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Rhodopseudomonas sphaeroides Membranes: Alterations in Phospholipid Composition in Aerobically and Phototrophically Grown Cells

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The effects of growth conditions on phospholipid composition in *Rhodopseudomonas sphaeroides* have been reexamined. The levels of phosphatidylethanolamine (27 to 28%), phosphatidylglycerol (23 to 24%), and phosphatidylcholine (11 to 18%) were very similar in cells grown aerobically or phototrophically at a high light intensity, consistent with findings for another member of *Rhodospirillaceae*. In addition, an unknown phospholipid species was detected which comprised 20 to 30% of the total phospholipid in these cells. In cells growing phototrophically at low-intensity illumination, the level of phosphatidylethanolamine increased by about 1.6-fold and that of the unknown phospholipid markedly decreased. Although the synthesis of photosynthetic pigments, light-harvesting protein, and intracytoplasmic photosynthetic membranes also increased markedly, the ratios of individual phospholipid species were essentially identical in photosynthetic membrane and cell wall fractions purified from these cells. Since a significant exchange of lipids apparently did not occur during the isolation of these fractions, it was suggested that the changes in cellular phospholipid accumulation were not due to a unique composition within the photosynthetic membrane. Instead, these phosphoglyceride changes were found to be related to overall phospholipid metabolism and could be accounted for principally by differences in biosynthetic rates. These results, together with studies in nutrient-restricted aerobic cells, suggested that the mechanism by which phospholipid levels are regulated may be related to radiant energy flux rather than cellular energy limitation.

The facultative photoheterotrophic bacterium *Rhodopseudomonas sphaeroides* has been a useful model system for the study of membrane biogenesis because alterations in oxygen tension and incident illumination levels result in dramatic changes in membrane structure, function, and protein composition (22, 27, 28). In aerobically (chemotrophically) grown cells, the cell envelope consists of outer and cytoplasmic membrane layers typical of gram-negative bacteria (5). When the oxygen tension is reduced sufficiently, an extensive system of intracytoplasmic membranes is elaborated which in *R. sphaeroides* appears to consist of a continuous vesicular network (16, 28, 34). Upon mechanical disruption, this structure gives rise to an essentially uniform (10, 25) population of sealed vesicles (chromatophores) which contain several major protein components not found in other cellular membranes (14, 32, 40). These chromatophore-specific proteins can be accounted for largely by the polypeptide components of pigment-protein complexes that function in the harvesting of light energy and primary photochemical events (3, 9, 30). The formation of these complexes and of the chromatophore membrane is related inversely to incident light intensity.

Although a role for phospholipid in the assembly of pigment-protein complexes has been established recently (4, 15), it is not yet clear whether the lipid composition of the chromatophore membrane is also different from that of the cell envelope. Despite early reports which suggested that the chromatophore membrane of *R. sphaeroides* does not have a unique phospholipid composition (18, 24), significantly elevated levels of phosphatidylglycerol have been reported for this organism in a more recent study (35). Furthermore, enrichment of both phosphatidylglycerol and phosphatidylethanolamine has been suggested for the chromatophore membrane of the closely related organism *R. capsulata* (37). In the case of *R. sphaeroides*, discrepancies may have been related to variations in growth conditions, methods of lipid analysis, or the lack of representative membrane preparations.

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The alterations in total membrane phospholipid ratios reported for *R. capsulata* (37) were correlated with conditions of lowered light intensity known to derepress (22) chromatophore membrane formation. Although it was suggested that this could be a consequence of a unique chromatophore phospholipid composition (37), the altered ratios could also reflect environmental effects on overall phospholipid metabolism. In this case, the phospholipid composition of both the chromatophores and the cell envelope could be essentially the same. In the present study, the composition and levels of phospholipid species have been critically assessed in membranes purified from *R. sphaeroides* grown aerobically and under phototrophic conditions at different levels of incident illumination. An unknown phospholipid species designated tentatively as PX was detected under some growth conditions. Alterations in cellular phospholipid composition at decreased light intensities of phototrophic growth were shown also to occur in *R. sphaeroides*. Despite the accumulation of considerable intracytoplasmic photosynthetic membrane under these circumstances, no significant differences in the ratios of phosphoglyceride species were detected between chromatophore and envelope fractions isolated from such cells. Studies of phospholipid metabolism suggested that differences in overall biosynthetic rates could largely account for the alterations in the phospholipid ratios.

