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Phospholipid Metabolism in *Ferrobacillus ferrooxidans*

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The lipid composition of the chemoautotroph *Ferrobacillus ferrooxidans* has been examined. Fatty acids represent 2% of the dry weight of the cells and 86% of the total are extractable with organic solvents. About 25% of the total fatty acids are associated with diacyl phospholipids. Polar carotenoids, the benzoquinone coenzyme Q-8, and most of the fatty acids are present in the neutral lipids. The phospholipids have been identified as phosphatidyl monomethylethanolamine (42%), phosphatidyl glycerol (23%), phosphatidyl ethanolamine (20%), cardiolipin (13%), phosphatidyl choline (1.5%), and phosphatidyl dimethylethanolamine (1%) by chromatography of the diacyl lipids, by chromatography in four systems of the glycerol phosphate esters derived from the lipids by mild alkaline methanolysis, and by chromatographic identification of the products of acid hydrolysis of the esters. No trace of phosphatidylserine (PS), glycerolphosphorylserine, or serine could be detected in the lipid extract or in derivatives of that extract. This casts some doubt on the postulated involvement of PS in iron metabolism. After growth in the presence of ^{14}C and ^{32}P, there was essentially no difference in the turnover of either isotope in the glycerol-phosphate ester derived from each lipid in cells grown at *p*H 1.5 or 3.5.

Ferrobacillus ferrooxidans has the capacity to grow at very acid *p*H values. However, the function of the electron transport system of this organism in cell-free preparations for both respiration and reversed electron transport has a *p*H optimum near 7.0 (M. I. H. Aleem and S. A. Short, Bacteriol. Proc., p. 140, 1968). This suggests that the internal *p*H is maintained near neutrality by an active process or that the membrane is impermeable to hydrogen ions. Since phospholipids may possibly play an active role in the transport systems used by the cell to maintain its internal environment (7), the phospholipid metabolism of *F. ferrooxidans* was examined. In addition, *F. ferrooxidans* has been reported to have a unique lipid composition, and phosphatidyl serine has been postulated to be involved in iron metabolism (8). In this report the phospholipids of *F. ferrooxidans* are identified and shown to be similar to those of the thiobacilli. The metabolism of the phospholipids will be shown to be relatively independent of the *p*H of the growth medium.

MATERIALS AND METHODS

Bacterial strain. The *Ferrobacillus ferrooxidans* used in this study was isolated from acid strip mine effluents in the Daniel Boone National Forest, McCreary County, Kentucky, in 1967. The bacterium was obtained in pure culture after isolation of individual colonies.

Isolation of individual colonies. The strain was isolated from individual colonies as follows. A volume of cells from which the precipitated iron had been removed (16) was combined with six volumes of 0.32 mM H_2SO_4 and passed through sterile cellulose acetate filters of 0.47 μm pore size (Millipore Corp., Bedford, Mass.). The filters were then transferred to agar surfaces and incubated for 48 hr at 26 C. The agar contained 0.865 mM $(NH_4)_2SO_4$, 0.495 mM KCl, 2.12 mM K_2HPO_4, 1.52 mM $MgSO_4 \cdot 7H_2O$, 0.043 mM $Ca(NO_3)_2$, 0.25 mM $FeSO_4 \cdot 7H_2O$, and 0.6% (w/v) purified agar (Difco). Colonies on the membrane surface are shown in Fig. 1.

Characteristics of the organisms. Organisms isolated from the individual colonies were gram-negative, motile rods measuring 0.6 to 1.0 μm wide and 1.0 to 1.6 μm long in the stained preparation. The organism oxidized ferrous sulfate (130 μmoles of oxygen utilized per min per mg of bacterial protein) but not thiosulfate (less than 1 μmole of oxygen uptake per min per mg of bacterial protein).

Growth of bacteria. Bacteria were grown in 20-liter carboys in a medium containing 34 mM $(NH_4)_2SO_4$, 20 mM KCl, 4.3 mM K_2HPO_4, 3.4 mM $MgSO_4 \cdot 7H_2O$, 0.092 mM $Ca(NO_3)_2$, and 195 mM $FeSO_4 \cdot 7H_2O$ in 18 liters of demineralized water. The carboys were inoculated with 300 ml of a 48-hr culture and were incubated at 28 C. Cultures were aerated with a mixture of air and CO_2 (3:1, v/v) at a rate of 8 liters per min

