Changes in the Acyl Lipid Composition of Photosynthetic Bacteria Grown under Photosynthetic and Non-Photosynthetic Conditions

By Nicholas J. RUSSELL and John L. HARWOOD

Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.

(Received 8 November 1978)

The acyl lipids and their constituent fatty acids were studied in the photosynthetic bacteria *Rhodospirillum rubrum*, *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides*, which were grown under photosynthetic and non-photosynthetic conditions. The major lipids were found to be phosphatidylethanolamine, phosphatidylglycerol and cardiolipin in each bacterium. The two *Rhodopseudomonas* species also contained significant quantities of phosphatidylcholine. Other acyl lipids accounted for less than 10% of the total. On changing growth conditions from non-photosynthetic to photosynthetic a large increase in the relative proportion of phosphatidylglycerol was seen at the expense of phosphatidylethanolamine. In *Rhodospirillum rubrum* the fatty acids of the major phospholipids showed an increase in the proportion of palmitate and stearate and a decrease in palmitoleate and vaccenate on changing growth conditions to photosynthetic. In contrast, the exceptionally high levels (>80%) of vaccenate in individual phospholipids of *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides* were unaffected by changing growth conditions to photosynthetic. Analysis of the lipids of chromatophores, isolated from the three bacteria, showed that these preparations were enriched in phosphatidylglycerol. The large increase in this phospholipid, seen during growth under photosynthetic conditions, appeared, therefore, to be due to a proliferation of chromatophore membranes. Possible roles for acyl lipids in the formation and function of the photosynthetic apparatus of bacteria are discussed.

Photosynthetic bacteria, such as members of the Rhodospirillaceae, provide a useful system in which to study the role of lipids in photosynthesis, because they can be cultured under chemoheterotrophic or phototrophic conditions. Moreover, the transition from non-photosynthetic to photosynthetic states, and vice versa, can be controlled easily. The photosynthetic apparatus is present in discrete membrane fractions within the cell, known as chromatophores, which can be isolated free of cytoplasmic and other membrane contaminants (Oelze & Drews, 1972; Drews, 1978).

There is a large number of studies on the synthesis of photosynthetic bacterial membranes during the switch from dark to light conditions, but these have concentrated almost exclusively on the co-ordination of protein and pigment synthesis (e.g. Lampe & Drews, 1972; Kerber *et al.*, 1972; Clayton & Haselkorn, 1972; Stanier, 1963; Yamashita & Kamen, 1969; Oelze & Drews 1972; Gibson *et al.*, 1972). Thus in contrast to the situation in higher plants, where we know in considerable detail the nature of chloroplast lipids (cf. Mazliak, 1977), there is much less information regarding the types and composition of acyl lipids of photosynthetic bacteria. In particular it is not clear whether photosynthetic bacteria, like green plants (Harwood, 1979), contain acyl lipids

that are characteristic of photosynthetic membranes. There is some evidence that in *Rhodopseudomonas* (*Rhps.*) *sphaeroides* and *Rhodospirillum* (*Rhps.*) *capsulata* the phospholipid composition of photosynthetic membranes differs from that of non-photosynthetic membranes (Lascelles & Szilagyi, 1965; Steiner *et al.*, 1970).

The present paper provides detailed information about the acyl lipid and fatty acid composition of three photosynthetic bacteria grown under photosynthetic or non-photosynthetic conditions, as well as data on isolated chromatophores. The results show that there are certain differences in the acyl lipid and fatty acid compositions of photosynthetic membranes compared with non-photosynthetic membranes. The present study is a preliminary to one on the role of specific lipids in bacterial photosynthesis.

Experimental

Materials

Fatty acid, triacylglycerol, and phospholipid standards were obtained from Sigma (London) Chemical Co. Ltd., Kingston upon Thames, Surrey KT2 7BG, U.K. Fatty acids were also purchased from Nu-Chek Prep., P.O. Box 172, Elysian, MN

56028, U.S.A. Lipid standards were found to be homogeneous by t.l.c. and the fatty acids were found to be homogeneous by g.l.c. Phosphatidylinositol, obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K., was purified by t.l.c. Phosphatidylglycerol and diacylsulphoquinovosylglycerol were isolated from broad-bean (*Vicia faba*) leaves as previously described (Harwood & James, 1975; Burns *et al.*, 1977). Other reagents were of the highest available grades and were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K., and from Sigma.