MATERIALS AND METHODS

Growth of organism. *R. sphaeroides* NCIB 8253 was grown in the MS-S medium of Lascelles (23). Cells were grown aerobically at 30°C in the dark in Fernbach flasks filled to 0.25 volume on a Gyrotory shaker (model G-25; New Brunswick Scientific Co., New Brunswick, N.J.) at 350 rpm. Cells were grown photoheterotrophically at 30°C under 95% nitrogen–5% CO_2 in 1-liter reagent bottles filled to capacity. Constant incandescent illumination was adjusted to provide 215, 2,690, and 21,520 lx, measured at the vessel surface with a Weston model 750 light meter. These illumination levels were found to support minimal, intermediate, and maximal growth rates, respectively. Growth was monitored from an optical density at 680 nm measured with a 1-cm light path on a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Cells were harvested at optical densities of 0.4 to 0.8.

Continuous culture studies at a submaximal growth rate were conducted in a Bioflo benchtop chemostat (model C-30; New Brunswick Scientific Co.) kindly provided by A. C. St. John. A generation time of 10 h was obtained by adjusting of the flow rate to 20 ml/h and limiting the supply of malate and glutamate in MS-S medium to 10 and 7 mM, respectively. The 300-ml chemostat culture was inoculated with exponentially growing aerobic cells and maintained at 30°C with aeration and stirring at 0.6 ml/min and 500 rpm, respectively.

Lipid analyses. Exponentially growing cells adapted to a medium containing 1 mM phosphate were transferred to fresh medium containing $^{32}P_i$ (0.5 μCi/ml; specific radioactivity, 0.5 μCi/μmol) and adjusted to an optical density of 0.1. Growth was continued for at least three generations. Cells were harvested by centrifugation at 8,000 × *g* and washed twice in 0.01 M Tris buffer, pH 7.2. Lipids were extracted by a modification of the method described in reference 1. Aqueous suspensions of whole cells and membranes were extracted at concentrations of 20 and 8 mg of protein per ml, respectively. The final respective proportions of methanol, chloroform, and water were 2:1:0.8 (vol/vol). Lipid extracts were separated from the precipitated residue after centrifugation at 10,000 × *g* for 10 min. One volume each of chloroform and 50 mM $MgCl_2$ was added to the extract, and the chloroform phase was washed three times with 0.25 volume of 0.15 M NaCl to remove unincorporated $^{32}P_i$.

Lipids were separated by a two-dimensional thin-layer chromatography procedure (33). The lipids were located by exposing developed plates to iodine vapors. ^{32}P-labeled phospholipids were detected by radioautography after exposure of plates to Kodak XRP film. Radioactivity was determined by scraping appropriate areas from the chromatogram and liquid scintillation counting (4). Phospholipid species were identified by their mobilities relative to those of standards (Sigma Chemical Co., St. Louis, Mo.; Supelco, Bellefonte, Pa.) and reactions with molybdenum blue (13), ninhydrin, and periodate-benzidine (8) sprays.

The total fatty acid content and acyl composition of the phosphoglycerides were determined by gas-liquid chromatography after preparation of methyl esters by mild alkaline methanolysis. Methyl esters were separated from photosynthetic pigments by thin-layer chromatography on Silica Gel G plates (Analtech Inc., Newark, Del.) predeveloped in hexane-diethyl ether (1:1, vol/vol) and developed in hexane-diethyl ether (95:5, vol/vol). The desired bands were located with rhodamine 6G spray (0.05% [wt/vol] in 95% ethanol), and methyl esters were eluted with the predevelopment solvent and concentrated under nitrogen. The methyl esters were separated on a Hewlett-Packard gas chromatograph (model 7106A; Hewlett-Packard, San Diego, Calif.) equipped with a hydrogen flame ionization detector. Fatty acid composition was expressed as weight percent of total fatty acids.