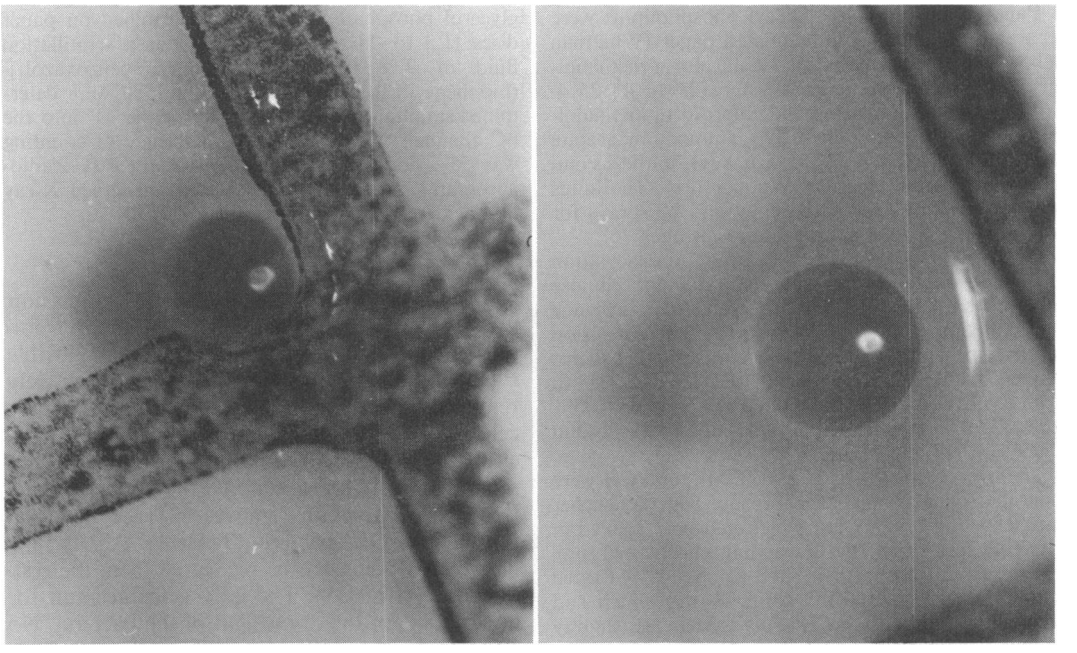

FIG. 1. *Colonies of F. ferrooxidans on the surface of cellulose acetate filters after incubation for 48 hr at 26 C. The colonies are 1 mm in diameter.*

per 18 liters of medium. Sterile $FeSO_4$ solution was added intermittently as described (3). After 72 hr, the cells were harvested with a De-Leval Gyrotest continuous-flow centrifuge (De Leval Separator Co., Chicago, Ill.). The cells were then separated from the iron precipitate as described (16).

Bacteria were grown in 600 ml of medium containing 1 mc of $NaH^{14}CO_3$ (New England Nuclear Corp., Boston, Mass.) and 1 mc of $H_3{}^{32}PO_4$ (Tracerlab, Waltham, Mass.). When radioactive bicarbonate was included in the medium, the flask after inoculation was gassed with oxygen for several minutes and then sealed; the $NaH^{14}CO_3$ solution was injected into the medium through a rubber septum. The turnover of the ^{14}C and ^{32}P in the lipids was measured as follows. The bacteria were grown in the presence of the isotopes for 2.3 divisions; the flask was flushed with air, and the $^{14}CO_2$ was trapped in $Ba(OH)_2$. The suspension was centrifuged at 12,000 $\times$ *g* for 20 min at 28 C, and the cells were then resuspended in 1,500 ml of nonradioactive medium. Cell suspensions containing 2.5 g of bacterial protein were inoculated into two flasks; in one flask the medium was *p*H 1.5 and in the other flask the *p*H was 3.5. The *p*H was maintained at these values by the addition of 5 N NaOH at 20-min intervals. Samples (200 ml) were withdrawn at 3-hr intervals into an equal volume of ice; the bacteria were harvested by centrifugation and the lipids were extracted. The lipids were then deacylated and separated chromatographically, and the radioactivity was determined.

Lipid extraction. The bacteria were withdrawn into an equal volume of ice, centrifuged, resuspended in 50 mM phosphate buffer (*p*H 7.6), and extracted by the Bligh and Dyer procedure (2, 19). This extraction procedure was reproducible.

Mild alkaline methanolysis. Glycerol phosphate esters were derived from diacyl phospholipids by mild alkaline methanolysis at 0 C as described by White (17). The reaction was complete in 2.5 hr. The alkali was neutralized with the weakly acidic cation-exchange resin, Biorex 70 (Bio-Rad Corp., Richmond, Calif.), as described by White (17). The abbreviations used for the glycerol phosphate esters derived from the phospholipids are: glycerolphosphorylethanolamine (GPE) from phosphatidyl ethanolamine (PE), glycerolphosphorylmonomethylethanolamine (GPME) from phosphatidyl monomethylethanolamine (PME), glycerolphosphoryldimethylethanolamine (GPDME) from phosphatidyldimethylethanolamine (PDME), glycerolphosphorylcholine (GPC) from phosphatidyl choline (PC), glycerolphosphorylglycerol (GPG) from phosphatidyl glycerol (PG), diglycerolphosphoryl glycerol (GPGPG) from cardiolipin (CL), and glycerolphosphorylserine (GPS) from phosphatidyl serine (PS).

