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THIOCAPSA FLORIDANA; A CYTOLOGICAL, PHYSICAL AND CHEMICAL CHARACTERIZATION

II. PHYSICAL AND CHEMICAL CHARACTERISTICS OF ISOLATED AND RECONSTITUTED CHROMATOPHORES

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SUMMARY

Equilibrium sucrose density gradient centrifugation of chromatophore membranes from *Thiocapsa floridana* resulted in the formation of a single pigmented band, having a density of 1.14 g/cm^3.

The chromatophore membranes contain 49 % protein, 47 % lipid and 3 % chlorophyll. Small quantities of carbohydrate, but no nucleic acids were found.

Complete membrane solubilization resulted in the release of small membrane subunits. Dialysis of the solubilized preparation against buffer containing Mg^{2+}, resulted in the reassociation of the subunits into structures that resembled the original complex not only in morphology but also in their ability to catalyze photophosphorylation.

INTRODUCTION

Sedimentable, pigmented particles, termed "chromatophores" have been isolated from a number of photosynthetic bacteria[1,2] and have been extensively described[3,7].

An extensive study of the chromatophore fraction from *Rhodospirillum rubrum* was undertaken early by COHEN-BAZIRE AND KUNISAWA[8], who found that sucrose density gradient centrifugation of cell-free extracts resulted in the formation of two distinct bands, according to their positions in the gradient. Although the light fraction had a higher specific bacteriochlorophyll content both fractions were capable of catalyzing photophosphorylation.

In order to elucidate the lowest level of structural organization still capable of supporting photosynthesis, several investigators have undertaken the fractionation of photosynthetic membranes by a wide variety of physical and chemical means[1,4,8-22].

Some fractions obtained after the fractionation of photosynthetic membranes into their subunits are active in simple photo-chemical electron transfer reactions such as the photooxidation of reduced phenazine methosulfate in the presence of ubiquinone. However, few reconstitution studies of the solubilized subunits have been reported, and none of these have shown photophosphorylating activity.

In this paper we describe the physciochemical properties of chromatophore membranes from *Thiocapsa floridana*, the complete solubilization of these membranes into identifiable subunits, and the reassociation of the subunits into structures that morphologically and functionally resemble the original membrane complex.

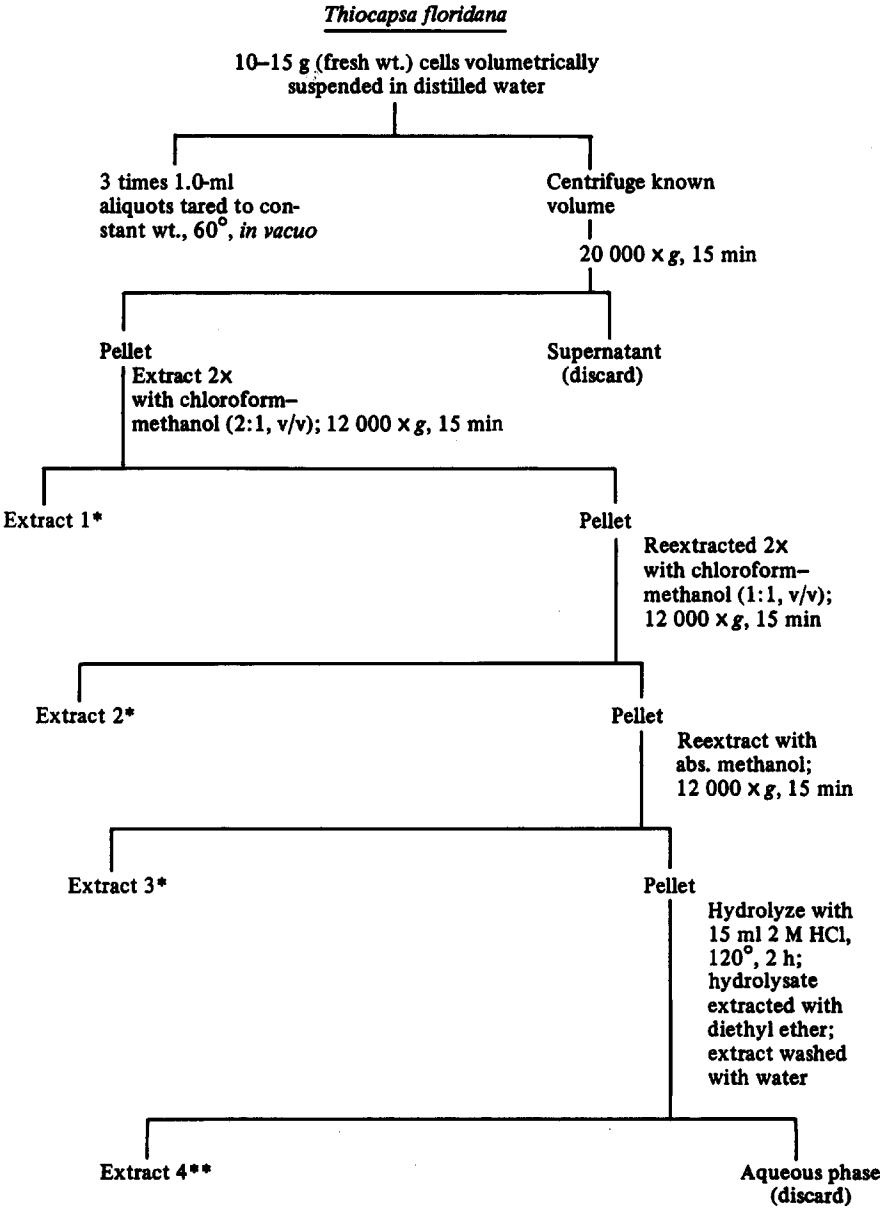


Fig. 1. Extraction of total lipids from *Thiocapsa floridana*. Extracts 1–3 combined as total extractable lipid (*). Extract 4 represents bound lipid fraction (**).

MATERIALS AND METHODS

T. floridana was grown at 30° in 500-ml screw capped bottles, containing either PFENNIG's medium[24], or our modified PFENNIG's medium as described previously[25].

Chemical assays

Bacteriochlorophyll a. Bacteriochlorophyll was extracted with acetone–methanol (7:2, v/v)[27], and quantitatively estimated as described by KETCHUM AND HOLT[9].

Protein. Protein content was determined by the method of LOWRY *et al.*[29].

Carbohydrate. The anthrone method of ASHWELL[29] was employed to determine total carbohydrate in subcellular fractions.