For detection of water-soluble deacylated products, phospholipids (1.5 to 2.0 mg) were evaporated to dryness and solubilized in an ultrasonic bath with 1 ml of 0.1 N methanolic KOH. Incubation at room temperature was continued for 15 to 25 min, and the deacylated products were recovered from the upper aqueous phase after the addition of 1 ml of water, 0.6 ml of chloroform, and 0.3 ml of methanol. The upper phase was neutralized by stirring with 0.5 ml of Amberlite IR-200 (H^+ form) and desalted by passage through a 2-ml column prepared with the same resin. The deacylated products were eluted with methanol-water (9:1, vol/vol) and dried under reduced pressure with a Savant concentrator (Savant Instruments, Inc., Hicksville, N.Y.). The samples were dissolved in methanol-ammonia (9:1, vol/vol) and chromatographed on an Eastman cellulose chromatogram (no. 6065; Eastman Kodak Co., Rochester, N.Y.) with the solvent system of reference 31. Glycerol phosphate

esters were detected with a phosphate ester-specific spray (20).

Membrane isolation. Chromatophores and a cell wall-enriched fraction were purified from phototrophically grown cells by differential and rate-zone sedimentation (27). For the isolation of cytoplasmic and outer membrane fractions from aerobically grown cells, French pressure cell extracts (27) in 0.01 M Tris-hydrochloride buffer (pH 7.2) were treated with 100 μg of lysozyme per ml for 30 min on ice, brought to 10 mM EDTA, and centrifuged at 250,000 × g_{av} for 60 min in a Beckman 60 Ti rotor (Beckman Instruments, Inc., Palo Alto, Calif.). The resuspended pellet was layered onto a gradient prepared with equal volumes of 20, 40, and 60% (wt/wt) sucrose (12) in 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid–0.01 M EDTA (pH 7.5) and centrifuged at 96,000 × g_{av} for 15 h in a Beckman SW27 rotor. The gradient profile of succinate dehydrogenase (succinate:phenazine methosulfate oxidoreductase [EC 1.3.99.1]) activity (32) and the polypeptide profiles in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (32) of the cytoplasmic and outer membrane fractions indicated that the separation was essentially complete.

Chemical analyses. The procedures used for determination of protein, bacteriochlorophyll *a* (Bchl), carotenoids, and phospholipids were those described previously (4).

RESULTS

Cellular phospholipid composition. An improved two-dimensional thin-layer chromatography procedure (33) proved to be a satisfactory method for the direct separation of the phospholipid components of *R. sphaeroides*. The autoradiogram in Fig. 1 shows a representative separation of ^{32}P-labeled phospholipids of aerobically grown cells. The phospholipids were identified by comparison with standard preparations and by the specific color reactions described above. Neither the single- nor the two-dimensional procedures described previously (18, 35) resolved an unknown phospholipid component (PX; see Addendum) from phosphatidylglycerol and phosphatidylethanolamine, respectively. In addition to PX, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, and cardiolipin, as well as three minor unidentified spots, were separated widely.

Although the structure of PX has not yet been elucidated completely, the following evidence indicates that this component is an endogenous phospholipid. The ^{32}P labeling characteristics (see below) and the similarity in fatty acid composition of PX to that of the other phospholipids isolated from *R. sphaeroides* (J.C. Onishi, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1980) suggest that the unknown component is a phosphoglyceride synthesized in a common pathway. Despite the comigration of PX with phosphatidate and lysophosphatidylethanolamine, the unknown is not a product of phospholipase degradation since it stained specifically with periodate-benzidine (8), indicating the presence of α-glycol groups. Furthermore, determinations of ester content as described previously (38) gave an ester-to-phosphate ratio of 2.1:1 rather than the 1:1 expected of lysophospholipids. The unknown phospholipid does not appear to be an artifact of extraction or chromatography since no iodine staining spot corresponding to PX developed from a mixture of phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin standards subjected to these procedures. Thus, it is unlikely that PX represents a lipid-salt complex (21). Chromatographic analyses of the organic solvent (J.C. Onishi, Ph.D. thesis) and water-soluble products of mild alkaline hydrolysis are also consistent with the identity of PX as a phosphoglyceride. Similar to other O-acyl phosphoglycerides, PX was rendered quantitatively water soluble on the basis of ^{32}P radioactivity. The deacylated product of PX detected with a phosphate ester spray (20) did not cochromatograph with the glycerophosphate, glycerylphosphorylglycerol, or diglycerylphosphorylglycerol resulting from the hydrolysis of phosphatidic acid, phosphatidylglycerol, and cardiolipin standards, respectively. On the basis of all of the above evidence, radioactivity associated with PX was included in the calculated phospholipid compositions.

