Biochimica et Biophysica Acta, 546 (1979) 236—247

BBA 47656

CHARACTERISATION OF REACTION CENTERS AND THEIR PHOSPHOLIPIDS FROM *RHODOSPIRILLUM RUBRUM*

MARIO SNOZZI and REINHARD BACHOFEN

Institute of Plant Biology, University of Zürich, Zolikerstrasse 107, CH-8008 Zürich (Switzerland)

(Received July 10th, 1978)
(Revised manuscript received November 17th, 1978)

Key words: Reaction center; Phospholipid composition; Chromatophore membrane; Bacterial photosynthesis; (Rhodospirillum rubrum)

Summary

1. Reaction centers from *Rhodospirillum rubrum* have been extracted with the zwitterionic detergent lauryl dimethyl amine oxide. Subsequent purification has been achieved by gel filtration and ion-exchange chromatography. The pure reaction centers are composed of three protein subunits (L, M, H), bacteriochlorophyll and bacteriopheophytin in the ratio 2 : 1 and phospholipids.

2. The phospholipid composition has been found to be similar to that of whole chromatophore membrane, except that diphosphatidyl glycerol is present in higher amount in the isolated complex. When the detergent treatment of the chromatophore membrane is done in the presence of NaCl, a lower phospholipid content in isolated reaction centers has been found together with a lower stability in the association among the protein subunits. In this complex, the largest subunit H is easily split off and a LM complex is obtained. It is concluded that the phospholipids play an important role in the stability of reaction center complexes.

Introduction

Photosynthetic energy transduction starts with the absorption of light in a set of light-harvesting pigments, mainly chlorophylls. The absorbed light energy is converted into chemical energy in form of negative oxido-reduction potentials at special sites, the photosynthetic reaction centers. Whereas green plants have at least two different reaction centers, one for each photosystem, it is generally believed that the photosynthetic bacteria contain only one reaction

Abbreviation: LDAO, lauryl dimethyl amine oxide.

center complex. Several attempts have been made to isolate the reaction centers of different photosynthetic bacteria [1,2] from the bulk of the light-harvesting bacteriochlorophyll. This has been first achieved by destroying the light-harvesting pigments with strong oxidants [3]. Later reaction center particles have been extracted from chromatophore membranes with the help of detergents [4]. Optimal results have been obtained using the zwitterionic detergent lauryl dimethyl amine oxide (LDAO) [5,6].

The isolated reaction center pigment protein complex is made up of three protein subunits with molecular weights between 21 000 and 29 000 [7,8]. Recently Clayton and Clayton [9] have isolated reaction centers from *Rhodopseudomonas gelatinosa* which contain only two subunits with molecular weights of 25 000 and 33 000. Absorption spectra of reaction centers from carotenoid-less mutants show bands near 535 and 757 nm which can be attributed to bacteriopheophytin, bands near 600, 802 and 865 nm attributable to bacteriochlorophyll, an unresolved soret maximum for both pigments near 365 nm and a band at 280 nm due to protein. The 865 nm band is bleached upon illumination or by chemical oxidation [10]. The reaction centers contain the two pigments bacteriochlorophyll and bacteriopheophytin in the ratio of 2 : 1 [11 -13]. In circular dichroism spectra at 77 K the bands at 757, 802 and 865 nm are each resolved into two separate maxima, indicating that reaction centers contain four bacteriochlorophyll and two bacteriopheophytin [14]. Reaction centers from the wild type of *Rhodospirillum rubrum* have an additional spirilloxanthin [15].

As primary acceptor for the electron a special ferro ubiquinone complex has been postulated by Okamura et al. [16] as integral part of the reaction centers from *Rps. spheroides* R 26.

In the present paper an isolation and purification procedure for reaction centers from *Rhs. rubrum* is described. The characterisation of the reaction centers covers besides pigments and proteins also phospholipid content of the complex. As shown in this paper, the phospholipids may play an important role in the function of the reaction centers and in the stability of the protein association.

Materials and Methods

Cells of *Rhs. rubrum* from the wild type strain S1 and from the carotenoid-less mutant $G9^+$ were grown in 10-l bottles at 30°C. The medium of Ormerod et al. [17] was used, omitting yeast extract and pepton. After inoculation (1.5—2.5%) the bottles were kept in the dark for 24 h. The following three days the culture was illuminated with light from tungsten lamps. During the last two or three days of growth, the bottles were further illuminated with additional light from fluorescent lamps. After harvesting, the cells were washed with distilled water and with 10 mM phosphate buffer and kept at —70°C.

