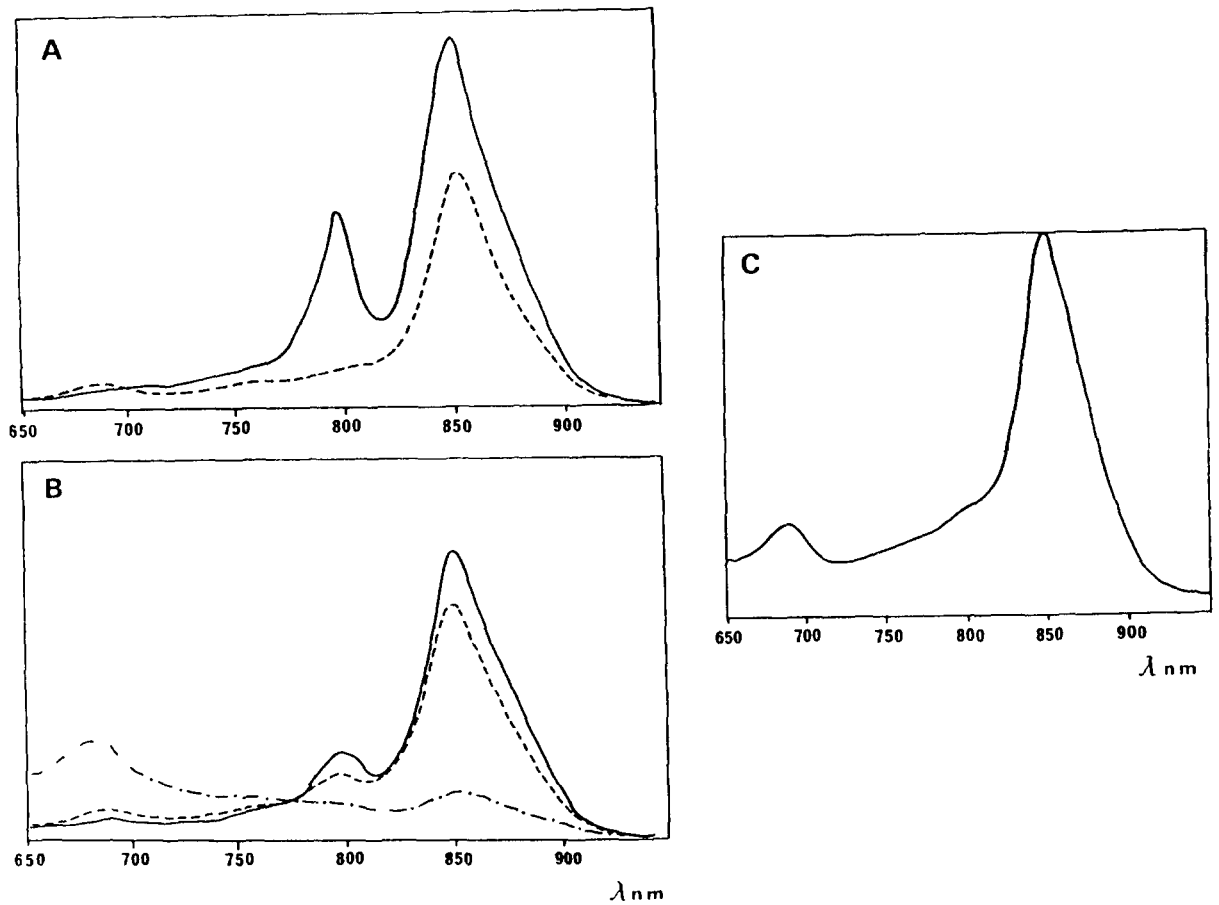

Fig 2. Infrared absorption spectra (A) Native chromatophores (———); PS-fusion band (- - - - -). (B) Soybean phospholipids fusion band 4 (———), band 2 (- - - - -); band 1 (– –) (C) Diphosphatidylglycerol fusion band 1.

Effects of fusion on antenna and reaction center properties

The stability of the different pigment-protein complexes, specially that of the 800 nm chromophore of B800–850 was modified by various fusogens. After fusion with soybean phospholipids the 800 nm absorption band was decreased in parallel with phospholipid enrichment (Fig. 2 and Table IV). After PS, diphosphatidylglycerol (Fig. 2) and phosphatidylglycerol fusion (not shown) the absorption band was practically absent. This absorption band was also decreased in the pH and freeze-thawed control experiments (Table IV). The same phenomenon was observed at liquid nitrogen temperature, where the chromophore (shifted to 798 nm) was already damaged by the simple mixture of chromatophores with diphosphatidylglycerol liposomes at pH 7.5, before the beginning of the fusion process (Table V). Degradation of BChl was noted, specially in the lightest band, with an increase in absorption at 690 nm (see in Fig. 2, diphosphatidylglycerol and soybean phospholipids band 1); in the case of phosphatidylglycerol fusion, some bacteriopheophytin was formed in bands 1 and 2 as evidenced by the absorption at 760 nm (not shown).