Estimates of phospholipid composition were performed on cells labeled uniformly with $^{32}P_i$.

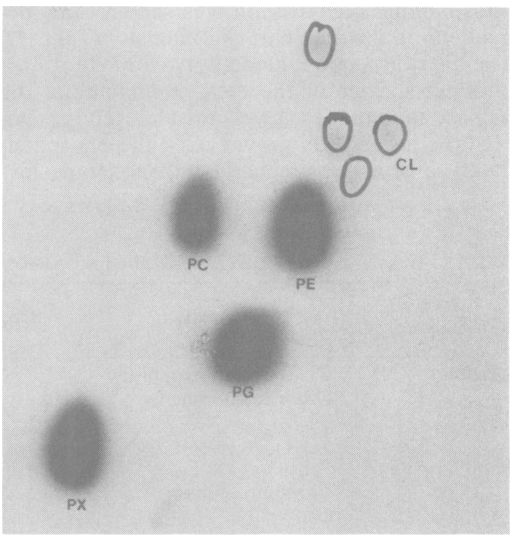


FIG. 1. Autoradiograms of two-dimensional thin-layer chromatographic separation (33) of ^{32}P-labeled lipids from aerobically grown cells. PX, Unknown phospholipid; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin.

TABLE 1. Phospholipid composition of aerobically and phototrophically grown cells

Growth condition	Light intensity (lx)	Specific Bchl[a]	Composition[b]				
			PX	PG	PC	PE	CL
Aerobic			31	24	11	28	0.2
Aerobic chemostat[c]			64	3.4	4.1	6.1	14
Phototrophic	215	34	7.1	27	16	43	1.8
	2,690	14	5.4	28	16	43	3.0
	21,520	3.1	21	23	18	27	2.6

[a] Micrograms of Bchl per milligram of protein.
[b] Abbreviations defined in the legend to Fig. 1. Expressed as percent ^{32}P in lipid extract.
[c] Generation time increased from 2.3 to 10 h by nutrient limitation.

Lipid composition was shown in aerobic cells to be dependent upon the growth medium. Although significant levels of PX were demonstrated in cells grown on a medium containing 1 mM phosphate (23), no PX was detected when the cells were grown as described previously (32); instead, the phosphatidylethanolamine and phosphatidylglycerol levels were elevated by 1.7- and 1.4-fold, respectively. Even though the phosphate concentration was 20-fold higher in the latter medium, significant levels of PX were also produced when the phosphate level was increased to 20 mM in MS-S medium (23). Because cells could be labeled with $^{32}P_i$ that was maintained at higher specific radioactivities, MS-S medium at the low phosphate concentration was used in subsequent experiments.

In phototrophically grown *R. sphaeroides*, the phospholipid composition was shown to be dependent on the incident light intensity (Table 1). The distribution of radioactivity within the phospholipid species of the cells maintained at the highest illumination level most closely resembled the aerobically grown cells; the phosphatidylglycerol and phosphatidylethanolamine levels were essentially identical, although some decrease in PX was observed in the former. In contrast, upon lowering illumination levels 8- to 100-fold in the phototrophically grown cells, the PX level was markedly diminished, and that of phosphatidylethanolamine increased by 1.6-fold.