Total phosphorus. This was determined by the method of BARTLETT[30].

Lipid analysis: (*1*) *Total lipids and fatty acids.* Total lipids were extracted as outlined in Fig. 1. The pooled extractable lipids (Extracts 1–3) and the bound lipids (Extract 4) were purified on G-25 fine Sephadex columns[31]. Methyl esters of fatty acids were prepared by the method of KANESHIRO AND MARR[32], and the esterified residues were purified on Silica Gel G thin-layer plates using hexane–diethyl ether–acetic acid (70:30:2, by vol.) as the developing solvent[33]. Unsaturated fatty acids were hydrogenated by the method of POUKKA *et al.*[34]. Fatty acids were analyzed on a dual column (Perkin–Elmer, Model 880) gas chromatograph, equipped with temperature programmer and a differential flame ionization detector, employing 9 cm polar (diethylene glycol succinate; 12% on Chromasorb P) and nonpolar (Apiezon L; 15% on gaschrom P) copper columns. Chromatography on Apiezon L (Fig. 5) and quantitative conversion of the monoenes to their saturated analogues after complete hydrogenation was used to confirm the identity of fatty acids (Fig. 4). The percent composition of compounds present and quantitative estimation of peak areas were determined by the method of CARROLL[35].

(*2*) *Phospholipids.* Phospholipids were extracted as the total extractable lipids (Fig. 1). The different classes of polar lipids were isolated and fractionated by the method of VÁCZI *et al.*[36]. For two-dimensional thin-layer chromatography the method of SKIDMORE AND ENTENMAN[37] was employed. One dimensional chromatography was performed by the method of WAGNER *et al.*[38]. Glycolipids were isolated by the method of GARDNER[39] and lipid sugars were analyzed by the method of PASTUSKA[40] and

TABLE I

SPRAY REAGENTS USED FOR THIN-LAYER CHROMATOGRAPHY OF VARIOUS LIPID CLASSES FROM *T. floridana*

Lipid class	*Reagents or method of detection*
Phosphate containing	Acid molybdate
Amino group containing	Ninhydrin
Glycerol	Ammoniacal $AgNO_3$
Phosphatidic acid	Ammoniacal $AgNO_3$
Glycolipids	Anthrone or diphenylamine
Non-specific lipid detection	I_2 vapor
	Ethanolic phosphomolibdate
	Rhodamine 6 G

LEVIN *et al.*[41]. The identification of phospholipids was based on their comparative R_F values with known lipid standards and on their colorimetric reaction when sprayed with specific reagents (Table I, ref. 42).

Amino acids. Samples for amino acid analysis were hydrolyzed in 6 M HCl for 18 h at 110°, evaporated to dryness under a stream of N_2 and the residue suspended in distilled water. Quantitative amino acid analysis was performed in an automatic amino acid analyzer.

Solubilization and reassociation of chromatophores

Isolated membranes were resuspended to a concn. of 2–3 mg protein per ml in 0.01 M sodium dodecyl sulfate (in either 0.05 M Tris–HCl buffer, pH 7.4, containing 0.01 M 2-mercaptoethanol, or in β-buffer[23]). Incubation was for 4 h at 4° with constant stirring. The complete dissolution of the membranes was assumed by the absence of a pellet after centrifugation at 165000 × *g* for 1 h. The resulting solubilized membrane fraction was dialyzed against 0.02 M Tris–HCl buffer (pH 7.4) containing 0.01 M 2-mercaptoethanol.

For reassociation the solubilized membrane fraction was dialyzed against 4 × 1 l of 0.02 M Tris–HCl buffer (pH 7.4) (containing 0.02 M $MgCl_2$; 0.01 M 2-mercaptoethanol) for 24 h at 4° and then centrifuged at 165000 × *g* for 1 h. The pellet or reassociated membrane fraction was then washed twice with Tris–Mg^{2+} buffer.

Photophosphorylation

All buffers (Table II) and assay solutions for photophosphorylation were prepared just before use and were degassed *in vacuo* then sparged with purified helium for 1 h prior to use.

The assay system of GELLER AND LIPMANN[26] was used with the following modifications: to Warburg flasks were added 2 μmoles ascorbate, 300 μmoles sucrose at pH 7.3, and the sample which contained 5–10 μg bacteriochlorophyll. In a typical run, the sample to be tested was resuspended in double strength assay buffer (Table II) lacking ADP. One ml of sample was delivered into Warburg flasks, followed by 1 ml of

TABLE II

COMPOSITION OF BUFFERS AND ASSAY SOLUTIONS FOR PHOTOPHOSPHORYLATION

	Buffer solutions (molar concn.)			
	Isolation (pH 8.0)	*Solubilization (pH 7.5)*	*Reassociation (pH 7.5)*	*Assay (double strength) (pH 7.1)*
Tris–HCl	0.02	0.05	0.02	—
Ascorbate*	0.0005	—	0.005	0.0001
$MgSO_4$	0.01	—	—	—
$MgCl_2$	—	—	0.02	0.01
2-Mercaptoethanol	0.01	0.01	0.01	—
Sucrose	0.03	0.3	0.3	0.6
Potassium phosphate	—	—	—	0.04
ADP	—	—	—	0.02
Sodium dodecyl sulfate	—	0.01	—	—

* pH is adjusted to desired value after the addition of ascorbate.