The 875 nm complex was less damaged by fusion, and the fusion was dependent on the nature of fusogenic phospholipid (Table V). Once again, the lighter the band was, the stronger was the absorption decrease of this chromophore (see diphosphatidylglycerol band 2 in Table V). These modifications at the level of the antenna were not accompanied by gross alterations at the level of the reaction centre. Difference spectroscopy during steady illumination demonstrated the presence of functional photochemical reaction centres in all fusion bands (except band 1 of PS, mainly composed of liposomes) (not shown). The size of the photosynthetic unit, expressed as total BChl/reaction center ratio, was similar or slightly smaller in fused vesicles than in chromatophores (see Table I). This originated probably from a loss in antenna BChl.

The formation of photoinduced membrane potential was tested only on the pH fusion fractions by the carotenoid shift and by the absorbance change at 845 nm. These effects were still present in the most dense band 4; their amplitudes were not constant throughout the band and decreased with the buoyant density (e.g. for PS fusion from 80 to 45% of the value relative to native chromatophores). In the lighter fusion bands, these effects were abolished.

Fusion with spheroplasts

We were not able to get a good yield of fusion products between spheroplasts and any of the acidic phospholipid assayed. With diphosphatidylglycerol and PS liposomes, some fusion took place, as shown by the appearance of weak fusion bands on a sucrose gradient; their analysis was however not possible due to lack of material.

TABLE IV

FUSION EFFECTS UPON B800 CHROMOPHORE, EXPRESSED AS $A_{800\,nm}/A_{850\,nm}$ (25° C)

Lipid	Freeze and thaw fusion	Low-pH fusion
PS [a]	0 31	0 32
Diphosphatidyl-glycerol [a]	0.21	0 21
Soybean PL band 2		0 28
Soybean PL band 4		0 345
Chromatophores control	0 45	0 46
Chromatophores native	0.56	

[a] Whole fused mixture

TABLE V

FUSION EFFECT UPON INFRARED ABSORPTION (77 K)

Fraction	$A_{798\,nm}/A_{855\,nm}$	$A_{885\,nm}/A_{855\,nm}$
Native chromatophores	0 65	0 60
Fusion with diphosphatidylglycerol		
Chromatophores + liposomes, (pH 7 5, before fusion)	0 40	0 61
band 4	0 23	0 58
band 3	0 21	0.55
band 2	0 145	0.37
Fusion with phosphatidylserine		
band 3	0 52	0 56
band 2	0 33	0 54

Discussion

The presence of several pigmented bands in a sucrose gradient with a lower buoyant density than that of chromatophores and the morphology of the vesicles they contain are consistent with a fusion between chromatophores and all the acidic phospholipids assayed. However, there are differences between the various fusogenic phospholipids reflected by the different lipid enrichments (cf. Table I) and the various increase in vesicle diameters (Table II).

Soybean phospholipids liposomes show the lowest capacity of fusion; here, chromatophore proteins and pigments are associated with the most dense band, where the vesicles have a diameter similar to that of chromatophores. The lipid enrichment is in quantitative agreement with previous results of Casadio and co-workers [6] for fusion with soybean phosphatidylcholine.

If we compare the enrichment in lipid for band 2 (which is present in all cases) it is similar for phosphatidylglycerol and soybean phospholipids, whereas PS liposomes are incorporated in a greater proportion, and diphosphatidylglycerol in a lower one. In contrast, the size of the vesicles in band 2 (cf. Fig. 1) is the biggest in diphosphatidylglycerol fusion. The large size of these fused vesicles might be attributed to the physical structure of diphosphatidylglycerol with its four hydrocarbon chains per molecule. The steric factor related to the relative size of hydrophilic and hydrophobic parts of the molecule would account for the formation of vesicles with a weak curvature radius.

In general, in all fused vesicles, the average density of intramembrane particles by surface area is lower than in chromatophores and their lateral distribution is symmetrical on both fracture-faces planes. However, the diphosphatidylglycerol-enriched vesicles showed a residual asymmetry when their diameter was lower than 150 nm: concave fracture faces being more particulate than convex ones, like in chromatophores. Intramembrane particles are generally uniformly distributed, which suggests that chromatophore membranes are naturally fluid to the extent that the integral membrane proteins can diffuse laterally in the lipid bilayer. A notable exception is provided by PS fused vesicles, where a clear coexistence of smooth and particulate regions was observed indicating a segregation of pure lipid, due to cristallization of the hydrocarbon chains. Thus a thermotropic phase transition took place at the membrane level. Such lipid-protein lateral separation is completely reversible above 17 °C, transition temperature of the spinal cord PS. It is not related to the phase transition of chromatophore phospholipids, which is known to be about −20 °C [27].