Cultural conditions

Rhodospseudomonas sphaeroides (N.C.I.B. 8253), *Rhodospseudomonas capsulata* (N.C.I.B. 8254) and *Rhodospirillum rubrum* (N.C.I.B. 8255), obtained from the Torrey Research Station, Aberdeen, Scotland, U.K., were grown photosynthetically and non-photosynthetically in the 'S medium' of Lascelles (1956). Bacteria were maintained in stab culture on S medium containing 2% (w/v) agar.

Bacteria were grown photosynthetically in screw-cap medical flat bottles filled with S medium, and incubated at 30°C in a constant-temperature room with a light intensity of approx. 2000 lx provided by 60 W light-bulbs. Stab cultures were used to inoculate medical flat bottles, which were incubated until the end of exponential growth (48–60 h), when they were used to inoculate fresh bottles, which were incubated for 24 h, which corresponded to the end of exponential growth, before harvesting bacteria.

Bacteria were grown non-photosynthetically at 30°C in conical flasks covered with aluminium foil and plugged lightly with cotton wool. The flasks were shaken at 120 rev./min in a Gallenkamp IH460 orbital incubator. Stab cultures were used to inoculate 50 ml of S medium in 250 ml conical flasks, which were incubated until the end of exponential growth (approx. 48 h), when they were used to inoculate fresh flasks, which were incubated for 24 h, which corresponded to the end of exponential growth, before harvesting bacteria.

Lipid extraction and analysis

Bacteria were harvested by centrifuging cultures at 10000g (r_{av} 7.2 cm) for 10 min at 15°C. Cell pellets were resuspended in methanol (0.5 ml of methanol per mg dry wt. of bacteria) and an equal volume of chloroform was added. The chloroform/methanol suspension was sonicated for 10 min by using an ultrasonic cleaning bath (model ME4.6; Mettler Electronics Corp., Anaheim, CA, U.S.A.) and left overnight at room temperature. The lipid extract was filtered by using a glass sinter (porosity 3) and washed as described by Garbus *et al.* (1963). The

purified lipid extract was washed with synthetic upper phase (Garbus *et al.*, 1963) before evaporating to dryness, and redissolving in a small volume of chloroform/methanol (2:1, v/v). Solvents contained 0.001% 2,6-di-*t*-butyl-*p*-cresol to prevent oxidation.

Lipids were separated by t.l.c. on silica-gel G (E. Merck, Darmstadt, Germany) by using chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) or chloroform/methanol/acetone/acetic acid/water (5:1:2:1:0.5, by vol.). The positions of lipid bands were revealed by spraying with aqueous 0.001% Rhodamine 6G. Lipids were provisionally identified by R_F values, by co-chromatography with standards and by differential colour reactions with phosphate, ninhydrin, sulphuric acid and Dragendorff sprays. Phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine were also eluted from t.l.c. plates with chloroform/methanol/acetic acid (200:100:1, by vol.) and were identified after paper chromatography of their deacylated derivatives (Dawson, 1960) and acid hydrolysates (Harwood & Stumpf, 1970). Amounts of acyl lipids were determined from their fatty acid content. Results for phospholipids were confirmed by phosphate analysis.

Fatty acid components were analysed by g.l.c. after transesterification with H_2SO_4 /methanol (2.5%, v/v). An internal standard of pentadecanoic acid was added for quantification. Separations were achieved in 15% (w/v) ethylene glycol succinate silicone (EGSS-X) on Chromosorb W AW (80–100 mesh; Supelco, Bellefont, PA 16823, U.S.A.) at 190°C. Glass columns 2 m × 5 mm (int.diam.) were used, and analysis was with a Perkin-Elmer F11 or Perkin-Elmer F33 gas chromatograph connected to a Varian CDS 111 integrator. Fatty acids were provisionally identified by co-chromatography with authentic standards. Confirmation was provided by g.l.c.-mass spectrometry with a Finnigan G.C/El CI mass-spectrometer system or by direct insertion of purified fatty acids into a Varian CHS D mass spectrometer with accelerating voltage of 3 kV, filament emission of 300 μ A and multiplier voltage of 1.9 kV.