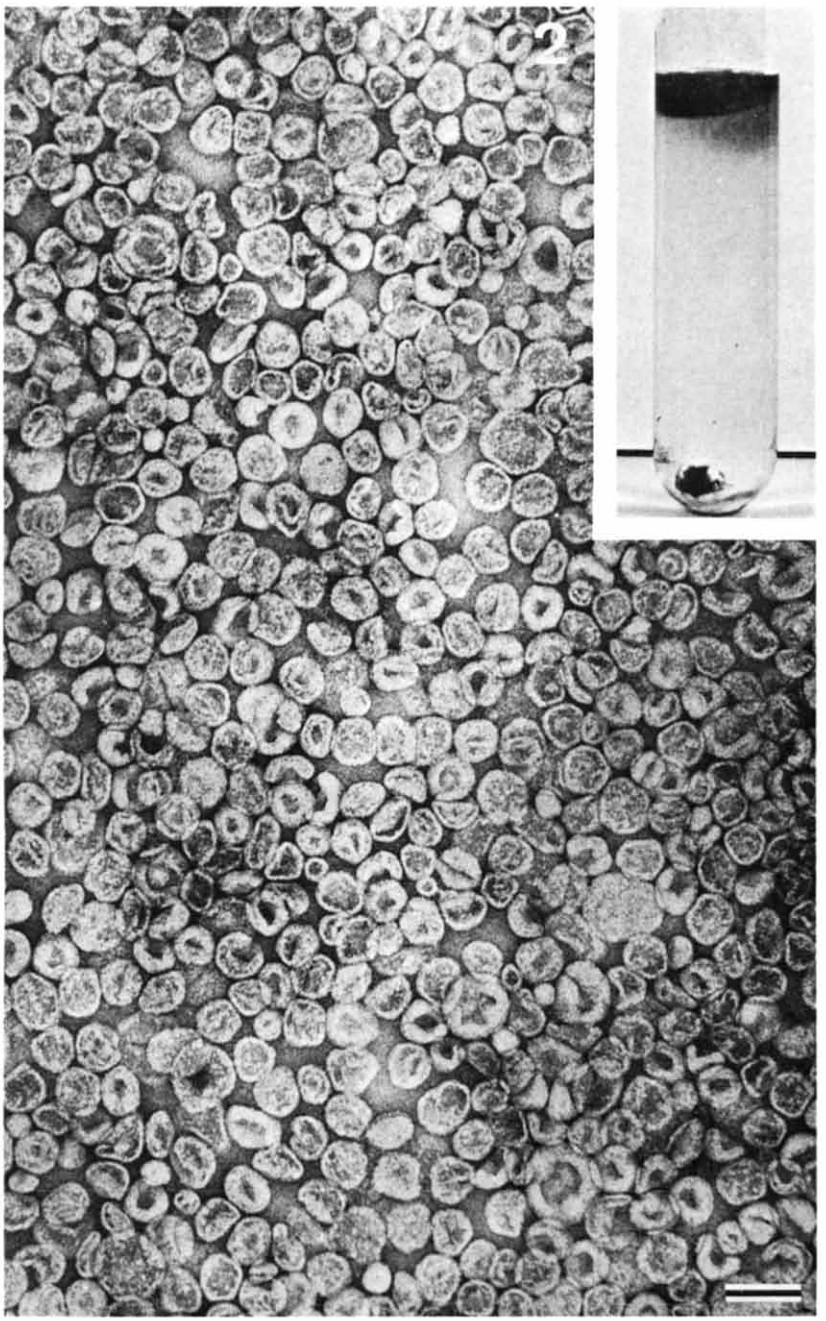


Fig. 2. Electron micrograph of negatively stained preparation of chromatophore membranes recovered from top of a RbCl isopycnic density gradient (insert). Note that the preparation is free from cell wall and ribosomal contamination. Stained with 1% phosphotungstic acid (pH 6.8). Bar represents 100 nm.

a double strength ADP solution. Warburg flasks were shaken at 120 cycles/min in a Gilson differential photosynthetic respirometer. The reaction vessels were kept anaerobic by constantly flushing the system with helium. A light intensity of 800 ft-candles incident on the vessels, sufficient to saturate the preparations with light, was used. The mixtures were shaken for periods of up to 30 min either in the dark or under constant illumination. The reaction was stopped by the addition of 1 ml of 30 % (v/v) perchloric acid; the suspension was left at 0° for 3 h in order to extract all ATP present, and then neutralized with 4 M KOH. The precipitate formed upon neutralization was removed by centrifugation and the ATP content of the supernate determined by the firefly luciferin-luciferase assay[43]. A Packard liquid scintillation spectrometer (Model 574), set for tritium counting, was employed. ATP content was determined from an ATP standard curve.

Physical methods

In vivo light absorption spectra. A Beckman DK-1A, Automatic Recording Spectrophotometer was used to record all absorption spectra.

Analytical ultracentrifugation. Analytical ultracentrifugation was performed using a Spinco Model E ultracentrifuge. Both Schlieren and absorption optics were employed. The samples were suspended either in distilled water or in 10 mM Tris-HCl buffer (pH 7.5). Isolated and reassociated chromatophore membranes were centrifuged at 18000 rev./min and solubilized membranes at 52000 rev./min. Temperature was maintained at 20°. Partial specific volume, and intrinsic viscosity were measured as described by SCHACHMAN[44].

Acrylamide gel electrophoresis. Electrophoresis in polyacrylamide gels was performed by the method of DAVIS[45], using a Canalco 66 disc electrophoresis apparatus as described by KETCHUM AND HOLT[9].

RESULTS

Purification of chromatophore membranes

Centrifugation in 30 % RbCl resulted in a sharp separation of the chromatophore membranes obtained from *T. floridana* (Fig. 2); resulting in an approximate 3-fold increase in the specific bacteriochlorophyll content (Table III). Electron microscopic examination of the banded material revealed the presence of spherical membranes with an average diameter of 800 Å (Fig. 2).

Centrifugation of the RbCl purified membranes through linear sucrose density gradients resulted in the separation of a single pigmented band, having a density of 1.14 g/cm^3 (Fig. 3A).

TABLE III

PURIFICATION OF THE CHROMATOPHORE MEMBRANES FROM *Thiocapsa floridana*

Preparation	*Specific bacteriochlorophyll content (μg bacteriochlorophyll/100 μg protein)*
Crude broken	230
13 000 rev./min supernate	240
Purified membranes	680

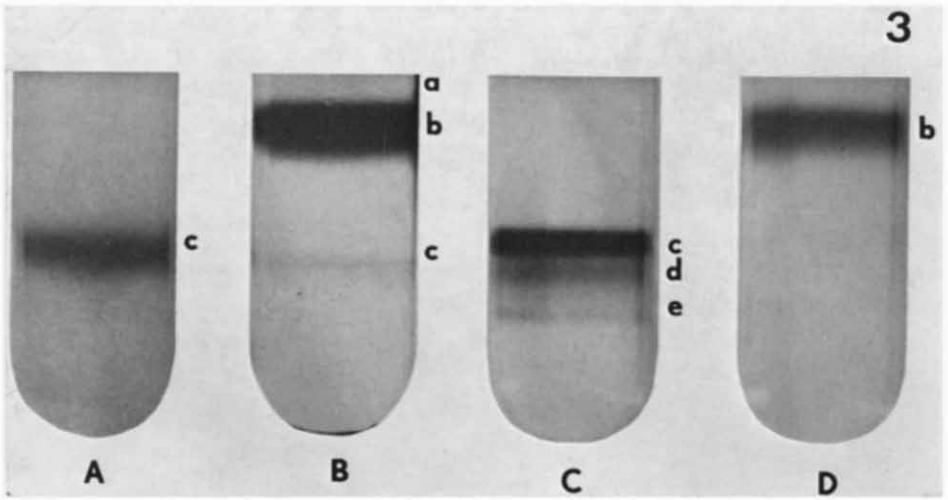


Fig. 3. Photographs of sucrose density gradients, showing the distribution of untreated chromatophores (A); solubilized chromatophores (B); reassociated chromatophores (C); and solubilized chromatophores that were extensively washed with Mg^{2+}- less buffer prior to solubilization. Small letters correspond to density distribution of a = 1.06 g/ml; b = 1.10 g/ml; c = 1.14 g/ml; d = 1.15 g/ml; e = 1.17 g/ml.