All the phospholipids used in the study were pure and acidic, except soybean phospholipids which are mainly constituted by phosphatidylcholine and a mixture of some acidic and lysophospholipids. In that case thin-layer chromatography showed that the main phospholipid incorporated was phosphatidylcholine. Probably lysolipids and acidic lipids promoted the fusion, carrying over PC. Indeed pure egg PC prepared by us was unable to fuse with chromatophores, in agreement with reports [22,23] showing that PC is a poor fusogen. However, a commercial sample of pure egg PC fused with high yield with chromatophores, with the low-pH method and the freeze-thaw one. Ca^{2+} ions were absent and no lyso or acidic contaminants were present in chromatograms of this sample. The reason for these contradictory results is not clear The composition of the hydrocarbon chains of these two PC samples was not determined; it is possible that the insaturation degree influences the process of fusion or exchange of membrane lipids [28]. One could remark that a better yield of fusion between *Rb. capsulatus* chromatophores and soybean phospholipid liposomes was obtained when these were enriched in phosphatidylcholine or neutral lipids [10]. Neutral PC vesicles are also able to fuse with thylakoids [2] and with plasma membrane of cultured cells [24,25].

It is interesting to note that very little (if any) fusion took place when acidic phospholipids were mixed with spheroplasts by identical procedures The larger curvature radius, of the spheroplasts and/or the different species of phospholipids exposed at the spheroplast surface may explain this lack of fusion.

The chromatophore proteins did not seem to be profoundly altered by the fusion process, as already observed by others [2,10,27]. At least in PS fusion, some loss of cytochrome c_2 occurred

However, cytochrome c_2 is an extrinsic membrane protein, easily released during fusion [6,27].

More profound modifications were found at the level of chromatophore lipids. The principal alterations were the massive incorporation of the fusogenic lipid (increasing from the denser to the lighter band) associated with a strong diminution of the endogenous phosphatidylglycerol and ornithine lipid content, and a relative decrease of PC. In each of these fusions, only PE seems unmodified. These observations indicate that fusion could involve a phospholipid exchange. Al-Bayatti and Takemoto [28] have shown that in chromatophore membranes, there is an asymmetrical distribution of phosphatidylglycerol and PE, where 73% of total phosphatidylglycerol is localized on the external monolayer (cytoplasmic face), a situation that allows the exchange of phosphatidylglycerol.

Birrell et al. [29] have published data suggesting that phosphatidylglycerol preferentially associates in vivo with bacteriochlorophyll-binding proteins of *Rb. sphaeroides*, and this was confirmed by Russel and Harwood [30]. Modifications at the level of phosphatidylglycerol could thus be related to alterations that we observed in these complexes. For B800–850, the 800 nm chromophore is the most drastically affected, specially after pH fusion. With soybean phospholipids that produce a low extent of fusion, it is still present but reduced up to one half. With the other stronger fusogens (Fig. 2 and Table IV), it is practically absent. This phenomenon is not only a result of the fusion process; it is found to a lesser degree in chromatophores after incubation at pH 6.5, or after a cycle of freezing and thawing and also after mixing with diphosphatidylglycerol liposomes at pH 7.5. Similar observations have been done by Takemoto et al. [10] in the low pH fusion between *Rb. capsulatus* chromatophores and soybean phospholipids. Nevertheless, Garcia and Drews [7] did not observe such a degradation after fusion of *Rb capsulatus* chromatophores with asolectin liposomes by the freezing and thawing method.

The B875 complex which is closely associated with the RC is less damaged by the fusion process; the extent of its degradation depends on the fusogenic lipid and on the phospholipid content (Table V). The RCs themselves are not affected by fusion, whatever the fusogenic lipid. The mean size of the photosynthetic unit (Table I) was only slightly modified by addition of phosphatidylglycerol or by moderate addition of diphosphatidylglycerol; in other cases (PS) its decrease can be attributed to the degradation of light harvesting BChls. Thus the massive addition of exogeneous lipid and the modifications in endogeneous one are acting mainly on the B800 chromophore, which is thought to be located at the periphery of the photosynthetic unit and near the cytoplasmic surface [31]; the chromophores of the B875-RC core are much less altered because there protein–protein interactions are probably dominant over protein–lipid ones.

The partial or total abolition of electrochromic band shifts is another consequence of the process of fusion, already observed in other fusion experiments with [5,9] or without [27] exogenous lipids. These shifts are confined to B800–850 pigments; their abolition may reflect the desorganization of this antenna (loss of B800 chromatophore, lack of proper orientation of the complex) as well as changes in ion permeability of the membrane.

Acknowledgments

The authors would like to thank J.D. Dedieu for excellent technical assistance in freeze-fracture electron microscopy, and Mrs. D Clérot and D. Vaubert for skillful assistance in growing bacteria.

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