To prepare chromatophores, the frozen cells were suspended in 10 mM phosphate buffer, pH 7.0, (about 60 g/150 ml suspension). The suspension was sonicated twice during 150 s. 1 mg DNAase/10 g of cells was added to digest the long DNA strands. The non-disrupted cells and large cell fragments were separated by low spin centrifugation (20 min, 13 000 rev./min, Beckman JA

20 rotor). The crude chromatophores were sedimented by high spin centrifugation (60 min, 55 000 rev./min, Beckman Ti 60 rotor). The chromatophores were washed with phosphate buffer and with a 5 mM EDTA solution (pH 7.0) to remove proteins loosely bound to the membranes.

Reaction centers were prepared using a modification of the method of Okamura et al. [18]. The absorbance at 870 nm of the purified chromatophores was adjusted to 85 (1 cm path length). Small portions of this suspension were mixed with equal volume of LDAO solutions (0.4–0.7% LDAO in 10 mM phosphate buffer). The incubation with LDAO was carried out in the dark at 4°C under a nitrogen atmosphere. After 10 min incubation, the non-solubilized chromatophore membranes were separated by high spin centrifugation (60 min, 55 000 rev./min). The accurate concentration of LDAO was determined from the optical spectra of the supernatants. For the quantitative preparation of reaction centers 150 ml of the chromatophore suspension were mixed slowly with 150 ml of the LDAO concentration determined before, at 4°C in the dark, under a nitrogen atmosphere. In those cases where sodium chloride was present during LDAO incubation, the salt was added to the chromatophore suspension in solid form before the LDAO addition.

For purification the reaction centers were first concentrated by ultrafiltration, using an Amicon UM 20 E filter. The concentrate was passed through a Sepharose 6B column, equilibrated with 10 mM Tris buffer, pH 8.0, containing 0.025% LDAO. Further purification was obtained using ion-exchange chromatography as follows: the reaction centers were absorbed on a DEAE-cellulose column preequilibrated with 10 mM Tris buffer, pH 8.0, 0.025% LDAO. The column was then washed with the same buffer containing 0.06 M NaCl until the blue band containing the reaction centers reached the bottom of the column. The reaction centers were then eluted using the Tris buffer with 0.12 M NaCl. The pure reaction centers were again concentrated by ultra filtration and dialysed against 10 mM Tris buffer, pH 8.0. All purification steps were carried out at 4°C, protected from strong illumination.

The concentrated reaction centers could be frozen in liquid nitrogen and stored at −70°C.

The total lipid content of reaction centers was determined after extraction in the dark with 20 vols. chloroform/methanol (2 : 1) 15 min at 20°C. The denatured proteins were sedimented by centrifugation. The supernatant, containing the lipids, pigments and part of the protein, was washed with 0.2 volumes of 0.04% $MgSO_4$ to remove inorganic phosphate. The quantitative determination of the phospholipids was done according to Fiske and SubbaRow [19] modified by Bartlett [20].

For the qualitative identification of the lipids two different thin-layer chromatographic methods were used. The two-dimensional system of Rouser et al. [21] did not separate phosphatidyl ethanolamine and phosphatidyl glycerol. This separation was achieved using the first solvent of the Lepage system [22]. This method needed very careful handling, because degradation products of the bacteriochlorophylls interfered with the phospholipids. The migration of the reaction center phospholipids was compared with the property of known phospholipids. For the quantitative determination of the different phospholipids, their corresponding spots were scratched out and submitted to the phosphate

determination. The recovery of phosphate after thin-layer chromatography was about 90%. The two-dimensional system gave slightly better results than the monodimensional.

The ubiquinone content was measured using the method of Takamiya and Takamiya [23]. Polyacrylamide gel electrophoresis in the presence of dodecyl sulphate was performed according to Laemmli [24]. Gels with acrylamide concentration of 10 and 12% were used. The gels were run at constant voltage (16 V/cm gel). They were stained with Coomassie Brilliant Blue R 250.