Acid hydrolysis. The glycerol phosphate esters derived from the diacyl phospholipids were hydrolyzed in 2 M HCl at 100 C for 1 hr (14). This reaction quantitatively splits the nitrogen-containing esters into α-glycerolphosphate (αGP) and ethanolamine (E)-hydrochloride, monomethylethanolamine (ME)-hydrochloride, dimethylethanolamine (DME)-hydrochloride, or choline chloride (CH), as described by White and Tucker (20). HCl was removed in a stream of nitrogen.

Paper chromatography. Diacyl phospholipids were separated on silica gel-impregnated paper (Whatman SG-81) with solvents of chloroform-methanol-diisobutyl ketone-acetic acid-water (23:10:45:25:4, v/v) in the first dimension and chloroform-methanol-diisobutyl ketone-pyridine-0.5 M ammonium acetate buffer, *p*H 10.4 (30:17.5:25:35:6, v/v), in the second dimension, as described by Wuthier (21). The lipids were detected by radioautography and by sprays for amines (9), phosphate, and amino nitrogen (6).

Glycerol phosphate esters derived by deacylation of the phospholipid were separated on aminocellulose paper (Whatman AE-81) with solvents of 3 M formic acid containing 0.4% pyridine in the first dimension and 1.15 M ammonium acetate containing 11.8 mM ethylenediaminetetracetic acid (EDTA) made to *p*H 5.0 with acetic acid and diluted 3:7 (v/v) with 95% ethanolic 0.26 M ammonium hydroxide in the second dimension (17).

The hydrochlorides of E, ME, DME, and CH were separated on acid-washed paper no. 589 (Schleicher and Schuell, Keene, N.H.) which had been soaked in 1 M KCl and dried, by descending chromatography with a solvent of phenol–*n*-butyl alcohol–2.3 N formic acid–water (100 g, 100 ml, 6 ml, 20 ml) which had been saturated with KCl. This system is slightly modified from that reported by Bremer et al. (4). Hydrochlorides were detected with the amine spray (9) or by radioautography.

Thin-layer chromatography. A method for separation of glycerol phosphate esters developed by R. L. Lester involved cellulose thin-layer plates (Eastman Chromagrams 6064, Rochester, N.Y.) and solvents of 3.8 mM EDTA and 0.7 M ammonium bicarbonate in 90 mM ammonium hydroxide containing 67% (v/v) ethyl alcohol in the first dimension and isobutyric acid-water-concentrated ammonium hydroxide (66:33:1, v/v) in the second dimension. The lipids were detected by radioautography. The spots corresponding to the radioactivity were cut out, and the esters were eluted in three 2-ml portions of water. The elution was quantitative. The quinone and phospholipids were separated on Silica Gel G thin-layer plates (19). The lipids were detected and eluted from the silica gel as described by White and Frerman (19).

Column chromatography. Neutral lipids and phospholipids were separated by silicic acid chromatography (19). Glycerolphosphate esters derived from the phospholipids were eluted from columns (3 mm by 81 cm) of Dowex 1–8 X (200–400 mesh) in the formate form with the ammonium formate-sodium borate gradient described previously (19) or with 20 mM ammonium formate, *p*H 9.0.

Analysis. The bacterial protein and the lipid phosphate were determined as described by White and Frerman (19). The bacterial and lipid extracts were saponified and the fatty acids were recovered as described by White (18). The fatty acids were determined colorimetrically (11) with palmitic acid as standard.

Measurement of radioactivity. Samples were assayed for radioactivity in a Packard scintillation spectrophotometer model 2311. The ^{32}P and ^{14}C in the glycerol phosphate esters were determined on paper discs (1.2 to 2.0 cm in diameter) in a scintillation fluid of 9.28 mM 2,5[2(5-terbutyl benzoxazol)]-thiophene in toluene. Where ^{14}C and ^{32}P were determined simultaneously, the overlap of the ^{32}P into the ^{14}C channels was 6%, and the efficiency of counting was 55% for the ^{14}C and 88% for the ^{32}P. Radioautograms were made with Kodak no-screen X-ray film as described previously (17).

RESULTS

Extraction of the lipids. A bacterial suspension was prepared and separated from the precipitated iron. The convenient and reproducible Bligh and Dyer extraction procedure effectively removed all the lipid-soluble phosphate from the cells (Table 1). This extraction was equally effective with small amounts of bacteria not separated from precipitated iron before extraction. This extraction removes 82% of the total fatty acids in the bacteria (Table 1). About 18% of the fatty acids can be recovered from the residue after extraction. The fatty acids account for about 1.8% of the dry weight of the bacteria. No additional phospholipid can be removed from the residue by repeated extractions. The phospholipids account for between 13 and 15 μmoles of lipid P per g of protein. The phospholipids identified in this study have fatty acid to phosphate ratios of 2 to 1. By using a 2:1 ratio, the phospholipids account for only 25% of the extractable fatty acids. This represents a somewhat lower yield of phospholipid than is found in other bacteria (17, 19).

