Metabolism of Phosphatidylglycerol and Bis(monoacylglycerol)-phosphate in Macrophage Subcellular Fractions*

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Bis(monoacylglycerol)phosphate (BMP) is synthesized from exogenous phosphatidylglycerol (PG) by macrophages (Cochran, F. R., Roddick, V. L., Connor, J. R., Thornburg, J. T., and Waite, M. (1987) J. Immunol. 138, 1877-1883). Previous work from our laboratory showed that arachidonic acid in BMP was released by the macrophages upon challenge of the cells with PMA (Cochran, F. R., Connor, J. R., Roddick, V. L., and Waite, M. (1985) Biochem. Biophys. Res. Commun. 137, 150-156). Here we extend these studies using a model cultured cell line of macrophages, RAW 264.7. When PG labeled with 32P and [3H]PG in both moieties was added to the culture medium, [3H]BMP was synthesized in a time-dependent manner. Fractionation of cell homogenates on a discontinuous sucrose gradient in which the light membranes were floated from dense sucrose showed an enrichment of [3H]BMP in light membrane fractions. The precursor [3H]PG was also found in the light fractions, but relative to the [3H]BMP, was more abundant in the denser membrane fractions. The appearance of [3H]PG and [3H]BMP in the light membrane fraction was time-dependent which suggested that the initial uptake and metabolism of [3H]PG was into the denser membrane. Incubation of the light membranes under conditions that are optimal for the lysosomal phospholipase A2 led to significant metabolism of [3H]PG. Both degradation of [3H]PG to water-soluble compounds and its conversion to acylphosphatidylglycerol occurred while no lysosomal PG was detected. On the other hand, little BMP was found to be degraded. From these studies we postulate that in lysosomes acylphosphatidylglycerol is a precursor of BMP and that the previously reported turnover of arachidonic acid by BMP may occur via transacylation rather than hydrolysis.

Bis(monoacylglycerol)phosphate (BMP)† is an unusual phosholipid that is reported to have the phosphate esterified to the sn-1 carbon of both glycerol moieties (1, 2) (Scheme I). In alveolar macrophages this phospholipid is not synthesized de novo, rather, it is derived from exogenous PG (3). It was postulated subsequently that the alveolar macrophage took up PG from lung surfactant (4) and that this would account for its high content of BMP, roughly 15-18% of the total cellular phospholipid (3, 5). Rat liver can be induced to synthesize BMP upon treatment of the animal with Triton WR1339, 4,4'-bis(diethy laminooxy)-a,α-diethy diphenyl ethano, or chloroquine (6-12). Treatment with these agents caused the BMP to be associated with light, lipid-rich, myelin-like bodies that were thought to be of lysosomal origin (9, 13). In most studies BMP is found primarily, if not exclusively, in lysosomes (6-9). Baby hamster kidney cells could also be induced to synthesize BMP concomitant with proliferation of lysosomes (14). Both PG and cardiolipin, when injected into rats, were converted into BMP (14) and a crude lysosomal preparation was shown to convert cardiolipin, PG, and lyso-PG to BMP (15). A number of studies have shown that BMP accumulates in tissues of patients with inherited lipid storage diseases (16-18). These studies, however, imply that the BMP thus formed is associated with myelin-like bodies derived from lysosomes that "represent the later stages in the lysosomal life span" (19) and may be metabolically effete.

Our studies on arachidonic acid turnover and leukotriene synthesis in alveolar macrophages indicated that BMP rapidly incorporated arachidonic acid which could be released upon stimulation of the cells with 12-0-tetradecanoylphorbol 13-acetate (3, 20). The turnover of BMP was related to the turnover of phosphatidylinositol, in keeping with the observation of others that synthesis of BMP occurs via a transacylation mechanism involving phosphatidylinositol (21) and other phospholipids (22). Interestingly, Dvorak et al. (23) showed that myelin-like cytoplasmic organelles in macrophages and mast cells rapidly turned over arachidonic acid. While detailed phospholipid analyses were not carried out in this study, it is possible that these organelles are the source or similar to those found by Brotherus et al. (13), Matsuzawa and Hostetler (7), and Somerharju and Renkonen (14). The study of Dvorak et al. (23) demonstrated that not only were these organelles metabolically active, but they also were translocated within the cell upon phagocytic stimulation.

It is the purpose of this paper to demonstrate that BMP synthesized in situ from PG by a cultured macrophage cell line (RAW 264.7) becomes associated with a light density organelle and that the lipids in this fraction are actively metabolized. Under conditions that are optimal for a phospholipase A1 in rat liver and macrophage lysosomes, PG, the precursor for BMP is either degraded or acylated to form APG. On the other hand, BMP is catabolized only slightly which suggests that the previously reported stimulation of...
arachidonic acid turnover in BMP upon phorbol 12-myristate 13-acetate stimulation may result from transacylation rather than hydrolysis. Here we postulate a pathway of BMP synthesis that includes APG as an intermediate and an alternative to a pathway that involves lyso-PG. We believe this study reflects the metabolism in situ of the alveolar macrophage since it normally has a high content of BMP that is postulated to be derived from PG in the lung surfactant (4).