The position of the double bond in monoenoic fatty acids was determined by using the $KMnO_4$ oxidation procedure of Downing & Greene (1968). Monoenoic fatty acids were isolated by t.l.c. of fatty acid methyl esters on silica-gel H (Merck) containing $AgNO_3$ (12.5%, w/w) by using light petroleum (b.p. 60–80°C)/diethyl ether (9:1, v/v). $KMnO_4$ -oxidation products were separated, and the dicarboxylic acids analysed by g.l.c. as described by Russell (1978).

Preparation of chromatophores

Chromatophores were prepared from the three bacterial species by a modification of the method of

Niederman *et al.* (1976). The cell pellet from 200 ml of culture was washed with 1 mM-Tris/HCl, pH 7.5, and finally resuspended in 25 ml of the same buffer containing 10 mM-MgCl₂, 250 µg of ribonuclease and 250 µg of deoxyribonuclease. Bacterial cells were disrupted by passing the suspension three times through a French pressure cell at 1.38 GPa. The broken cell suspension was centrifuged for 10 min at 10000g (r_{av} , 5.35 cm). The supernatant, containing the chromatophores, was extracted and washed by using the method of Garbus *et al.* (1963).

Results

Initial experiments were directed to finding, firstly, suitable chromatographic systems for complete separation of constituent acyl lipids and their individual fatty acids and, secondly, their identification. Details of the procedure for lipid identification are given in the Experimental section. All the major components of each of the three bacteria were identified.

In Table 1 the acyl lipid compositions of cells of *Rhsp. rubrum*, *Rhps. capsulata* and *Rhps. sphaeroides* are shown. The bacteria were grown under photosynthetic and non-photosynthetic conditions and analyses for both conditions are given. The major lipids for *Rhsp. rubrum* were found to be phosphatidylethanolamine, phosphatidylglycerol and cardiolipin. Small amounts of phosphatidylcholine were found, but the quantities of other lipid types were very small. On changing growth conditions from non-photosynthetic to photosynthetic, a significant change in lipid composition was seen, with a decline in the relative proportion of phosphatidylethanolamine and an increase in phosphatidylglycerol (Table 1). With the exception of the appearance of small amounts of neutral acyl lipids, no other lipid type, apparently, was affected.

Comparison of the acyl lipid compositions of *Rhps. capsulata* and *Rhps. sphaeroides* again revealed that phosphatidylethanolamine, phosphatidylglycerol and cardiolipin were major components. In addition, phosphatidylcholine was present in appreciable amounts, and *Rhps. sphaeroides* contained 2-4% of the glycolipid diacylsulphoquinovosylglycerol. When the growth conditions were altered to photosynthetic, there was again a decrease in the relative proportion of phosphatidylethanolamine and an increase in phosphatidylglycerol. Photosynthetic growth therefore appeared to result in an increased proportion of phosphatidylglycerol in all three bacteria.

To gain further information about the marked changes in acyl lipid compositions that accompanied the alteration from non-photosynthetic to photosynthetic growth conditions, we analysed the fatty acid composition of individual lipids in the three

Table 1. Acyl lipid composition of *Rhodospirillum rubrum*, *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* grown under photosynthetic or non-photosynthetic conditions