The cells contain a large amount of neutral

TABLE 1. *Extraction of F. ferrooxidans lipid*

F. ferrooxidans	Fatty acid[a]	Lipid phosphate
Intact bacteria	145.0	
Extract	106.0	13.5
Residue after extraction	26.2	<0.2[b]

[a] Expressed as micromoles per gram of protein. The protein was determined with the biuret procedure (19). Bacteria representing 23.0 mg of protein were extracted by the Bligh and Dyer procedure (2) and the residue was collected. Fatty acids were determined after extraction in both the residue and the extract. After saponification, fatty acids were measured colorimetrically with palmitic acid as standard (18). These values were compared to the fatty acids released after saponification of the intact bacteria. Lipid phosphate was determined (19) in the extract.

[b] After extraction, the residue was reextracted and the phosphate was determined in the second extraction procedure.

lipid. Yellow and orange carotenoids are prominent in the neutral lipid fraction.

There are essentially no glucolipids as determined by the anthrone reaction (19) in the lipid extract.

Isolation of the quinone. The neutral lipids and phospholipids were separated by silicic acid chromatography (18). The neutral lipid fraction was deposited as a band along the bottom edge of a Silica Gel G thin-layer plate. The plate was chromatographed in a solvent system of chloroform-isooctane (2:1, v/v) and dried, and the center portion was covered with Saran wrap (4). The exposed ends of the plate were sprayed with KBH_4 followed by neotetrazolium (19). A single red band appeared at an R_F value of 0.45. The portion of the band that had been under the Saran wrap was recovered from the silica gel and dissolved in absolute ethyl alcohol. The ultraviolet spectrum of the quinone showed a single maximum at 273 nm. After reduction with KBH_4, the hydroquinone spectra showed a maximum at 287 nm. The reduced-minus-oxidized difference spectrum showed a maximum at 271 nm and a minimum at 295 nm. These are the spectral properties of coenzyme Q (CoQ). The purified CoQ was chromatographed on paper impregnated with Dow Corning 550 with a solvent of *n*-propanol–water (4:1) as previously described (10). The R_F value of the CoQ of *F. ferrooxidans* has the chromatographic mobility of CoQ-8 (Fig. 2). Neither other isoprenologues of CoQ nor vitamin K_2 could be detected in the lipid extracts.

Separation of the phospholipids. The diacyl phospholipids were separated by two-dimensional chromatography over silica gel-impregnated paper. A radioautogram of the separated lipids is illustrated in Fig. 3. Spot 1 represented 67% of the lipid phosphate and contained amine- and phosphate-reacting components as determined with spray reagents. The spot had the chromatographic mobility of PG and PME. Spot 2 contained 21% of the total lipid phosphate, reacted for phosphate and amine, and had the chromatographic mobility of PE. Spot 3 represented 11.8% of the lipid phosphate, reacted for phosphate, and had the chromatographic mobility of CL.

Separation of the deacylated phospholipids. Mild alkaline methanolysis quantitatively splits the diacyl phospholipids into lipid-soluble methyl esters of the fatty acids and water-soluble glycerol phosphate esters (17). These glycerol phosphate esters were separated and identified by paper, thin-layer, and column chromatography. A radioautogram of the esters

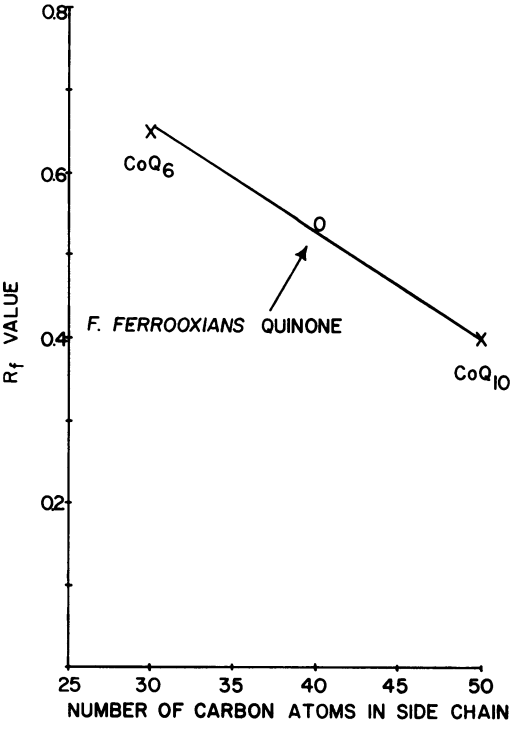

FIG. 2. *Chromatographic mobility of CoQ from F. ferrooxidans compared with CoQ-6 and CoQ-10 after separation by ascending reversed-phase paper chromatography (10).*

obtained after chromatography on aminocellulose paper is illustrated in Fig. 4. Esters can be detected with the chromatographic mobility of GPGPG, GPG, GPE, and GPME + GPDME + GPC. A radioautogram of the esters after separation on a cellulose thin-layer plate is illustrated in Fig. 5. This radioautogram shows spots which represent compounds which correspond to the chromatographic mobilities of GPGPG, GPE, GPME, GPDME, and GPC. Glycerol phosphate esters derived from the lipid by mild alkaline methanolysis were applied to Dowex 1 columns and were eluted with a gradient of ammonium formate and sodium borate. The elution volumes of the esters are illustrated in Fig. 6. Radioactive components with elution volumes corresponding to GPC, GPME, GPE + GPDME, GPG, and GPGPG were detected. Elution of the glycerol phosphate esters from Dowex-1 columns with 20 mM ammonium formate (*p*H 9.0) separated esters with the elution volumes illustrated in Fig. 7.