The purified lipid content of whole cells of *T. floridana* was about 15 % of the dry weight, while the isolated chromatophore membranes contained as much as 47 % pure lipid (Table IV). The major lipids of both were lysophosphatidylethanolamine, phosphatidylethanolamine, phosphatidyl glycerol (cardiolipin) and glycolipid. Upon acid hydrolysis the glycolipid moiety yielded glucose and rhamnose.

TABLE IV

TOTAL LIPID COMPOSITION OF *T. floridana* (%)

Lipid class	*Whole cells*		*Isolated chromatophore*		*Reassociated chromatophore*	
	Crude lipid	*Pure lipid*	*Crude lipid*	*Pure lipid*	*Crude lipid*	*Pure lipid*
Extractable	21.46	13.10	50.50	45.45	42.91	34.91
Bound	1.62	1.31	6.02	2.02	2.73	0.87
Total	23.08	14.41	56.56	47.47	45.64	35.78

The major fatty acids both of whole cells and chromatophore membranes were identified as palmitate, palmitoleate, and oleate, representing 20, 28 and 45 %, respectively (Fig. 5).

Although almost one-half the dry weight of *T. floridana* whole cells was found to be carbohydrate, it constituted less than 2 % of the dry weight of isolated chromatophore membranes indicating, most probably, that it was not a structural or functional component of the membranes.

Amino acid analysis of isolated chromatophore membranes from *T. floridana*

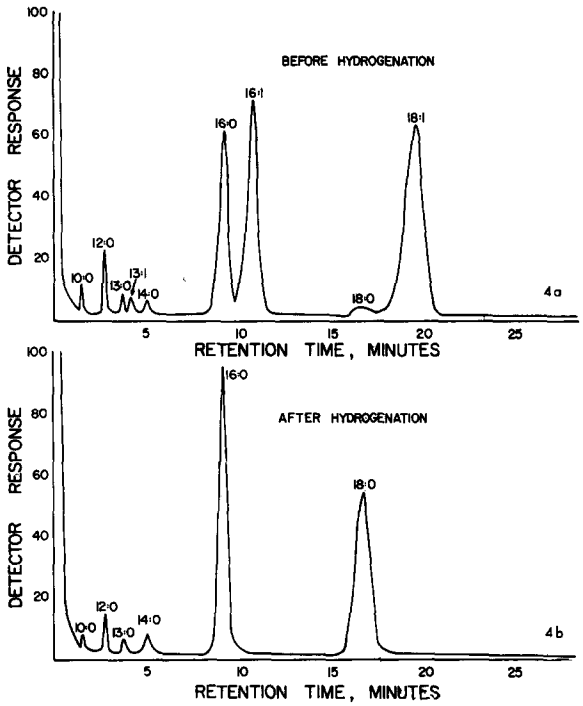


Fig. 4. Gas–liquid chromatograms of fatty acid methyl esters extracted from *T. floridana* and analyzed on a 9 foot diethylene glycol succinate (DEGS) column, before (a) and after (b) catalytic hydrogenation. Temp., 190°.

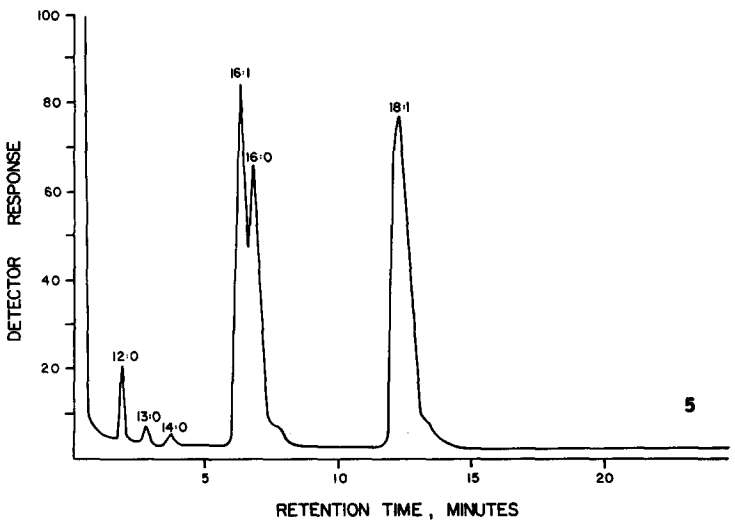


Fig. 5. Gas–liquid chromatogram of fatty acid methyl esters extracted from *T. floridana* and analyzed on a 5-ft Apiezon L column. Temp., 250°.

were compared with the amino acid composition of two other photosynthetic bacteria (Table V). A close agreement between the amino acid composition of *T. floridana*, *Rps. spheroides* and *R. rubrum* was noteworthy. Using the "polar" and "apolar" amino acid groupings of HATCH[46] we found that the chromatophore proteins from these organisms contained a relatively large amount of non-polar amino acid residues. The ratio of polar to apolar amino acid residues in *T. floridana* chromatophores was 1.18,

TABLE V

AMINO ACID COMPOSITION OF CHROMATOPHORE MEMBRANES FROM *T. floridana*, *Rps. spheroides* *, *R. rubrum* *

Constituent	*Amino acid* (%)		
	T. floridana	*Rps. spheroides* *	*R. rubrum* *
Ala	14.69	18.17	11.31
Arg	2.57	3.33	4.43
Asp	7.27	6.67	8.56
Cys**	0.65	—	—
Glu	10.23	10.83	10.70
Gly	7.15	9.33	9.94
His	2.77	2.00	1.99
Ile	5.44	2.50	5.35
Leu	10.58	10.00	9.63
Lys	3.99	4.50	4.28
Met	1.69	2.67	1.99
Phe	6.77	3.67	6.12
Pro	6.73	6.33	3.97
Ser	4.00	5.50	5.96
Thr	5.13	6.17	6.57
Tyr	2.37	2.50	1.83
Val	7.97	5.83	7.34

* Ref. 47.
** Cysteine and tryptophan were not determined.