LDAO was purchased as Ammonyx LO from the Onyx Chemical Company, NJ. The Ammonyx LO solution was freeze-dried and twice recrystalized. Later, the pure LDAO was obtained from Fluka Switzerland.

Results

The effect of LDAO on the chromatophore membrane is summarized in Fig. 1A. An initial concentration of the detergent (0.225% in this experiment) was needed to solubilize proteins from the membrane. A further increase of the LDAO concentration from 0.25 to 0.275% had no effect on the amount of protein released. Higher detergent concentrations (above 0.3%) resulted in

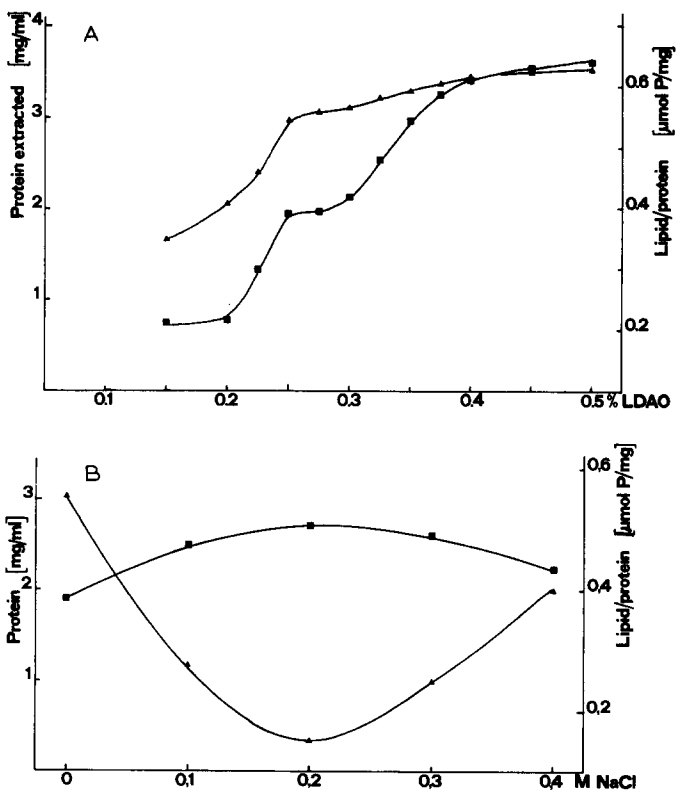

Fig. 1. (A) Effect of LDAO on the chromatophore membrane. ■, protein extracted; ▲, specific lipid content. (B) Effect of NaCl concentration on the solubilisation of proteins with 0.275% LDAO. ■, protein extracted; ▲, specific lipid content.

denaturating the reaction centers and the specificity of LDAO in solubilizing reaction centers was lost. The supernatants of these samples contained large amounts of light-harvesting complexes, whereas in the region between 0.25 and 0.275% LDAO the reaction centers accounted for about 70% of the released protein.

The EDTA treatment of the chromatophores removed several of loosely bound surface proteins. Omitting this step, the crude reaction centers have also to be separated from these proteins.

A good test for the quality of the reaction centers was the ratio of the absorbance at 757 nm to 802 nm. In native preparations this ratio was between 2.2 and 2.3. It is known that the absorbances at 865 nm and 802 nm decrease and a new band at 770 nm appears when the native chlorophyll orientation in the complex is changed.

The presence of sodium chloride increased the amount of protein extracted from the chromatophore membrane by LDAO compared to the effect of the same detergent concentration without NaCl. In Fig. 1B the dependency of the LDAO effect on the ion concentration is shown. The presence of 0.2 M NaCl seems to be optimal in solubilizing the reaction centers with LDAO. The effect of the concentration of LDAO is only slightly influenced by the presence of NaCl.

The yield of isolated crude reaction centers was in the absence of NaCl about 45–55% of the theoretical value. This theoretical value was calculated using the fact, that about 4% of the absorbance at 870 nm can be bleached either by actinic light or by chemical oxidation. The yield of the pure reaction centers was about 25% of the theoretical value. With the help of NaCl, 70% of the reaction centers could be extracted from the membrane (Table I).