Comparison of the proportions of the lipid phosphate and identification of the lipids or glycerol phosphate esters from their chromato-

graphic mobility on paper, thin-layer, and column chromatographic systems is given in Table 2. The data are compatible with the phospholipid of *F. ferrooxidans* containing PE, PME, PDME, PC, PG, and CL.

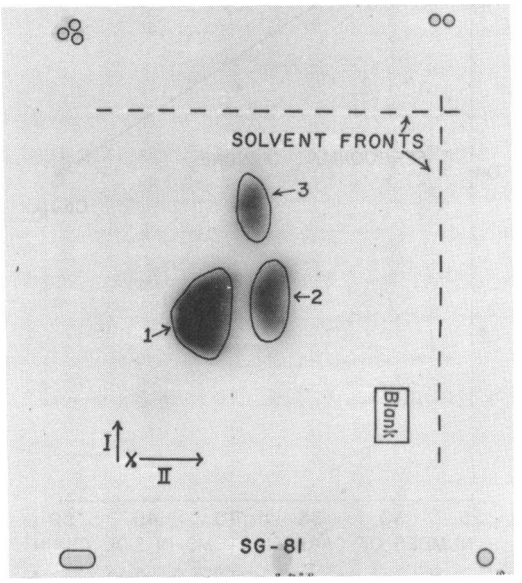

FIG. 3. *Radioautogram of F. ferrooxidans lipids containing ^{32}P after separation on silica gel-impregnated paper (20).*

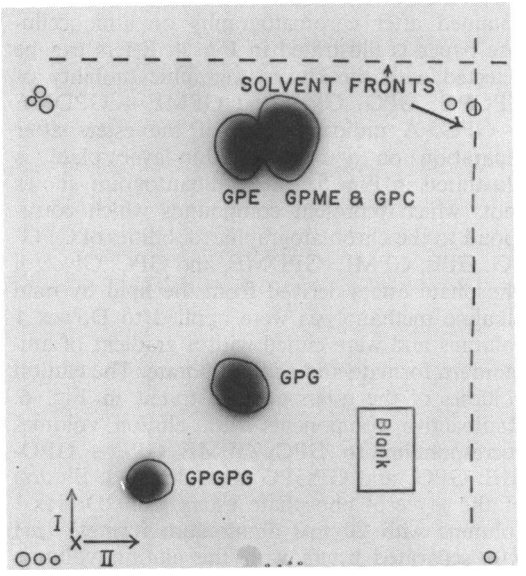

FIG. 4. *Radioautogram of glycerol phosphate esters derived from the phospholipids of F. ferrooxidans grown with ^{32}P after separation on aminocellulose paper (17).*

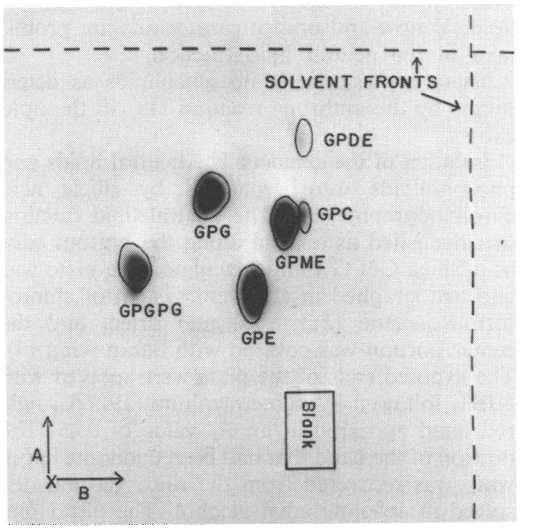

FIG. 5. *Radioautogram of glycerol phosphate esters derived from the phospholipids of F. ferrooxidans grown with ^{32}P and after separation over cellulose thin-layer plates.*

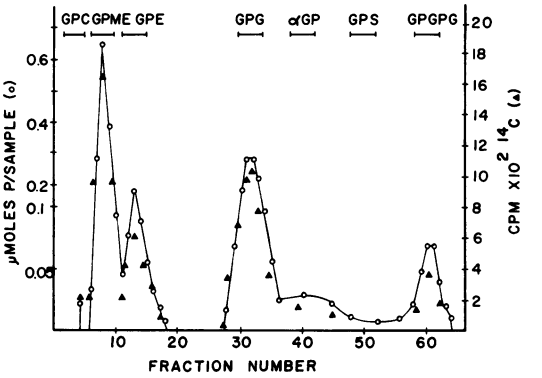

FIG. 6. *Separation of the glycerol phosphate esters derived from the phospholipids of F. ferrooxidans grown with ^{14}C by elution from a Dowex-1 column with an ammonium formate-sodium borate gradient (19). The elution volumes of authentic esters are illustrated at the top of the figure.*