Species	Conditions (or significance)	<i>Rhsp. rubrum</i>			<i>Rhps. sphaeroides</i>			<i>Rhps. capsulata</i>		
		Non-photosynthetic (n=5)	Photosynthetic (n=4)	P	Non-photosynthetic (n=4)	Photosynthetic (n=3)	P	Non-photosynthetic (n=4)	Photosynthetic (n=2)	P
Cardiolipin	...	12.2 ± 1.0	11.3 ± 1.9	n.s.	6.2 ± 0.5	11.3 ± 3.8	< 0.05	10.5 ± 2.1	8.1 ± 4.0	n.s.
Phosphatidylglycerol	...	25.2 ± 5.1	37.3 ± 4.0	< 0.005	37.0 ± 1.4	44.4 ± 7.2	< 0.10	39.3 ± 2.1	62.5 ± 7.7	< 0.005
Phosphatidylcholine	...	3.4 ± 1.3	5.1 ± 0.8	n.s.	14.8 ± 2.0	11.9 ± 4.8	n.s.	10.0 ± 1.1	7.0 ± 0.5	< 0.005
Phosphatidylethanolamine	...	53.5 ± 8.0	37.8 ± 3.5	< 0.02	35.0 ± 1.4	21.4 ± 0.9	< 0.005	33.8 ± 1.9	18.7 ± 1.4	< 0.001
Ornithine lipid	...	1.6 ± 0.6	1.4 ± 0.3	n.s.	2.8 ± 1.0	2.3 ± 2.3	n.s.	2.9 ± 0.4	0.9 ± 0.4	< 0.005
Diacylglycerol	...	1.7 ± 1.1	2.6 ± 0.2	n.s.	2.3 ± 1.8	4.2 ± 2.1	n.s.	2.1 ± 1.3	0.7 ± 0.3	n.s.
Diacylphosphatidylglycerol	...	tr.	2.1 ± 0.9	—	tr.	2.8 ± 2.7	—	tr.	1.0 ± 0.5	—
Neutral acyl lipids	...	2.4 ± 0.5	2.4 ± 0.5	n.s.	1.9 ± 0.3	1.7 ± 0.3	n.s.	1.4 ± 0.2	1.1 ± 0.1	n.s.
Other polar acyl lipids	...	2.4 ± 0.5	2.4 ± 0.5	n.s.	1.9 ± 0.3	1.7 ± 0.3	n.s.	1.4 ± 0.2	1.1 ± 0.1	n.s.

bacteria. The results for *Rhsp. rubrum* are shown in Table 2 and for *Rhps. capsulata* and *Rhps. sphaeroides* in Table 3. Only the major acyl lipids are detailed. The fatty acid content of the lipids of *Rhsp. rubrum* is characterized by appreciable quantities of palmitic and palmitoleic acids in contrast to *Rhps. sphaeroides* and *Rhps. capsulata*, where vaccenic (*cis*-11-octadecenoic) acid represented over 80% of the fatty acids in all lipids with the exception of diacylsulphoquinovosylglycerol. In *Rhsp. rubrum* the fatty acid compositions of the three major phospholipids were remarkably similar, with vaccenate, palmitoleate, palmitate and stearate, in decreasing order, being the major fatty acids. On changing from non-photosynthetic to photosynthetic growth conditions, a decrease in palmitoleate and vaccenate and an increase in palmitate and stearate was seen in the three major phospholipids. In diacylsulphoquinovo-

sylglycerol the amounts of C₁₆ acids only were altered by growth conditions (Table 2). In contrast, the exceptionally high content of vaccenic acid in the major lipids of *Rhps. capsulata* and *Rhps. sphaeroides* was unaffected by changing growth conditions (Table 3). The most noticeable difference between *Rhps. capsulata* and *Rhps. sphaeroides* was the higher stearate content in the latter.

One possible explanation for the changes seen in lipid composition on changing growth conditions could be that they were due to differences in bacterial structure. During photosynthetic growth it is known that membranes proliferate and there are defined, isolatable structures (the chromatophores) present (Lascelles, 1968; Oelze & Drews, 1972). Accordingly chromatophore preparations were made from *Rhsp. rubrum* and *Rhps. sphaeroides* and then analysed for their lipid content (Table 4). The results showed

Table 2. Fatty acid composition of the major acyl lipids of *Rhodospirillum rubrum* grown under photosynthetic or non-photosynthetic conditions

Oxidative degradation of fatty acids indicated that C_{16:1} consisted of >95% palmitoleic acid and C_{18:1} was >95% vaccenic acid. Abbreviations used: N, non-photosynthetic; P, photosynthetic; tr., trace.

Lipid	Growth	Fatty acid ...	Fatty acid (% total)					
			C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	Others
Cardiolipin	N		2.4	11.8	27.2	2.8	48.9	6.9
	P		1.0	17.6	20.8	9.9	42.3	8.4
Phosphatidylethanolamine	N		1.8	13.8	30.1	1.7	50.2	2.4
	P		1.3	17.9	20.3	11.2	42.7	6.6
Phosphatidylglycerol	N		3.4	20.6	29.3	1.7	38.5	6.5
	P		0.2	20.1	22.0	11.9	40.2	5.6
Diacylsulphoquinovosylglycerol	N		tr.	12.8	24.4	16.5	38.5	7.8
	P		tr.	25.3	14.5	13.3	41.5	5.4

Table 3. Fatty acid composition of the major acyl lipids of *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides* grown under photosynthetic or non-photosynthetic conditions

For abbreviations see Tables 1 and 2.