Lipid composition of purified membranes. For a determination of whether the light-dependent quantitative phospholipid alterations were due to the synthesis of a chromatophore membrane differentiated in phospholipid content, representative membranes were purified (27) from the various cells. In general, the phospholipid compositions of the chromatophore and cell wall-enriched fractions were quite similar, independent of the incident illumination levels during growth (Table 2). When the membranes from phototrophically grown cells are compared, it is noteworthy that chromatophore and cell wall fractions each comprised about 28% of the lipid phosphorus in cells grown under low-intensity illumination (1,828 lx), with much of the remainder present in small membrane fragments (C. W. Radcliffe, and R. A. Niederman, unpublished

TABLE 2. Phospholipid composition of purified membrane fractions

Growth condition	Fraction	Specific Bchl[a]	$^3H/^{32}P$[b]	Composition[c]			
				PX	PG	PC	PE
Aerobic	Cytoplasmic membrane			26	26	14	29
	Outer membrane			23	28	19	22
Phototrophic							
215 lx	Chromatophore	102	0.015	6.2	26	21	36
	Cell wall	8.1	0.215	4.5	27	19	40
21,520 lx	Chromatophore	57	0.061	18	30	21	23
	Cell wall	19	0.186	14	26	22	33

[a] Micrograms of Bchl per milligram of protein.
[b] Contamination of chromatophores by cell envelope phospholipids (details in the text).
[c] Abbreviations defined in the legend to Fig. 1. Expressed as percent ^{32}P in lipid extract.

data). In contrast to the suggestion that the increased phosphatidylethanolamine levels in *R. capsulata* grown at a low light intensity may be due to the formation of a chromatophore membrane with an elevated content of this phospholipid (37), no such enrichment was observed here in the chromatophore fraction from *R. sphaeroides* grown under this condition. Moreover, the phospholipid composition of the cytoplasmic and the outer membranes from aerobically grown cells did not differ greatly (Table 2).

The previous demonstration that the membranes of aerobically grown cells and the nonchromatophore membranes of phototrophic cells exhibit similar sedimentation properties (27) was used to test the possibility that significant randomization of phospholipids may have occurred during the membrane isolation procedures. Aerobic cells were labeled to equilibrium with [2-^{3}H]glycerol (0.27 μCi/μmol) and mixed with an equivalent amount of phototrophic cells labeled with $^{32}P_i$ (0.25 μCi/μmol). The cells were passed through the French press, and the chromatophore and cell wall were fractionated (27). Since only the aerobic cell envelope phospholipids were labeled with [2-^{3}H]glycerol, the degree of contamination of the isolated chromatophore fractions was estimated by the $^3H/^{32}P$ ratio. The $^3H/^{32}P$ ratio of the lipid extracts (Table 2) was consistent with the possibility that in the cells grown at a low light intensity, the isolated chromatophores were contaminated with phospholipids of nonchromatophore origin by less than 10%, whereas the figure approached 30% in the high-light-intensity chromatophores.

In agreement with previous reports (19, 35), the total cellular fatty acid composition (J. C. Onishi, Ph.D. thesis) did not depend upon the growth mode. In general, *cis*-vaccenic acid ($C_{18:1}$ *cis*-Δ^{11}) comprised about 80 to 90% of the total fatty acids, with stearic acid ($C_{18:0}$) accounting for most of the remainder (6 to 11%), independent of both oxygen tension and light intensity during growth. With regard to the fatty acid composition of the isolated membrane fractions, *cis*-vaccenic acid again comprised the vast majority of fatty acyl species, but some enrichment in palmitic acid ($C_{16:0}$) levels of outer membrane-enriched fractions was observed; this fatty acid accounted for 4.4 and 6.1% of the total in the aerobic and phototrophic fractions, respectively, compared with 2.3% in the cytoplasmic membrane and 3.5% in chromatophores. Palmitic acid enrichment has also been reported in the outer membranes of *Rhodospirillum rubrum* (11, 29) and *Escherichia coli* (41). For the phospholipids purified from the cytoplasmic and chromatophore membranes, the composition of fatty acyl moieties was essentially identical (J. C. Onishi, Ph.D. thesis), in agreement with results reported for the phospholipids obtained from *R. sphaeroides* Ga cells (26). This is consistent with the theory that the various phospholipid species originated from a common pool of phosphatidic acid.