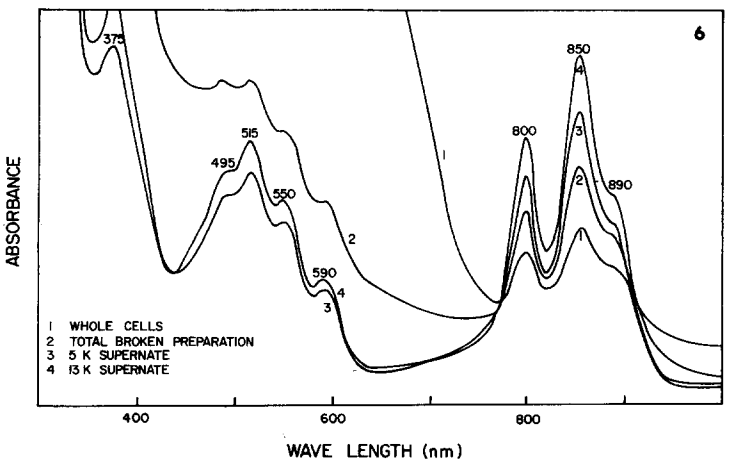


Fig. 6. DK 1-A absorption spectra of various fractions obtained in the purification of chromatophore membranes from *T. floridana* in Tris–HCl buffer.

the same as the value obtained for *R. rubrum* chromatophores[47]. The significance of the high content of alanine was not immediately clear, although alanine seemed to be the major amino acid present not only in the chromatophores of photosynthetic bacteria[48], but also in the chloroplasts of higher plants[49].

Absorption spectra of whole cells and of isolated chromatophore membranes from *T. floridana* are shown on Fig. 6. Above 700 nm the isolated membranes and whole cells showed identical absorption spectra, indicating that the pigment complex remained intact during purification. Absorption maxima at 375, 590, 900, 850 nm, as well as a shoulder at 890 nm are characteristic of bacteriochlorophyll *a*. The carotenoid absorption region of the spectrum, between 435 and 630 nm, indicated that the carotenoids of this organism belong to the spirilloxanthin series[49]. The absorption spectra of acetone–methanol (7:2, v/v) extracts from *T. floridana* showed the expected single, near-infrared absorption band at 750 nm (Fig. 6).

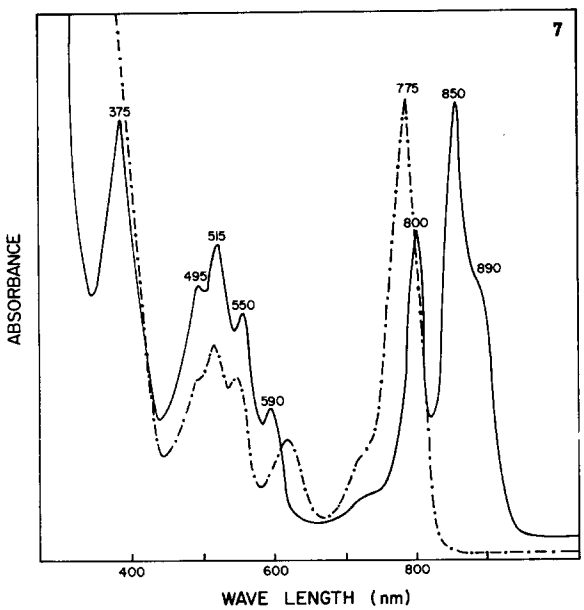


Fig. 7. DK 1-A absorption spectra of isolated chromatophore membranes in Tris–HCl buffer (———) and an acetone–methanol (7:2, v/v) extract (—·—).

Analytical ultracentrifugation of purified monodispersed chromatophore membranes, extrapolated to infinite sample dilution, gave a sedimentation constant of 150 S (Fig. 8), similar to that reported for the "light" fraction of *Rps. spheroides*[2], and mol. wt. of $12.6 \cdot 10^6$, similar to the value obtained by BERGERON[4] for chromatophore membranes of *Chromatium*.

The calculated radius[44] of the spherical particle was 272 Å while the value was 325 Å as determined by electron microscopy of negatively stained preparations.

Solubilization and reassociation of chromatophores

Isolated membranes of *T. floridana* were solubilized with 10 mM sodium dodecyl sulfate in β-buffer. Centrifugation at 165000 × *g* for 1 h, resulted in a red supernatant.

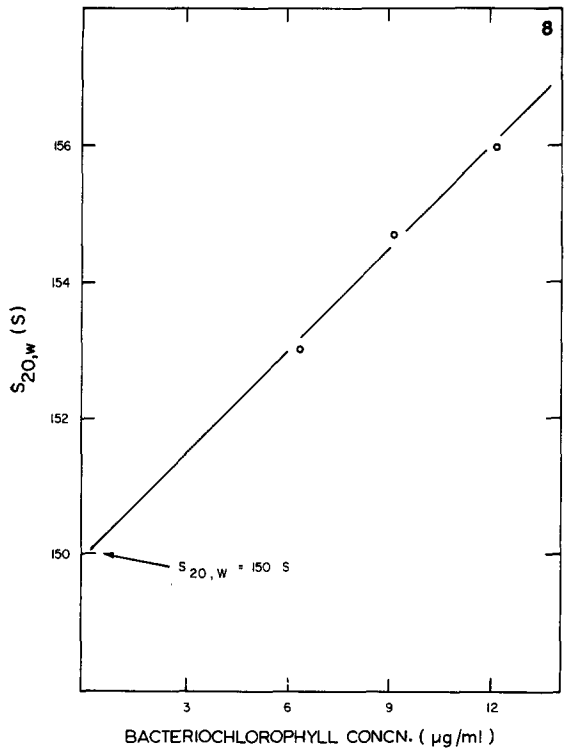


Fig. 8. Extrapolation of the sedimentation constant of isolated chromatophore membranes to infinite dilution at 20°. Rotor speed 18000 rev./min.

Dialysis of the supernatant against β-buffer containing Mg^{2+}, and subsequent centrifugation resulted in the sedimentation of all the pigmented material. Electron microscopic examination revealed the presence of small sheets or vesicles, bearing little resemblance to the original membrane fraction (Fig. 9a). When NaCl was omitted from the solubilization and reassociation buffers, the resulting reaggregated membranes more closely resembled the original chromatophore membranes not only in size but also in gross morphology (Fig. 9b). This suggests that monovalent cations might interfere with the proper re-alignment of the membrane subunits. The most satisfactory results, however, were obtained when sucrose was incorporated into the solubilization and reassociation buffers, or when the solubilized material was banded on a sucrose density gradient prior to reassociation (Figs. 10c, d).