Fig. 2 shows the absorption spectrum of the pure reaction centers prepared without NaCl. The purity measured by the ratio of absorbance at 280 nm to 802 nm gave a value of 1.25 ± 0.04. The activity of the complex was controlled by photooxidation of the 865 nm band with actinic light at 368 nm.

The purity of these reaction centers was further checked by dodecyl suphate gel electrophoresis. As shown in Fig. 3 pure reaction centers, prepared without NaCl, showed only three bands after gel electrophoresis. From the

TABLE I

Amount of reaction centers in different stages of the preparation, given in absorbance at 865 nm multiplied with the volume in ml (AV_{865}). For chromatophores, the theoretical reaction center content (4% of the 865 nm absorption) is given in parenthesis. The yield of each stage is given in percent of the theoretical content of chromatophores.

	AV_{865}	Percent of total content
Chromatophores	12 000 (480)	100
Crude reaction centers, without NaCl	220–270	45–55
Reaction centers after the Sepharose column, without NaCl	200	40
Pure reaction centers, without NaCl	120	25
Crude reaction centers, with 0.2 M NaCl	340	70
Reaction centers after the Sepharose column, with 0.2 NaCl	310	65
Pure reaction centers (LM complex), with 0.2 M NaCl	180	38

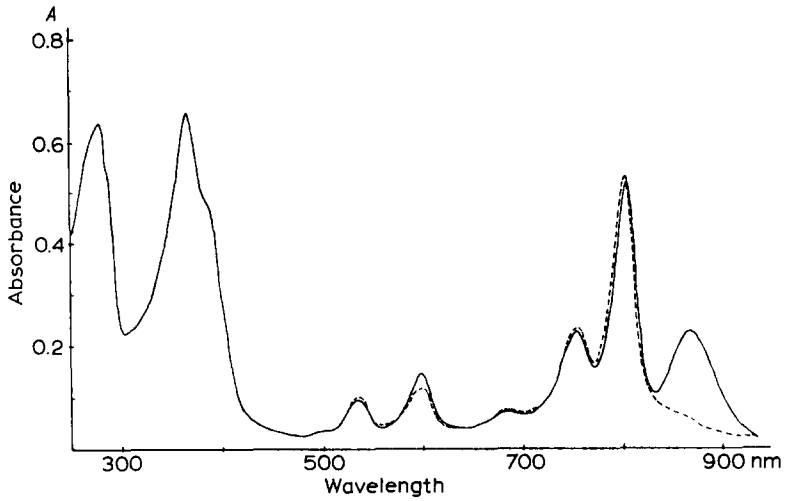

Fig. 2. Absorbance spectrum of pure reaction centers in reduced and oxidised state. Oxidation was done by illumination with actinic light of 368 nm and an intensity of $8 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}.

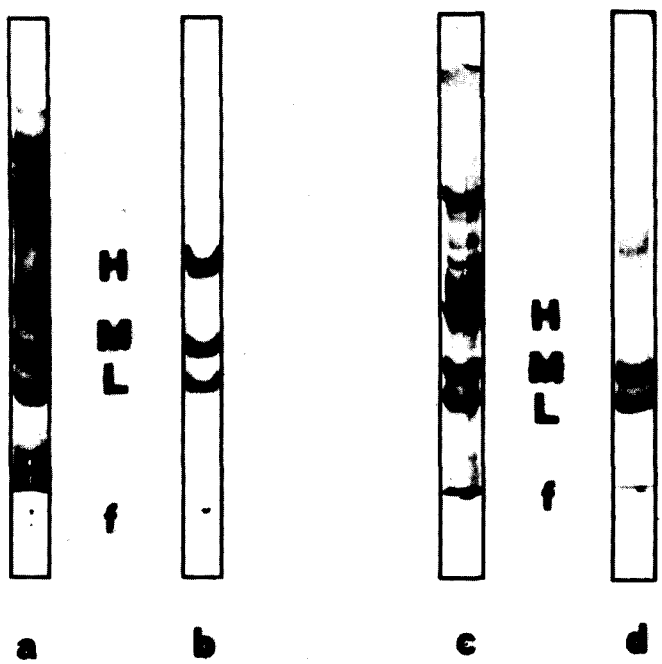

Fig. 3. Dodecyl sulphate gels of reaction centers stained with Coomassie Brilliant Blue R 250. a, Crude reaction centers after extraction with 0.275% LDAO in 12% gels; b, Pure reaction centers without NaCl in gels with acrylamide concentration of 12%; c, Crude reaction centers after extraction with 0.275% LDAO in the presence of 0.2 M NaCl in gels with acrylamide concentration of 10%; d, NaCl reaction centers after purification. The H subunit of NaCl reaction centers is split off during the purification procedure.

mobility in the gel the molecular weight of these three subunits was determined to be 21 000 for L, 24 000 for M and 29 000 for the H subunit.