Phospholipid metabolism. Although changes in levels of individual phospholipid species as a function of growth conditions were found here, no major differences were observed between their levels in the chromatophore and their levels in the other cellular membranes. This suggested that these changes were due to differences in overall phospholipid metabolism. The relative rates of phospholipid biosynthesis and degradation were therefore examined in cells grown aerobically and under phototrophic conditions at a low light intensity. In Fig. 2, the incorporation of $^{32}P_i$ into each phospholipid species is plotted as a function of changes in cell mass in the respective exponentially growing cultures. Therefore, the various slopes represent the biosynthetic rates. After an initial lag in each culture, the rates of phosphatidylethanolamine and phosphatidylglycerol biosyntheses were increased about 2.4- and 2.6-fold, respectively, in the phototrophic cells relative to those grown aerobically. In contrast, the biosynthetic rate of PX was decreased about 6.6-fold in the phototrophic culture. In addition to these differences in the relative rates of biosynthesis, the stability of the ^{32}P-labeled phospholipids also differed under the two growth conditions. For comparisons of phospholipid stability, homogeneously labeled cells were shifted to unlabeled media, and the phosphoglycerides were analyzed periodically for about 0.5 generation during this chase. $^{32}P_i$ that was incorporated into each phospholipid species of the aerobic culture and phosphatidylcholine and PX in the phototrophic cells remained quite stable despite a small initial dilution (Fig. 3). In the slowly growing phototrophic culture, however, the initial turnover of phosphatidylethanolamine and phosphatidylglycerol was more marked, amounting to about 30 and 40%, respectively, during the first 0.08 generation. Thereafter, these phospholipids also remained quite stable, and the small increase in their radioactivity during prolonged phototrophic growth may reflect reincorporation of the isotope from degraded phospholipid.

Although no detailed analysis of phospholipid turnover was performed, the overall differences in biosynthesis rates, rather than changes in turnover, are generally sufficient to account for the observed alterations in the relative levels of the phospholipid species. The initial decrease in the level of phosphatidylethanolamine in the phototrophic cells is most likely accounted for by phosphatidylcholine biosynthesis. The more

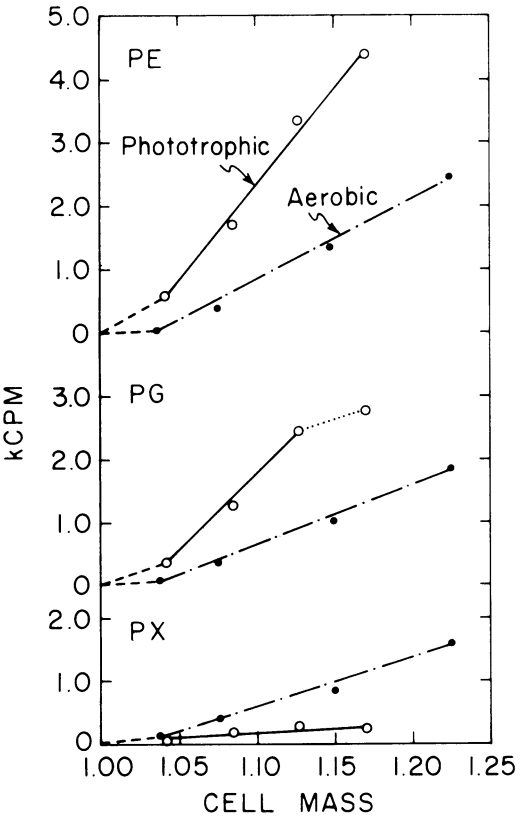
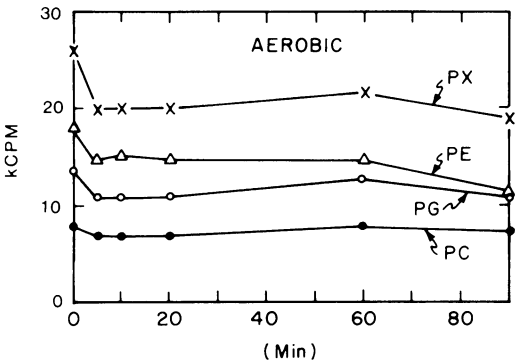