Solubilized membrane subunits were approx. 60 Å in diameter and each was made up of five spherical subunits with an average diameter of approx. 20 Å (Fig. 10b).

Equilibrium sucrose density gradient centrifugation of the solubilized material resulted in one major pigmented band (Fig. 3b) at a density of 1.10 g/cm^3; some yellowish material (found to be glycolipid, ρ = 1.06 g/cm^3) remained at the top of the gradient. Dialysis of the pigmented band against buffer containing Mg^{2+} (Table II), resulted in reconstitution of the subunits to chromatophore-like membranes (Fig. 3c). The reconstitution was Mg^{2+} dependent. Sucrose density gradient centrifugation of this reassociated material resulted in the formation of one major pigmented band, at a

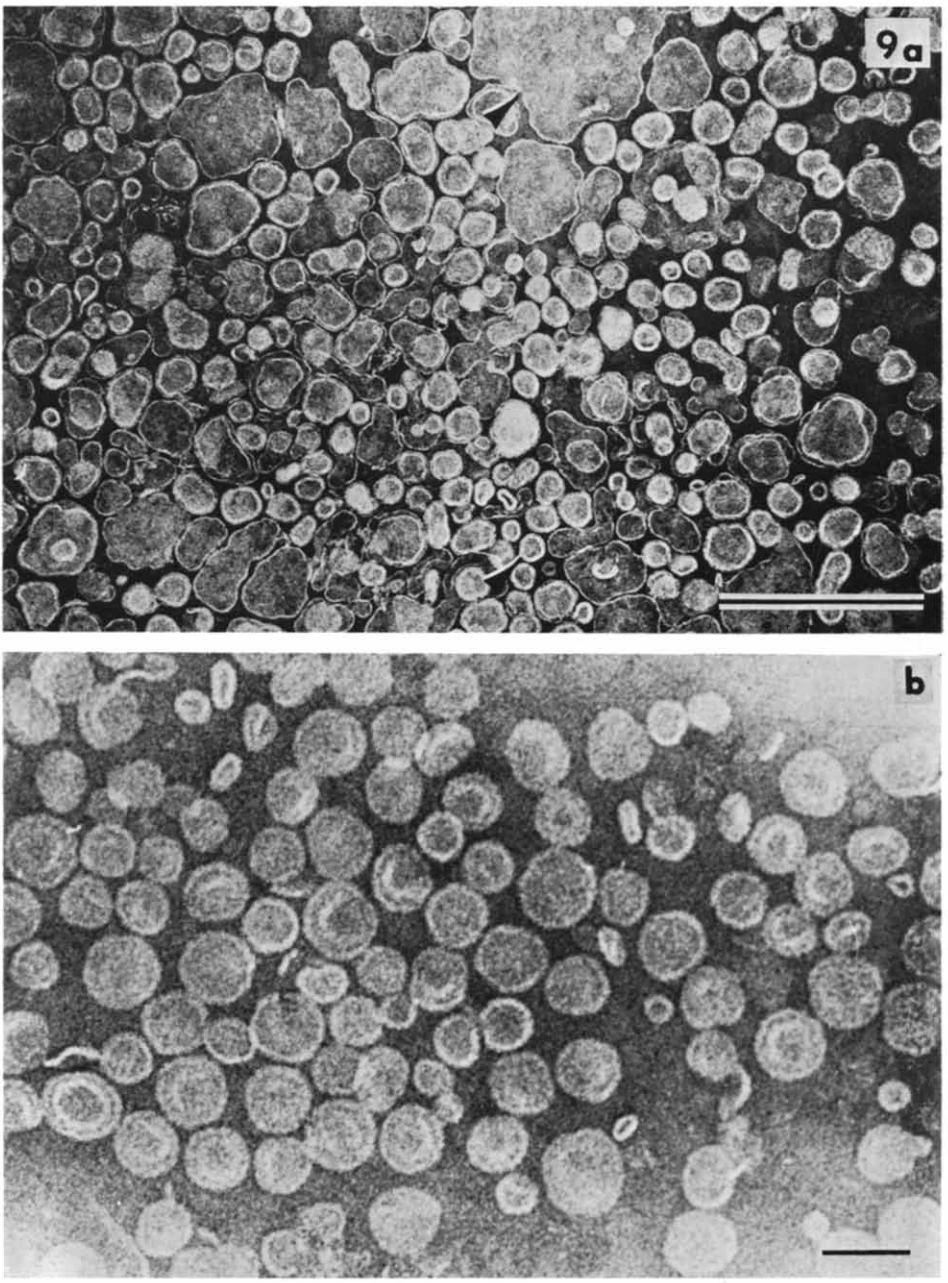
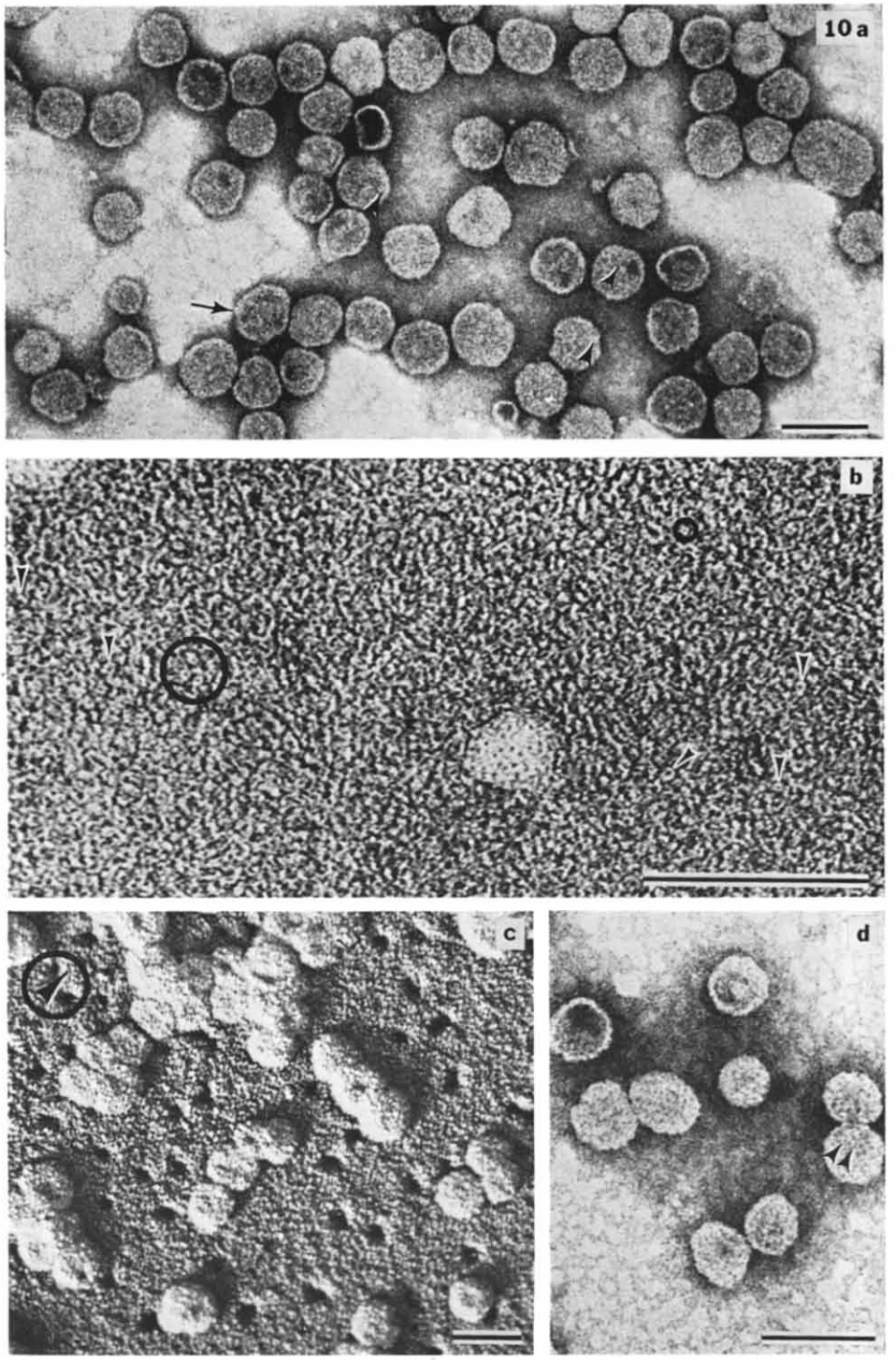