Identification of the ethanolamine bases. The glycerol phosphate esters derived from the phospholipids were separated as illustrated in Fig. 5. The esters were located by radioautography, and the region containing each ester was cut out and eluted quantitatively with three volumes of water. The absorbant was separated from the water after each centrifugation between extractions. The glycerol phosphate esters were then hydrolyzed in 2 M HCl for 1 hr at 100 C, and the HCl was removed in a stream of nitrogen. The hydrochlorides were then applied to paper and subjected to descending chromatography. A

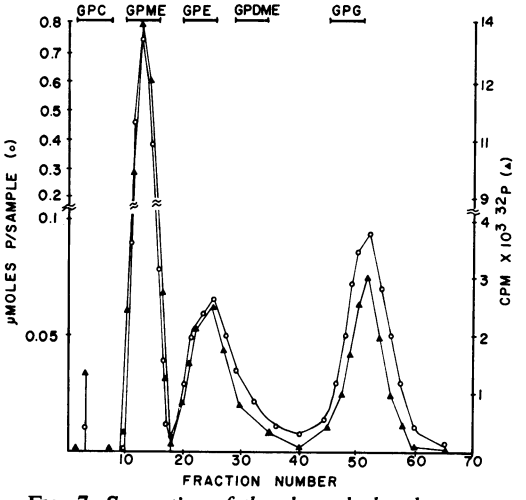

FIG. 7. *Separation of the glycerol phosphate esters derived from the phospholipids of F. ferrooxidans grown with ^{32}P by elution of a Dowex-1 column with 20 mM ammonium formate, pH 9.0. The elution volume of authentic esters is illustrated at the top of the figure. GPGPG was not eluted from the column.*

radioautogram after chromatography of the hydrolysates is illustrated in Fig. 8. The mobility in relation to CH was 0.84 for DME, 0.60 for ME, 0.15 for E, 0.06 for serine-hydrochloride, and αGP 0.00 when using authentic samples in this system. The hydrolysis of GPC yielded material with a mobility of 1.04 relative to CH; hydrolysis of GPDME yielded material with a mobility of 0.84 (DME); hydrolysis of GPME yielded material with a mobility of 0.64 (ME); hydrolysis of GPE yielded material with a mobility of 0.15 (E) after chromatography. Glycerol phosphate remains at the origin. Mild acid hydrolysis of glycerol phosphate esters derived from diacyl phospholipids quantitatively yields the base and αGP (14, 20).

Material eluted from the cellulose thin-layer plates with the mobility of GPG and GPGPG was mixed with authentic nonradioactive GPG or GPGPG and rechromatographed over aminocellulose, as in Fig. 4. The radioactive glycerol phosphate esters from *F. ferrooxidans* and the unlabeled authentic glycerol phosphate esters co-chromatographed in both cases.

The phospholipids of *F. ferrooxidans* have been reported to contain large amounts of PS (8). This lipid would give rise to GPS after mild alkaline methanolysis. No ^{32}P-labeled GPS was detected in the various chromatographic procedures of Fig. 4–7. A sample of the lipid from bacteria grown in the presence of $^{14}CO_2$ was deacylated and hydrolyzed in 2 M HCl for

TABLE 2. *Proportions of the phospholipids of F. ferrooxidans separated by different methods*

Lipid	Separation method[a]					
	Ester	SG-81[b]	AE-81[c]	TLC[d]	Column	
					A[e]	B[f]
PC	GPC			1.5	1	1
PME	GPME	67	49	42	42	50
PDME	GPDME			1		
PE	GPE	21	17	20	18	17
PG	GPG		23	23	23	20
Cl	GPGPG	11.8	8.4	13	9	

[a] Figures show percentage of lipid phosphate recovered from chromatography.

[b] Separation of the diacyl lipids on silica gel-loaded paper, as in Fig. 3. Spots were located by radioautography and eluted, and the phosphate was determined; recovery was 99%. The PME fraction contained PG, PDME, and PC.

[c] Separation of the glycerol phosphate esters derived from the lipids on aminocellulose paper, as in Fig. 4. Recovery was 97%. The GPME contains GPC and GPDME.

[d] Separation of the glycerol phosphate esters derived from the lipids on cellulose thin-layer plates as in Fig. 5. Recovery was 98%.

[e] Separation of the glycerol phosphate esters on Dowex-1 columns with an ammonium formate-sodium borate gradient as in Fig. 6. Recovery was 96%. The GPE fraction contains the GPDME.

[f] Separation of the glycerol phosphate esters on Dowex-1 columns with ammonium formate as in Fig. 7. Recovery was 90%, as the GPGPG was not eluted with this solvent. The GPDME is in the GPE fraction.