Lipid	Growth	n	Fatty acid	...	Fatty acid (% total)				
					C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	Others
<i>Rhodopseudomonas capsulata</i>									
Phosphatidylethanolamine	N	2			5.2±0.7	4.1±1.0	2.9±0.5	84.0±0.9	3.8±0.4
	P	2			4.5±0.4	4.2±0.7	2.3±0.4	83.5±3.4	5.5±0.7
Phosphatidylcholine	N	2			2.9±1.1	4.4±1.0	3.2±1.0	85.7±0.6	3.8±0.7
	P	2			4.8±2.4	2.2±1.1	4.1±0.7	84.2±3.5	4.7±0.6
Phosphatidylglycerol	N	2			1.9±0.5	4.4±1.2	4.0±0.8	83.9±2.2	5.8±0.4
	P	2			4.5±0.1	4.8±0.3	3.0±0.2	84.6±1.8	3.1±0.4
<i>Rhodopseudomonas sphaeroides</i>									
Phosphatidylethanolamine	N	2			3.7±0.2	1.2±0.1	8.8±0.7	84.1±0.2	2.2±0.3
	P	3			4.2±0.8	0.9±0.2	8.4±3.1	82.0±1.7	4.5±0.5
Phosphatidylcholine	N	2			2.8±0.7	1.2±0.3	6.3±1.9	84.5±3.0	5.2±0.6
	P	3			3.2±1.6	1.5±0.3	6.8±0.5	85.5±1.5	3.0±0.2
Phosphatidylglycerol	N	2			5.5±0.4	1.3±0.1	10.7±0.5	80.1±0.5	2.4±0.3
	P	3			4.9±0.8	1.0±0.1	9.7±0.4	80.8±2.7	3.6±0.4
Diacylsulphoquinovosyl-glycerol	N	2			20.1±1.3	1.5±0.5	15.4±2.0	61.0±4.2	2.0±0.3
	P	3			20.2±1.3	0.9±0.3	15.4±2.0	62.4±5.3	1.1±0.3

that the major acyl lipids of the chromatophores were those expected from analysis of whole cells as recorded in Table 1. As with whole cells, the most noticeable difference between chromatophores of the two bacteria was the greater amount of phosphatidylcholine in *Rhps. sphaeroides*. The chromatophores of *Rhsp. rubrum* had a smaller proportion of phosphatidylethanolamine and a higher proportion of phosphatidylglycerol compared with lipids of whole cells. In addition, the increased amounts of palmitate and stearate that were seen at the expense of palmitoleic acid in photosynthetically as against non-photosynthetically grown *Rhsp. rubrum* (Table 2) were also reflected in the fatty acid compositions of the acyl lipids of isolated chromatophores. The latter had an even higher content of saturated acids and less palmitoleate than photosynthetically grown cells (Table 4). When the chromatophores from *Rhps. sphaeroides* were examined their acyl lipid composition, although as high in phosphatidylglycerol as photosynthetically grown cells, was not as low in phosphatidylethanolamine. On the other hand, contents of the remaining 'major' acyl lipids (Table 4) were similar to those in photosynthetically rather than non-photosynthetically grown cells

(Table 1). Since the fatty acid compositions of the acyl lipids of *Rhps. sphaeroides* did not change with altered growth conditions (Table 3), it was natural that the fatty acids of the bacteria's chromatophores merely reflected the values for whole cells. It is worth emphasizing that the fatty acids of diacylsulphoquinovosylglycerol in chromatophores (Table 4), as well as in whole cells (Table 3), differ noticeably from the pattern for other major acyl lipids.