Extraction of reaction centers in the presence of sodium chloride showed no difference in the subunit composition compared to reaction centers extracted without NaCl. However, in the pure reaction centers, after the two purification steps, the largest subunit H was largely missing when the reaction centers were extracted in the presence of NaCl (Fig. 3). The resulting LM complex had a similar absorption spectrum to normal reaction centers, except that the ratio of the absorbance at 280 nm to 802 nm decreased to 1.08 ± 0.03 (Fig. 4). This LM complex was more sensitive to degradation by light and temperature.

Reaction centers contained the two pigments bacteriochlorophyll and bacteriopheophytin in the ratio 2 : 1.

In this context it is interesting to note, that the tetrapyrrole ring of pheophytin is esterified with phytol, whereas chlorophyll is a geranyl geraniol ester [25]. The ubiquinone content was determined to be 0.97 ± 0.1 ubiquinone/reaction center. This ratio did not depend on the LDAO concentration. The ubiquinone content of crude and pure reaction centers was found to be the same.

In contrast to the amount of protein solubilized, the specific phospholipid content did not vary much at different LDAO concentrations tested (Fig. 1A). However, in a few chromatophore preparations, the phospholipid content of the supernatants of all LDAO concentrations were higher (0.7 μmol lipid/mg protein) compared to about 0.55 μmol lipid/mg protein measured usually.

The presence of NaCl during the incubation with LDAO lowered the phospholipid content of the isolated reaction centers (Fig. 1B). The concentration of NaCl (0.2 M) which lead to minimal phospholipid content (about 0.15 μmol lipid/mg protein) coincided with optimal extraction of protein.

Table II shows the total content of phospholipids in chromatophores and in

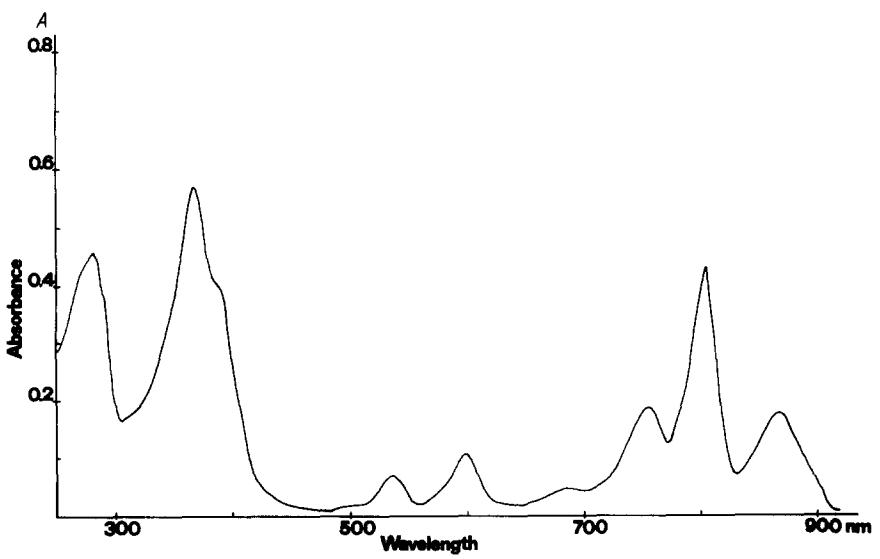

Fig. 4. Absorption spectrum of LM complex obtained after purification of NaCl reaction centers.

TABLE II

Phospholipid content of reaction centers and composition isolated without and in the presence of 0.2 M NaCl, followed by the normal purification procedure. The phospholipid composition is given in percent of the total lipid. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PEA, phosphatidyl ethanolamine.