2 hr at 100 C. The hydrolysate was chromatographed in the system employed for Fig. 8. No ^{14}C-serine-hydrochloride could be detected by radioautography and there was no trace of amino nitrogen-containing material with the chromatographic mobility of serine-hydrochloride. Another portion of the lipid was partitioned twice against 4 M KCl at *p*H 2.0, recovered, and applied as a band to a Silica Gel G thin-layer plate. The lipid was chromatographed with a solvent of chloroform-methanol-6.7 M ammonium hydroxide (33:18.2:2.5, v/v). After drying in a vacuum oven, the center of the thin-layer plate was covered with Saran wrap and the exposed ends were sprayed with rhodamine, ninhydrin, and acid molybdate (6). A band containing both amino nitrogen and phosphate was detected at an R_F value of 0.60. The part of this band protected by the Saran wrap was recovered from the Silica Gel G and deacylated by mild alkaline methanolysis. These purification procedures re-

move small basic peptides from the phospholipids (19). The glycerol phosphate esters containing 0.27 μmole of phosphate were then hydrolyzed in 2 M HCl, and the HCl was removed in a stream of nitrogen. The hydrolysate was then applied to the c-2 type resin of a 75-cm column Technicon amino acid analyzer. The hydrolysis product was then eluted with a *p*H gradient between 2.75 and 6.10 in 5 hr, as described in *Technicon Instruction Manual AAA-1* (p. 38, 1967). One ninhydrin-positive component, containing 0.05 μmole with the elution volume of E, was detected. This corresponds to 18% of the total lipid phosphate, the expected proportion of GPE in the hydrolysis mixture. Less than 0.1 nmole of serine was detected in the lipid sample, although this concentration of authentic serine could be detected in the standard. Neither DME nor ME reacted significantly with ninhydrin under the assay conditions used.

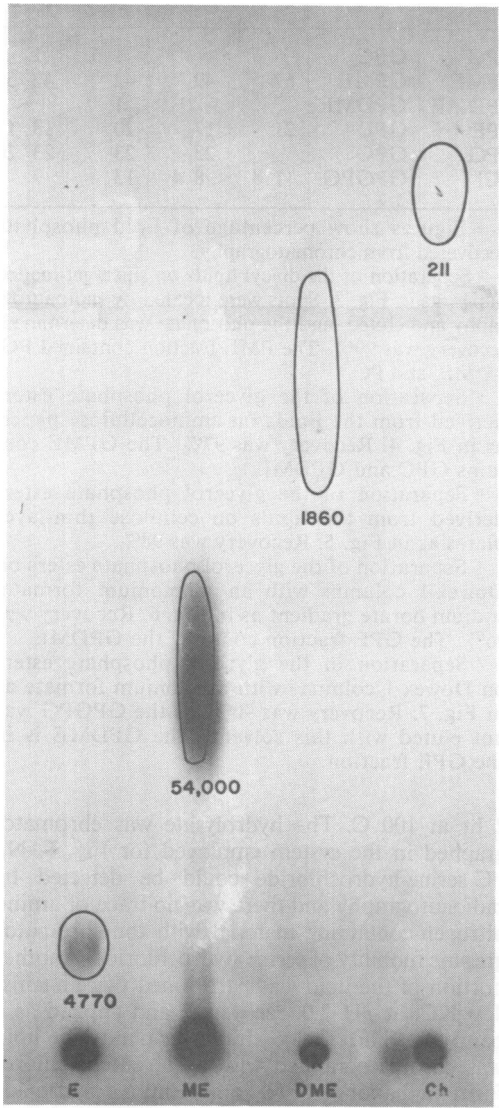

FIG. 8. *Radioautogram of the separation of the hydrochlorides of CH, DME, ME, and E that were derived from each glycerol phosphate ester by acid hydrolysis. The lipids of F. ferrooxidans grown with $^{14}CO_2$ were deacylated and the esters were separated as in Fig. 5. The areas of the chromatogram corresponding to the radioactivity were cut out and the esters were eluted and hydrolyzed. The hydrolysate was chromatographed on acid-washed paper, the areas corresponding to dark spots on the radioautogram were cut out, and the radioactivity was determined. The counts/minute above background for each hydrochloride are shown.*

Turnover of lipid ^{14}C and ^{32}P during growth at pH 1.5 and 3.5. The turnover of the glycerol phosphate esters derived from the diacyl phospholipids was examined. The bacteria were grown for 2.3 doublings with ^{32}P and ^{14}C and were washed by centrifugation. Equal portions were inoculated into nonradioactive medium at *p*H 1.5 and 3.5. The *p*H was maintained at these values by addition of 5 M NaOH at 20-min intervals throughout the growth cycle. At intervals, a sample of cells was harvested; the lipids were extracted, deacylated, and separated on cellulose thin-layer plates; the radioactivity in each glycerol phosphate ester was determined (Fig. 9). Since formation of large amounts of a reddish-brown suspension of $Fe(OH)_3$ precluded turbidimetric measurements, growth was assumed to follow the metabolic activity which was measured in terms of the amount of NaOH necessary to neutralize the H_2SO_4 generated as the end result of bacterial metabolism. The growth rates measured at *p*H 1.5 and 3.5 were similar (Fig. 9, A). In different experiments, the ^{32}P incorporated into bacteria was measured. After the cells were collected on a membrane filter (Millipore Corp., Bedford, Mass.), the ^{32}P was determined with a gas-flow counter. The ^{32}P-incorporation rate indicated a doubling time of approximately 6 hr for cultures grown at *p*H 1.5. At *p*H 3.5, the formation of ferric phosphate obscured the ^{32}P uptake as a measure of growth.