The chromatophores whose analysis was shown in Table 4 were isolated from a separate batch of bacteria from those analysed in Table 1, and it was thought desirable, in view of variations between individual samples of bacteria, to carry out an analysis of cells and chromatophores derived from the same batch culture. The results of such an analysis for *Rhps. sphaeroides* are shown in Table 5. In this case an increase in phosphatidylglycerol and a decrease in phosphatidylethanolamine was found in chromatophores compared with photosynthetically grown cells. A similar but more marked result was obtained with *Rhps. capsulata*. The data therefore indicate that chromatophores prepared from all three bacterial types contain a higher proportion of phosphatidylglycerol and a lower proportion of

Table 4. Lipid composition of chromatophores isolated from *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*

Bacterium	Lipid	Percentage of total lipid	Fatty acid ...	Fatty acid composition (% of total)				
				C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	Others
<i>Rhodospirillum rubrum</i>	Cardiolipin	13.3		28.7	17.2	8.4	43.6	2.1
	Phosphatidylethanolamine	29.5		25.3	16.3	7.2	46.4	4.8
	Phosphatidylcholine	2.4		34.1	17.5	7.4	39.1	1.9
	Phosphatidylglycerol	43.6		27.5	20.0	9.4	39.8	3.3
<i>Rhodopseudomonas sphaeroides</i>	Cardiolipin	13.0		6.9	0.8	14.2	78.0	2.1
	Phosphatidylethanolamine	28.3		7.2	1.6	15.8	71.2	4.2
	Phosphatidylglycerol	44.4		9.3	1.4	16.3	72.9	2.1
	Phosphatidylcholine	10.0		10.6	1.5	13.8	71.9	2.2
	Diacylsulphoquinovosylglycerol	3.7		21.5	0.6	14.5	54.4	9.0

Table 5. Comparison of the acyl lipid composition of whole cells of *Rhodopseudomonas sphaeroides* with that of chromatophore preparations

Preparations	Lipid	Percentage of total	Fatty acid ...	Major fatty acids (% of total)			
				C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}
Cells	Cardiolipin	10.2		9.8	3.4	14.6	73.2
	Phosphatidylethanolamine	28.3		6.2	1.5	14.4	77.9
	Phosphatidylglycerol	47.9		6.7	1.0	15.0	77.3
	Phosphatidylcholine	11.2		8.2	1.6	15.0	75.2
Chromatophores	Cardiolipin	9.6		7.2	1.0	13.8	78.0
	Phosphatidylethanolamine	23.7		5.8	0.7	13.7	79.8
	Phosphatidylglycerol	56.2		5.6	1.6	10.6	82.2
	Phosphatidylcholine	10.2		4.8	1.8	11.6	81.8

phosphatidylethanolamine than their respective photosynthetically grown cells.

Discussion

The acyl lipid compositions that we obtained for the three bacteria (Table 1) agree with published data in that the major acyl lipids in these organisms were phosphatidylethanolamine and phosphatidylglycerol. There are some variations in the relative proportions or, indeed, the presence of different acyl lipids for these bacteria quoted in the literature. For example, Wood *et al.* (1965) failed to find phosphatidylcholine in *Rhsp. rubrum*, whereas Haverkate *et al.* (1965) reported that it represented 6% and Hirayama (1968) 10% of the total lipids for anaerobically light-grown cells; we found 5.1% (Table 1). In addition, the lipid composition for *Rhps. sphaeroides* we obtained agrees well with that found by Haverkate *et al.* (1965) and Steiner *et al.* (1970), but differs from that reported by Lascelles & Szilagyi (1965), who found low amounts of phosphatidylglycerol and very large quantities of phosphatidic acid. The elevated amounts of the latter lipid are perhaps indicative of degradation during their analytical procedure. Small amounts of what was, apparently, the ornithine-containing lipid first identified by Gorchein (1964) (cf. also Brooks & Benson, 1972), were found in all three bacteria. In agreement with Radunz (1969), we provisionally identified diacylsulphoquinovosylglycerol in the bacteria, though the amounts in *Rhps. capsulata* were rather small. The composition of *Rhps. capsulata* (Table 1) also agreed well with available published data, although no complete quantification had been made previously (Goldfine, 1972; Oelze & Drews, 1972; Wood *et al.*, 1965).