Fig. 9. Electron micrographs of negatively stained (1 % phosphotungstic acid; pH 6.8) preparations of reassociated membranes. (a) reassociated in the presence of NaCl; (b) reassociated without NaCl. Bars represent: (a) 500 nm, (b) 100 nm.


10 a
b
c
d

density of 1.14 g/cm^3, identical to the density of the original preparation. Two minor pigmented bands, having densities of 1.15 and 1.17 g/cm^3, were also obtained (Fig. 3c). These very minor bands were not examined further.

The lipid content of the reassociated membranes was found to be 36 % of the dry weight, a loss of approx. 12 % as compared to the lipid content of the original chromatophore membranes. The major portion of this lost lipid was glycolipid.

The chlorophyll, protein, and carbohydrate content of the reassociated membranes were quite similar to the values obtained for the original chromatophore preparation (Table VI).

TABLE VI

CHEMICAL COMPOSITION OF ISOLATED CHROMATOPHORES AND REASSOCIATED MEMBRANES FROM *T. floridana*

n.d. stands for not detectable.

Component	% *dry weight*	
	Chromatophores	*Reassociated chromatophores*
Protein	49	46
Lipid	47	36
Chlorophyll	3	2.5
Carbohydrate	1.8	1.5
DNA	n.d.	n.d.

The only noticeable change in absorption spectrum, caused by sodium dodecyl sulfate solubilization was the disappearance of the 890 nm shoulder (Fig. 11). Similar detergent effect on the long wavelength bacteriochlorophyll center of other photosynthetic bacteria is well documented[50]. Removal of the detergent, and reassociation of the membrane subunits to chromatophore-like structures did not result in the reconstitution of the B 890 center.

In the analytical ultracentrifuge the solubilized membranes demonstrated a single, symmetrical Schlieren peak and a sedimentation constant of 3.3–3.5 S.

The electrophoretic mobility and structural homogeneity of the sodium dodecyl sulfate solubilized membrane subunits were determined by polyacrylamide gel electrophoresis. Only one fast-moving band which migrated just behind the indicator stain (bromo phenol blue) was found. Since identical results were obtained when sodium dodecyl sulfate impregnated gels were used, we can conclude that the solubilized preparation consisted of homogeneous protein subunits.

Photophosphorylation

Crude cell-free extracts, isolated and purified chromatophore membranes, and reassociated membranes from *T. floridana* exhibited a light-dependent phosphory-

Fig. 10. Electron micrographs of (a) intact chromatophore membranes (control); (b) solubilized chromatophore membranes; (c, d) reassociated chromatophore membranes. Notice the presence of membrane subunits (arrows), having an approx. diameter of 6 nm, and their spherical substructure with an average diameter of 2 nm. (c) shadowed 15°, Pt-C; (d) negative stain 1 % phosphotungstic acid (pH 6.8).

lation of ADP to ATP. Cell-free extracts esterified ADP at rates of up to 170 μmoles/h per mg bacteriochlorophyll (Fig. 13) while isolated chromatophore membranes had low and variable photophosphorylative activities, unless they were isolated in the presence of ascorbate (Table II). Even under these conditions the purified chromatophore

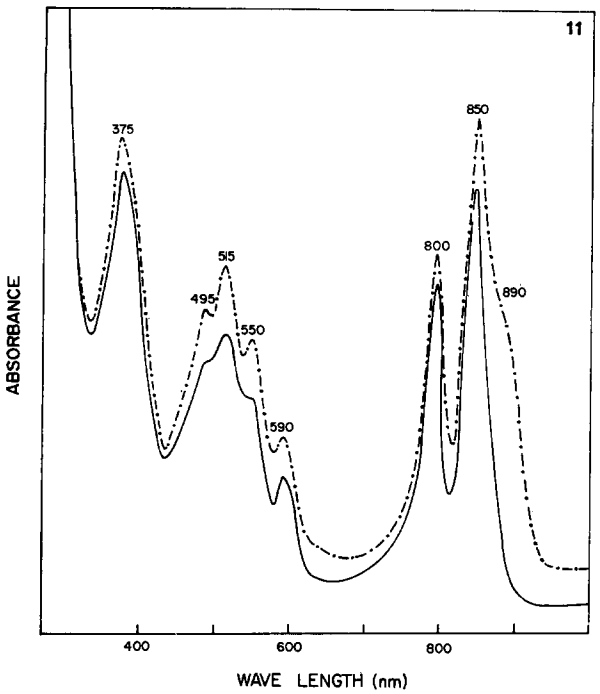


Fig. 11. DK 1–A absorption spectra of intact (—·—) and reassociated (———) chromatophore membranes from *T. floridana* in Tris–HCl buffer.

membranes had approx. 50 % less activity than cell-free extracts, (90 μmoles ADP/h per mg bacteriochlorophyll, Fig. 13).