There was essentially no turnover of the ^{14}C or ^{32}P in GPC or GPME at either *p*H (Fig. 9, B and G). These lipids account for 43% of the total lipid phosphate. The most active phospholipid metabolism involves loss of both ^{32}P and ^{14}C from GPG and GPE in cells grown at *p*H 3.5 (Fig. 9, E and F). The most rapid turnover was the loss of half the isotope in 11.2 hr (about two doublings). This turnover rate was about one-half that seen with GPG in other gram-negative bacteria (20). In the GPG, both the ^{14}C and ^{32}P turned over at the same rate. In *Haemophilus parainfluenzae*, the nonacylated glycerol and phos-

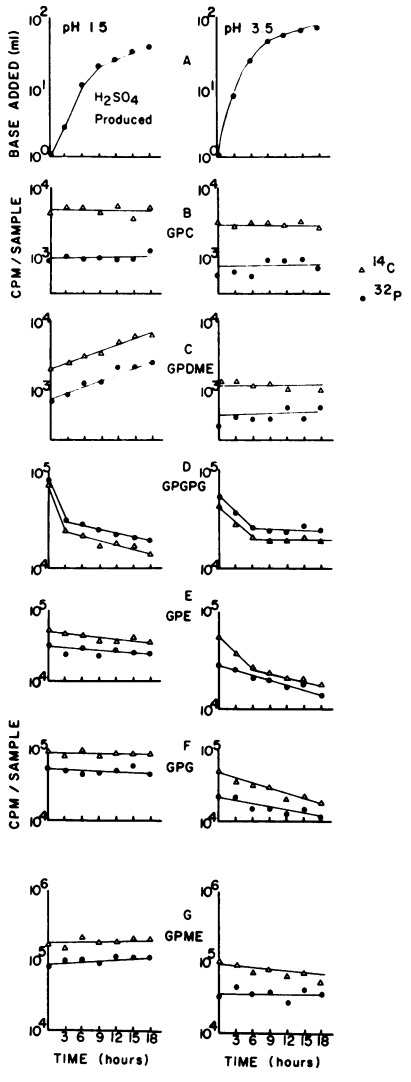

FIG. 9. *Turnover of ^{14}C and ^{32}P in the glycerol phosphate esters derived from the phospholipids of F. ferrooxidans during growth at pH 1.5 and 3.5.*

phate turned over at three times the rate of the diacylated glycerol (20). The turnover of GPGPG seems to be biphasic during growth at both *p*H values (Fig. 9, G). The apparent increase in radioactivity of GPDME may not have been significant, as this lipid represented 1% of the phospholipid.

DISCUSSION

In this study, a strain of *F. ferrooxidans* picked from a single isolated colony was used. The strain was recently isolated from a typical habitat and had biochemical and morphological characteristics of the species (16). The preliminary report of a unique phospholipid composition for *F. ferrooxidans* (8) could not be confirmed by using this strain. These authors claim that 20% of the dry weight of the cell is lipid. Since bacterial membranes contain 30% lipid (13), the membrane should account for about 47% of the dry weight of cells. Electron micrographs of *F. ferrooxidans* do not show that half the cell volume is occupied by membrane (12). *F. ferrooxidans* reportedly contains PS as 49% of its phospholipid (8). This diacyl phospholipid had the chromatographic mobility of PS on silica gel paper, and the authors claim that the deacylated derivative GPS could be identified chromatographically. No analysis for lipid serine was reported. The authors also claim the lysophospholipids were present in the bacteria. From this study of *F. ferrooxidans*, the total fatty acids of this strain represented only 2% of the dry weight, and essentially no lysophospholipids, PS, GPS, or serine-hydrochloride could be detected in the lipid extract. The phospholipid composition of this strain of *F. ferrooxidans* was similar to that reported for the thiobacilli (1, 15). *F. ferrooxidans* was reported to contain vitamin K_2 (5). The strain used in this study contained CoQ-8 as the only quinone present in detectable amounts in the lipid extract.

F. ferrooxidans grows at a hydrogen ion concentration seven orders of magnitude below the *p*H optimum for the function of its electron transport system. From the data illustrated in Fig. 9, the phospholipid metabolism of *F. ferrooxidans* is relatively inactive when compared to gram-negative organisms growing at *p*H 7 (20). Lowering the external *p*H by a factor of two does not significantly increase the slow rate of phospholipid metabolism. Changing the *p*H of the growth medium also does not affect the rate of phospholipid synthesis significantly, because the proportions of the phospholipids are remarkably similar during bacterial growth at either *p*H value.

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