Wood *et al.* (1965) isolated and identified the double-bond positions of the C₁₈ monoenoic fatty acid isolated from photosynthetic bacteria grown anaerobically in the light. Their results showed that 99% or more of this acid was vaccenic. Our data agree entirely with their finding, and, in addition, that the C₁₆ fatty acid present in *Rhps. rubrum* is palmitoleic acid. Total fatty acid patterns for the three bacteria that have been reported previously agree excellently with our data (Tables 2 and 3) in that *Rhsp. rubrum* contains large amounts of palmitic and palmitoleic acids in addition to vaccenic acid, which represents by far the major acid of *Rhps. capsulata* and *Rhps. sphaeroides* (Haverkate *et al.*, 1965; Schmitz, 1967; Schröder *et al.*, 1969; Wood *et al.*, 1965). Moreover, it has been found also that *Rhps. sphaeroides* is relatively richer in stearic acid (Table 3) than the two other bacteria (c.f. Oelze & Drews, 1972). A detailed analysis of the fatty acids of individual lipids has not to our knowledge been

carried out previously with these bacteria, though Wood *et al.* (1965) examined three phospholipids in *Rhps. capsulata*. It is noticeable that, in contrast to the fatty acid compositions of mammalian (White, 1973) or plant (Mazliak, 1977) phospholipids, there was remarkably little difference between the major phospholipids. The alterations in the proportions of the fatty acids of *Rhsp. rubrum* that were observed for all lipids on changing the growth conditions (Table 2) were also found by Wood *et al.* (1965) when they examined the total fatty acids of that bacterium.

The lipid compositions and fatty acid content of the chromatophore preparations that we obtained (Tables 4 and 5) are in agreement with the small amount of data already published (Collins & Niederman, 1976; Michels & Konings, 1978). They also confirmed the suggestion, based on the analyses shown in Table 1, that production of chromatophore membranes during the switch to photosynthetic growth was accompanied by an increase in phosphatidylglycerol content. Indirect confirmation for this idea also comes from the work of Lascelles & Szilagyi (1965), who found a relationship between phospholipid synthesis and pigment production in *Rhps. sphaeroides*. In particular, phosphatidylglycerol was synthesized selectively during the transition from non-photosynthetic to photosynthetic growth conditions. Klein & Mindich (1976) extended this work to demonstrate an obligatory dependence of pigment synthesis with phospholipid formation. Moreover, experiments with photosynthetic bacteria have demonstrated the importance of phospholipids for several aspects of bacterial photosynthesis (Klemme *et al.*, 1971; Myers & Guillory, 1974).

The changes that occurred in the relative saturation of the fatty acids of *Rhsp. rubrum* (but not in the highly vaccenate-enriched *Rhps. sphaeroides* and *Rhps. capsulata*) during the switch to photosynthetic growth may also be important in relation to chromatophore function. The evidence we obtained for an association between phosphatidylglycerol and the photosynthetic apparatus of bacteria is noteworthy in view of the absence of phospholipids other than phosphatidylglycerol from the thylakoid membranes of higher plant chloroplasts (cf. Harwood, 1979).

Since the lipid and fatty acid patterns of photosynthetic bacteria are relatively simple, they offer a good experimental system for examining, firstly, the influence of the lipid environment on photosynthesis and electron transport and, secondly, the co-ordination of lipid synthesis with membrane biogenesis. In the latter connection the observation by Leuking *et al.* (1978) that, in *Rhps. sphaeroides* growing synchronously, total phospholipids are synthesized discontinuously, is important. Further study is required to answer these questions of co-ordination of

synthesis and of the function of specific lipids in the bacterial photosynthetic apparatus.

Since this paper was originally submitted, Birrell *et al.* (1978) have published data that suggests that the bacteriochlorophyll-binding proteins of *Rhps. sphaeroides* preferentially associate with phosphatidylglycerol *in vivo*. This confirms our suggestion that phosphatidylglycerol may be involved in the photosynthetic function of this bacterium, in the above case in the light-harvesting reaction.

The mass-spectrometric analysis was kindly carried out by Dr. D. Games and Dr. M. Rossiter of the Department of Chemistry, University College, Cardiff.