Preparations	Phospholipid content (μmol PO_4/mg protein)	% of total lipid		
		PG	PEA	DPG
Chromatophores	0.62	22	65	13
Crude reaction centers (—NaCl)	0.55	24	52	24
After Sepharose column (—NaCl)	0.50	23	53	24
Pure reaction centers (—NaCl)	0.48	24	51	25
Pure reaction centers (+NaCl)	0.26	27	45	28

reaction center complexes. These phospholipids seem to be firmly bound to the reaction centers, since they are still attached to the complex after the two purification steps. Addition of 25% ammonium sulfate did not give any precipitation, but the blue colour of the reaction centers appeared in a lipid film on the surface. The phospholipid content of this film was identical with the soluble reaction centers. A precipitation of the reaction centers with trichloroacetic acid in the presence of LDAO was not possible, this treatment gave only a milky suspension. However, pure reaction centers could be precipitated by dialysis against distilled water. The phospholipid content of the precipitate was again not changed.

When reaction centers were put on a discontinuous sucrose gradient (0—20%) they formed after centrifugation (120 min, 55 000 rev./min, Beckman Ti 60 rotor) a band in the region of 10% sucrose. These reaction centers still had an unchanged phospholipid content.

The content of phospholipids in the reaction centers can be almost entirely accounted for by diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Lysophosphatidylethanolamine in different amounts was also found in chromatophores as well as in reaction centers (Fig. 5). Since in freshly harvested cells no lysophosphatidylethanolamine could be found, this

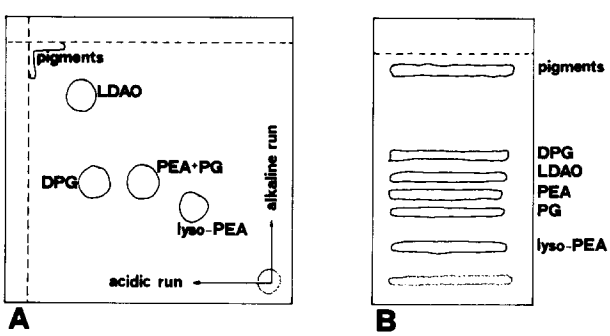

Fig. 5. Separation of the reaction center phospholipids in the two thin-layer chromatography systems used. The phospholipids were extracted from the reaction centers with chloroform/methanol (2 : 1). (A) System of Rouser et al. [21]: alkaline solvent (v/v): 65 chloroform, 25 methanol, 5 conc. ammonia: acidic solvent (v/v): 4 acetone, 3 chloroform, 1 methanol, 1 acetic acid, 0.5 water. (b) System of Lepage [22] solvent (v/v): 65 chloroform, 25 methanol, 4 water.

compound must be formed from phosphatidylethanolamine during storage of the cells and during the preparation of chromatophores. Therefore any detected lysophosphatidylethanolamine was computed as phosphatidylethanolamine.

Previous work gave evidence that whole cells of *Rhs. rubrum* contain also small amounts of phosphatidylcholine [26]. In the chromatophores of this organism no phosphatidylcholine was detectable. When the lipid extract of the chromatophores was mixed with phospatidylcholine and run on the thin-layer plate, a new spot could be seen. Therefore it is concluded, that chromatophores and reaction centers do not contain phosphatidylcholine.

The phospholipid composition varied slightly in the different preparations. However there was always an increase of diphosphatidylglycerol in crude reaction centers compared with the chromatophores. This tendency was again found during the purification of the reaction center complex. The portion of phosphatidylglycerol was somewhat higher in reaction centers than in chromatophores, but remained at the same level during purification. Phosphatidylethanolamine on the other hand was present in smaller amounts in reaction centers than in the membrane (Table II).

The lipid composition of reaction centers isolated in the presence of NaCl was different from the normal ones. The amount of phosphatidylethanolamine was smaller, whereas phosphatidylglycerol and diphosphatidylglycerol were present in higher quantities (Table II).

Similar results were obtained starting with the wild type of *Rhs. rubrum.* In this case the reaction center pigments contained also spirilloxanthin besides bacteriochlorophyll and bacteriopheophytin. These reaction centers were less sensitive to light compared to the mutant, indicating that spirilloxanthin protects the reaction centers from light. This effect has been shown previously by Boucher et al. [27].