FIG. 2. Rates of phospholipid biosynthesis in aerobically and phototrophically grown cells. Phototrophic growth was at 215 lx. The increase in ^{32}P-labeled phospholipid is plotted as a function of cell mass measured from the optical density at 680 nm, with that at the start of the labeling set arbitrarily at 1.0. The generation times were 2.2 and 11.8 h for the aerobic and phototrophic cultures, respectively. Cells were labeled as described in the text with 0.5 μCi of $^{32}P_i$ per ml of medium. No radioactivity was detected in cardiolipin in either culture, and phosphatidylcholine became labeled only after approximately 1.2 doublings. The lines through the points were calculated by linear regression analyses. Abbreviations are defined in the legend to Fig. 1.

substantial turnover of phosphatidylglycerol in the slowly growing phototrophic culture may also be reflected in the apparent decrease in the rate of $^{32}P_i$ incorporation into this phospholipid after 1.1 generations (Fig. 2, dotted portion). This high turnover rate may also account for the lack of accumulation of anionic phospholipids under these circumstances (see below).

It had been suggested (37) that qualitative alterations in phospholipid composition in *R. capsulata* were due to changes in adenosine nucleotide levels. An alternative theory is that such changes are related more directly to radiant energy fluxes. As an approach to this problem, the nutrient supply was limited to an aerobic culture in a chemostat such that the generation time was increased to 10 h. This doubling time was essentially equivalent to that of the phototrophic cells grown at 215 lx. Although the nutritional states of both of these slowly growing cultures are not necessarily equivalent, the energy supply of the aerobic cells should also be reduced significantly.

After the optical density of the chemostat culture had reached a plateau (33 h), $^{32}P_i$ was added and labeling was continued for an additional 32 h. These results are also shown in Table 1. Instead of the decrease in PX and the increase in phosphatidylethanolamine levels ob-



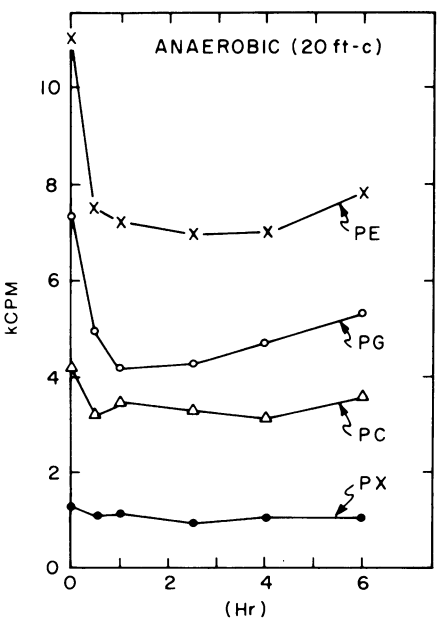


FIG. 3. Phospholipid turnover in aerobically and phototrophically grown cells. Phototrophic growth was at 215 lx. Cells were labeled with 0.5 μCi of $^{32}P_i$ per ml of medium. Abbreviations are defined in the legend to Fig. 1.

served for the cells grown phototrophically at a low light intensity, a two-fold increase in PX levels and a marked decrease in the other phospholipid species were observed. In addition, significantly elevated levels of cardiolipin were also seen in this energy-limited culture.