References

- Birrell, G. B., Sistrom, W. R. & Griffith, O. H. (1978) *Biochemistry* **17**, 3768–3773
- Brooks, J. L. & Benson, A. A. (1972) *Arch. Biochem. Biophys.* **152**, 347–355
- Burns, D. D., Galliard, T. & Harwood, J. L. (1977) *Phytochemistry* **16**, 651–654
- Clayton, R. K. & Haselkorn, R. (1972) *J. Mol. Biol.* **68**, 97–105
- Collins, M. L. P. & Niederman, R. A. (1976) *J. Bacteriol.* **126**, 1316–1325
- Dawson, R. M. C. (1960) *Biochem. J.* **75**, 45–53
- Downing, D. T. & Greene, R. S. (1968) *Lipids* **3**, 96–100
- Drews, G. (1978) *Curr. Top. Bioenerg.* **8**, 161–207
- Garbus, J., De Luca, H. F., Loomans, M. E. & Strong, F. M. (1963) *J. Biol. Chem.* **238**, 59–63
- Gibson, K. D., Segen, B. J. & Niederman, R. A. (1972) *Arch. Biochem. Biophys.* **152**, 561–568
- Goldfine, H. (1972) *Adv. Microb. Physiol.* **8**, 1–58
- Gorchein, A. (1964) *Biochim. Biophys. Acta* **84**, 356–358
- Harwood, J. L. (1979) in *Biochemistry of Plants* (Conn, E. E. & Stumpf, P. K., eds.), vol. 4, Academic Press, New York and London, in the press
- Harwood, J. L. & James, A. T. (1975) *Eur. J. Biochem.* **50**, 325–334
- Harwood, J. L. & Stumpf, P. K. (1970) *Plant Physiol.* **46**, 500–508
- Haverkate, F., Teulings, F. A. G. & Deenen, L. L. M. (1965) *Proc. K. Ned. Acad. Wet. Ser. B* **68**, 154–159
- Hirayama, O. (1968) *Agric. Biol. Chem.* **32**, 34–41
- Kerber, N. L., Garcia, A. F., Vernon, L. P. & Raveed, D. (1972) *Biochim. Biophys. Acta* **256**, 108–119
- Klein, N. C. & Mindich, L. (1976) *J. Bacteriol.* **128**, 337–346
- Klemme, B., Klemme, J. H. & San Pietro, A. (1971) *Arch. Biochem. Biophys.* **114**, 339–342
- Lampe, H.-H. & Drews, G. (1972) *Arch. Mikrobiol.* **84**, 1–19
- Lascelles, J. (1956) *Biochem. J.* **62**, 78–93
- Lascelles, J. (1968) *Adv. Microb. Physiol.* **2**, 1–42
- Lascelles, J. & Szilagyi, J. F. (1965) *J. Gen. Microbiol.* **38**, 55–64
- Leuking, D. R., Fraley, R. T. & Kaplan, S. (1978) *J. Biol. Chem.* **253**, 451–457
- Mazliak, P. (1977) in *Lipids and Lipid Polymers in Higher Plants* (Tevini, M., Lichtenthaler, H. K., eds.), pp. 48–74, Springer-Verlag, Berlin
- Michels, P. A. M. & Konings, W. N. (1978) *Biochim. Biophys. Acta* **507**, 353–368
- Myers, D. E. & Guillory, R. J. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1338
- Niederman, R. A., Mallon, D. E. & Langan, J. J. (1976) *Biochim. Biophys. Acta* **440**, 429–442
- Oelze, J. & Drews, G. (1972) *Biochim. Biophys. Acta* **265**, 209–239
- Radunz, A. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 411–417
- Russell, N. J. (1978) *Biochim. Biophys. Acta* **531**, 179–186
- Schmitz, R. (1967) *Arch. Mikrobiol.* **56**, 238–247
- Schröder, J., Bidermann, M. & Drews, G. (1969) *Arch. Mikrobiol.* **66**, 237–280
- Stanier, R. Y. (1963) in *General Physiology of Cell Specialisation* (Mazia, D. & Tyler, A., eds.), pp. 242–252, McGraw-Hill, New York
- Steiner, S., Sojka, G. A., Conti, S. F., Gest, H. & Lester, R. L. (1970) *Biochim. Biophys. Acta* **203**, 571–574
- White, D. A. (1973) in *Form and Functions of Phospholipids* (Ansell, G. B., Dawson, R. M. C. & Hawthorne, J. N., eds.), pp. 441–482, Elsevier, Amsterdam
- Wood, B. J. B., Nichols, B. W. & James, A. T. (1965) *Biochim. Biophys. Acta* **106**, 261–273
- Yamashita, J. & Kamen, M. D. (1969) *Biochem. Biophys. Res. Commun.* **34**, 418–429