Reconstituted membranes that were solubilized and reassociated under the same reduced conditions, esterified 54 μmoles of ADP/h per mg bacteriochlorophyll, which represented the reconstitution of approx. 60 % of the photophosphorylative ability of the original isolated chromatophore membranes (Fig. 13).

Solubilized membrane subunits did not display any photophosphorylative ability.

DISCUSSION

The sedimentation of chromatophore membranes from *T. floridana* in a sucrose density gradient resulted in the formation of only a single pigmented band at a density of 1.14 g/cm^3. The absence of any "heavy" membrane suggests that in this organism there may be a membrane continuum which includes the peripheral (cytoplasmic) membrane rather than the two different types of membrane as has been demonstrated for *R. rubrum*[9].

Little work has been done to elucidate the macromolecular architecture of the photosynthetic membranes from the purple-sulfur bacteria, or for that matter bacterial phototrophs in general. One approach to establishing the way in which molecular complexes interact to establish the chromatophore (or photosynthetic membrane) is

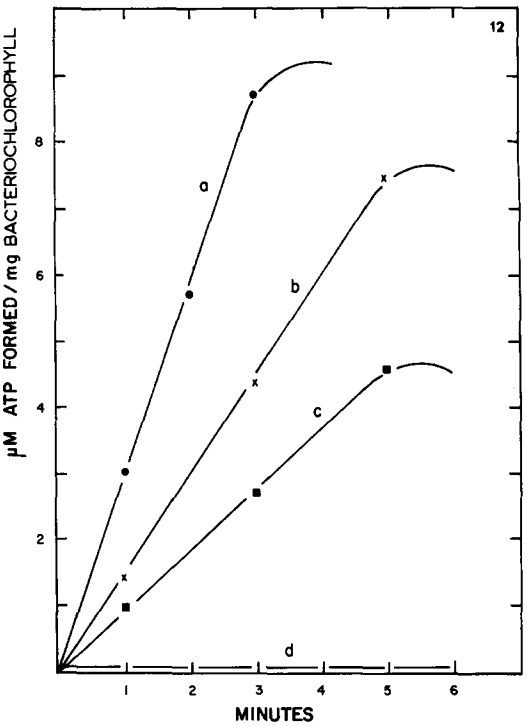


Fig. 12. Rates of photophosphorylation as function of time; (a) using cell-free extracts; (b) isolated chromatophore membranes; (c) reassociated chromatophore membranes; and (d) solubilized chromatophore preparation.

to subject the purified chromatophore membrane to (*e.g.*) anionic detergent solubilization and examine the resultant solubilized material. Ultimately this material must be reassociated into structures that resemble the starting material not only in morphology but also in their ability to catalyze photophosphorylation.

Complete chromatophore membrane solubilization from *T. floridana* resulted in the release of small homogeneous pentagonal subunits (3.3–3.5 S). Similar to the values reported for membrane subunits of *Mycoplasma laidlaui*[23] and *Micrococcus lysodeikticus*[51].

Sucrose density gradient centrifugation of this material resulted in the separation of lipid from the protein–pigment complex. However, not all of the membrane lipid is separated under these conditions; glycolipids are released preferentially. Whether the unreleased phospholipids form micellar aggregates with the protein–pigment subunits is not clear. The chromatophore membranes from *T. floridana* appeared as spheres after negative staining, with an average radius of 325 Å. Calculation of the radius of the membrane from the results of analytical ultracentrifugation

indicated a radius of 272 Å. These values are in excellent agreement with those expected for the existence of a hydrated sphere. Even though the particles were exposed to the perturbations of electron microsocopy (staining, drying, vacuum, electron bombardment) there appeared to be very little of the distortion and heterogeneity reported for *Chromatium* chromatophores[52].

The absorption spectrum of sodium dodecyl sulfate-solubilized membranes reveals that the shoulder at 890 nm is lost during solubilization, indicating the probable sensitivity of this chlorophyll component to the action of the detergent, or photo or chemical oxidation. The loss is apparently irreversible since reconstituted membranes do not display the 890 nm shoulder. The studies by BRIL[50] of the spectral properties of detergent-treated chromatophores from *R. rubrum*, *Chromatium* and *Rsp. spheroides* indicated that even low detergent concentrations blocked the energy flow between the 850 and 890 nm forms of chlorophyll. This was interpreted as showing that the 890 nm shoulder actually represents an aggregated state of the bacteriochlorophyll–protein complex which is disrupted by detergent action.

Since the reassociation of the membrane subunits into recognizable chromatophore membranes does not require the incorporation of all the solubilized membrane lipid, the structural integrity of the membrane does not seem dependent upon all of its normal chemical constituents. This implies that certain lipids within the chromatophore membranes of *T. floridana* may play a functional role(s) rather being solely involved in a structural capacity.

Examination of the lipid and phospholipid content revealed a pattern similar to that reported for other photosynthetic bacteria[53–57]. Of significance was the finding that cardiolipin (phosphatidyl glycerol) and the glucosyl and rhamnosyl diglycerides constituted the major phospholipids found in the chromatophore membranes from *T. floridana*. This finding is thus compatible with the suggestion by CONSTANTOPOULOS AND BLOCH[54] that the glycolipids may play a role in photosynthesis, although they were unable to detect any glycolipids in *Rsp. palustris*.

Of significant note is the demonstration that reassociated membranes were capable of performing respectable rates of photophosphorylation when compared to the rates for cell-free extracts. Not only are the rates of the reassociated chromatophore membranes significant (60 % of the starting chromatophore membranes), but they are comparable to other systems[5,7,3].

Fruitful approaches for the biochemical and morphological clarification of biological membranes have come from solubilization and reassociation procedures such as these. Solubilization of membranes into subunits, characterization of the subunits and reassembly of subunits into structures resembling the original preparation, may prove to be the choice approach for the elucidation of the functional and structural organization of biological membranes.

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