Discussion

The described isolation and purification method is faster and less complicated than others described before [18,28]. As a great advantage large quantities of reaction centers can be produced in one batch. As judged from the ratio of absorbance at 280 nm to 802 nm the purity of our reaction center preparation is as good as that reported by Okamura et al. [18]. Pigment and protein composition have been the same as reported in other publications. The finding of Walter [25] that in whole cells as well as in reaction centers of *Rhs. rubrum* only the pheophytin is esterified with phytol, whereas the chlorophyll is a geranyl geraniol ester, is another argument for the original composition of isolated reaction centers regarding the pigment content.

The determination of the molecular weight of the protein subunits is problematic, because of the lipophilic behaviour of L and M. The reported values must be considered as estimations.

Okamura et al. [16] found in reaction centers from *Rps. spheroides* R 26 two ubiquinones/reaction center. One of these is loosly bound and can be removed by LDAO treatment. The firmly bound ubiquinone is regarded as primary electron acceptor, since the removal of this ubiquinone gave an

inactive complex. In our preparations we found only one ubiquinone/reaction center. This ubiquinone may correspond to the firmly bound one found by Okamura et al. [16]. Some difference in the reaction centers of these two organisms was also found immunologically by Steiner et al. [29].

In contrary to our results Morrison et al. [30] showed that in chromatophores of *Rhs. rubrum* only 0.5 ubiquinone/reaction center are required for full bleaching activity of *P*-870. However, in their model a reaction center dimer with one firmly bound and one loosly bound ubiquinone was proposed. Our measurements of one ubiquinone/reaction center are well in agreement with their view since the two types of ubiquinone were not separately analysed. In addition results from gel filtration suggest that our reaction centers exist as di- or tetramers rather than as monomers.

So far, little information on the lipid content of reaction centers has been published yet. As shown here for the first time, they are present in considerable amounts. This suggests that they may play an important role either in the function of the reaction centers or for their orientation in the membrane.

If these phospholipids were not closely attached to the protein, one would expect that the ion-exchange chromatography would separate them [31]. The fact that the phospholipid content remained unchanged after centrifugation in a sucrose gradient, is another indication that the lipids are firmly bound to the reaction center complex [32]. The composition of the phospholipids in the isolated complex is in the whole similar to the one found in chromatophore membranes. In spite that LDAO seems to extract reaction centers quite selectively, the detergent seems rather to cut a patch out of the membrane. This phenomenon was also reported by Wildenauer and Khorana [33], who isolated the light-driven proton pump from *Halobacterium halobium* with LDAO. In both cases, this detergent seems to solubilize membrane proteins together with a small lipid core. The lipid content of the reaction centers isolated in the presence of NaCl is smaller. From this it may be assumed that NaCl either allows the detergent to attack the membrane closer to the protein or favours the substitution of phospholipids by LDAO.

The composition of the lipids in the isolated complexes reflects probably the original environment of the proteins in the membrane. If this were true, the increased amount of diphosphatidylglycerol in the pure reaction centers leads to the conclusion, that the negatively charged diphosphatidylglycerol is accumulated in the neighbourhood of the proteins of the reaction centers. This trend is even more accentuated, when reaction centers are extracted in the presence of NaCl.

Cytochrome oxidase is associated with several molecules of diphosphatidylglycerol. These few molecules of cardiolipin can be dissociated from the enzyme only under conditions that denature the protein and dissociate it into its component polypeptides [34,35]. Since the LM complexes still contain phospholipids it may well be that the lipids could play an important role in the oxidation of the *P*-865. This possibility has to be studied further.

Reaction centers with a low phospholipid content are less stable and the largest subunit H is easily split off. The resulting LM complex has similar properties as the LM band described by Okamura et al. [18]. Following his preparation, treatment of the pure reaction centers with LDAO and dodecyl

sulphate, followed by separation on a sucrose gradient, we found, that the amount of phospholipids attached to the complex is decreased to one-fourth of the original content. This corresponds roughly to the lipid content in LM complexes after LDAO incubation in the presence of NaCl. From these results it can be concluded, that the phospholipids stabilize the three subunit reaction center complex, LDAO cannot substitute the phospholipids without loosing the H subunit.