DISCUSSION

The evidence presented here has confirmed that the phospholipid composition of *R. sphaeroides* is dependent upon growth conditions. In accord with results obtained with *R. capsulata* (37), both the qualitative and the quantitative phospholipid compositions were essentially identical in cells grown aerobically and phototrophically at a high light intensity. This is consistent with the suggestion that in *Rhodospirillaceae*, differences in levels of phospholipid species do not result from changes in oxygen tension (37), but further studies under conditions of varying oxygen levels are necessary to establish this point. Furthermore, the relation between individual phosphoglyceride levels and incident light intensity during phototrophic growth observed for *R. capsulata* (37) has also been generally corroborated by the present results. In addition, the improved resolution of the chromatographic procedure (33) used here has permitted the detection of an unknown phospholipid species, designated PX, which accounted for more than 30% of the total phospholipid in aerobically grown cells. In thin-layer chromatography, this component behaved as an acidic and amphipathic compound. It was shown that the content of PX was dependent upon the growth medium and that the levels of this component were markedly decreased at low light intensities in phototrophically growing cells. Under these circumstances, phosphatidylethanolamine levels increased about 1.6-fold compared with cells grown aerobically or phototrophically at high incident illumination levels. Although on a protein basis, the decrease in light intensity was accompanied by a 10-fold increase in Bchl levels, the differentiation in phospholipid composition is apparently not due to the synthesis of chromatophore membrane with a unique phospholipid composition, as had been suggested for both *R. sphaeroides* (35) and *R. capsulata* (37). This was shown here for *R. sphaeroides* by the similarity in phospholipid composition between purified chromatophore and cell wall fractions in which neither significant exchange of phospholipids nor hybrid membrane formation during isolation apparently occurred. The recent finding that an apparent in vivo intermembrane phospholipid transfer in *R. sphaeroides* is not specific for phospholipid species (6) is also in accord with our results.

Changes in biosynthetic rates for individual phospholipid species were generally sufficient to account for the different cellular phospholipid compositions. This may be the basis for increases in PX in the aerobic cells and phosphatidylethanolamine in cells grown phototrophically at 215 lx. Although phosphatidylglycerol synthesis is also accelerated under the latter circumstances, the extensive and prolonged turnover may account for the lack of excessive accumulation of this phospholipid in these slowly growing phototrophic cells. Substantial phosphatidylglycerol turnover is also observed in *E. coli* and this is thought to be related mainly to the mobilization of the head group for membrane-derived oligosaccharide synthesis (36). In *R. sphaeroides* grown at 2,690 lx in which the generation time was decreased more than twofold, a 1.3-fold increase in total levels of phospholipids containing glycerophosphate head groups (phosphatidylglycerol plus cardiolipin) was observed in comparison with aerobically grown cells (Table 1); increases as high as 1.5-fold have also been observed (Radcliffe and Niederman, unpublished data). Although the total levels of these phosphoglycerides do not approach those reported previously (35), the magnitude of the differences between the aerobic and these phototrophic cells was similar. This provides some support for the suggestion from spin-label studies (2) that negatively charged phospholipids preferentially associate with the light-harvesting complex enriched in cells grown at low illumination. In the present study, a 2.4-fold enrichment in light-harvesting protein was observed in chromatophores over a 100-fold decrease in light intensity which is consistent with other results (39).

The phospholipid composition of aerobic cells grown in this study in a chemostat with a generation time equivalent to that of cells grown at a low illumination level suggests that energy limitation is not the basis for the changes observed in the ratios of the phospholipid species during phototrophic growth. Instead, the overall metabolism of phospholipids during phototrophic growth may be related by some other means to the flux rate of quanta absorbed by the Bchl and carotenoids of the light-harvesting apparatus. This is known to strongly influence the rates of both growth and membrane synthesis (17). The mechanism by which such changes in radiant energy flux might regulate the levels of the polar phospholipid head groups within the membrane is unknown and requires further investigation. Speculations include changes in the oxidation-reduction state of the crucial regulatory element as proposed for Bchl synthesis (27) and changes in membrane structure affecting enzyme activity (7, 15). However, the latter is not consistent with a recent report on *R. sphaeroides* in which

an in vivo intermembrane transfer of phospholipids to the chromatophore membrane was proposed (6). Although this suggested that phospholipids are synthesized at a site distinct from the intracytoplasmic membrane, direct assays of enzymes of phospholipid biosynthesis have recently demonstrated substantial activity in purified chromatophores (C. W. Radcliffe, G. M. Carman, and R. A. Niederman, unpublished data). Further studies on this question are in progress here.

ACKNOWLEDGMENTS

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ADDENDUM

While this manuscript was in preparation, the presence of this apparent phospholipid species was also reported in *R. sphaeroides* by Cain et al. (6).

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