From the bleaching kinetic of the LM band at cryogenic temperature, Okamura et al. [18] suggested, that the H subunit may contain the primary acceptor or at least be responsible for the right orientation of this electron acceptor to the reaction center. As Zürrer et al. [36] showed, the H subunit is the only part of the reaction centers located at the cytoplasmic side of the chromatophore membrane, giving evidence that the H subunit might be responsible for the correct orientation of the reaction center complex in the membrane.

Acknowledgements

We thank A. Bruni (Institute of Pharmacology, University of Padova, Italy) for his introduction to the lipid technique and his helpful discussion of the results. This work has been supported by the Swiss National Foundation for Scientific Research (grant 3.041.76).

References

1 Sauer, K. (1974) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 139—144, Academic Press, New York
2 Loach, P.A. (1976) Prog. Bioorg. Chem. 4, 89—192
3 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312—323
4 Reed, D.W. and Clayton, R.K. (1968) Biochem. Biophys. Res. Commun. 30, 471—475
5 Feher, G. (1971) Photochem. Photobiol. 14, 373—387
6 Clayton, R.K. and Wang, R.T. (1971) Methods Enzymol. 23, 696—704
7 Clayton, R.K. and Haselkorn, R. (1972) J. Mol. Biol. 68, 97—105
8 Feher, G., Okamura, M.Y., Raymond, J.A. and Steiner, L.A. (1971) Biophys. J. 11, 38a
9 Clayton, B.J. and Clayton, R.K. (1978) Biochim. Biophys. Acta 501, 470—477
10 Clayton, R.K. (1962) Photochem. Photobiol. 1, 201—210
11 Straley, S.C. (1972) PhD Thesis, Cornell University, Ithaca, NY
12 Beugeling, T., Slooten, L. and Barelds-Van de Beck, P.G.M.M. (1972) Biochim. Biophys. Acta 283, 328—333
13 Mauzerall, D.C. (1972) Fed. Proc. 31, Abs. 885
14 Reed, D.W. and Ke, B. (1973) J. Biol. Chem. 248, 3041—3045
15 Van der Rest, M. and Gingras, G. (1974) J. Biol. Chem. 249, 6446—6453
16 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3491—3495
17 Ormerod, J.G., Ormerod, K.S. and Gest, H. (1961) Arch. Biochem. Biophys. 94, 449—463
18 Okamura, M.Y., Steiner, L.A. and Feher, G. (1974) Biochemistry 13, 1394—1403
19 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375—385
20 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
21 Rouser, G., Yamamoto, A. and Fleischer, S. (1970) Lipids 5, 494—496
22 Lepage, M. (1964) J. Chromatogr. 13, 99—103
23 Takamiya, K. and Takamiya, A. (1969) Plant Cell Physiol. 10, 363—373
24 Laemmli, U.K. (1970) Nature 227, 680—685
25 Walter, E. (1978) Thesis, ETH Zürich, Switzerland
26 Haverkate, F., Teulings, A.G. and Van Deenen, L.L.M. (1965) Kon. Ned. Akad. Wet. 68, 154—159
27 Boucher, F., Van der Rest, M. and Gingras, G. (1977) Biochim. Biophys. Acta 461, 339—357
28 Noel, H., Van der Rest, M. and Gingras, G. (1972) Biochim. Biophys. Acta 275, 219—230

29 Steiner, L.A., Okamura, M.Y., Lopes, A.D., Moskowitz, E. and Feher, G. (1974) Biochemistry 13, 1403–1410
30 Morrison, L., Runquist, J. and Loach, P. (1977) Photochem. Photobiol. 25, 73–84
31 Jacobs, E.E., Kirkpatrick, F.H. and Andrews, E.C. (1966) Biochem. Biophys. Res. Commun. 25, 96–104
32 Engelman, D.M., Terry, T.M. and Morowitz, H.J. (1967) Biochim. Biophys. Acta 135, 381–390
33 Wildenauer, D. and Khorana, G. (1977) Biochim. Biophys. Acta 466, 315–324
34 Robinson, N.C. and Capaldi, R.A. (1977) Biochemistry 16, 375–381
35 Awasthi, Y.C., Chuang, T.F., Keenan, T.W. and Crane, F.L. (1971) Biochim. Biophys. Acta 226, 42–52
36 Zürrer, H., Snozzi, M., Hanselmann, K. and Bachofen, R. (1977) Biochim. Biophys. Acta 460, 273–279