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Isolation**—RAW 264.7 (macrophage, Abelson leukemia virus-transformed, BALB/c) cells, passages 5–20, were used in all experiments (American Type Culture Collection). RAW 264.7 cells were cultured in Dulbecco’s minimal essential medium with high glucose (Gibco), 10 mM Hepes that included 10% heat-inactivated fetal bovine serum, and 50 µg/ml gentamicin sulfate. The cells were routinely split every 3–4 days at 1:2. Two 75-cm² flasks were used for every 245 × 245-mm sterile culture plate. Incubation was at 37 °C in 5% CO₂, 95% air. After 3.5 days the medium was changed and new media plus 40 µM [3H]glycerol-PG or [32P]PG were added (20-h incubation, plus PG). For cells cultured with [3H]- or [32P]PG for 3 h, new medium with 40 µM [3H]- or [32P]PG was added after 4 days. At the end of labeling incubations the cells were scraped into media, immediately resuspended in cold isotonic saline, and centrifuged at 100 × g for 5 min. A cell count was done and one 245 × 245-mm plate yielded approximately 2.5 × 10⁶ cells. The cells were then washed twice in saline and centrifuged. Finally, cells were resuspended in 1 mM NaHC0₃ and put on ice.

**Cell Homogenization and Fractionation**—The cells were homogenized 30 times with a Dounce apparatus which produced 90–95% cell disruption. The disrupted cells were centrifuged at 1000 × g for 10 min. Both supernatant and the resuspended pellet were saved and the supernatant mixture was used for separation of the BMP fraction. The most satisfactory gradient used was a discontinuous floatation sucrose gradient (34). The 1000 × g supernatant mixture was made 45% (w/v) with saturated sucrose and brought to a volume of 12 ml. Over 11 ml of the supernatant (fraction 8) was pipetted, 9 ml of 40% (fraction 6), 7 ml of 35% (fraction 4), 7 ml of 30% (fraction 2), and 2 ml of 9% sucrose (fraction 1), all in 0.001 M Tris-HCL pH 7.4 (Scheme II).

These tubes were centrifuged at 64,000 × g for 290 min in a Beckman L5-50 centrifuge in a Beckman SW 27 rotor and the fractions were collected by hand. The membrane-rich fractions were at the interfaces where visible bands showed fractions 1, 3, 5, 7 and fraction 5, the original 1,000 × g supernatant fluid. After fractions were collected and volumes recorded, the fractions were rapidly freeze/thawed using cold acetone and dry ice (25).

**Enzyme Assays and Protein and Lipid Analysis**—Lysozyme was measured by the decrease in optical density at 450 nm with Micrococcus lysodeikticus as a substrate using a modification of the method of Kass et al. (28) as described by Borregaard et al. (27). Nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome c reductase and cytochrome c oxidase were measured by the method of Sottocasa et al. (28), β-Glucuronidase was determined by a modification of the method of Talay et al. (29), as described by Canonico and Bird (30). Acid phosphatase was determined by the method of Gianetto and de Duve (31), using β-glycerophosphate as a substrate. Sialyl transferase was measured by the method of Clark and Smith (32).

Protein was determined by the method of Peterson (33) and the phosphorus content of lipids was determined by the method of Chalvardjian and Rudnicki (34).

**Lipid Extraction and TLC**—Lipids were extracted by a modification (4) of the method of Bligh and Dyer (35). The chloroform layer was dried under a stream of N₂ and resuspended in a small amount of chloroform and spotted on pre-spread Silica G or GHL TLC plates (Analtech). The plates were developed first in chloroform/methanol/glacial acetic acid (90:30:12, by volume) to 15 cm and dried and, second, in hexane/ether/formic acid (90:60:4, by volume) to 17 cm. The radioactive bands were identified by a Bioscan System 2000 imaging scanner and the mass localized by iodine vapor. These bands of silicic acid that contained the lipids were scraped into vials, 300 µl of methanol added to wet the silica and Ecollume (ICN Radiochemicals) scintillant was added for scintillation counting.

**Phospholipase Assays**—After isolating the gradient fractions the radioactivity in each was determined. Approximately 10,000 dpm of each fraction were used per assay in duplicate. The pH was adjusted to 4.5 with a 10-µl aliquot per ml of 1 M sodium acetate, pH 4.0, that contained 20 mM EDTA. Controls were boiled for 5 min and samples were also run without adjustment of the pH. The samples were incubated at 37 °C for periods of time up to 2 h. The reaction was stopped by the addition of methanol/chloroform (2:1, by volume) and extracted. Aliquots from chloroform and methanol water layers were counted and the lipids in the CHCl₃ layer were separated by TLC.

The phospholipid A₁ activity on micelles of pure lipids was carried out at various pH values as described by us previously (25, 36) except the concentration of substrate was 2 nM in 4 mM Triton X-100.

**Radiolabeled Lipids**—The substrate for phospholipase A₁, 1-[3H] acyl-DAPG, was prepared by chemical acylation of 1-[3H]PG with oleoyl anhydride in pyridine. The 1-[3H]PG was produced from 1-[14C]-9,10-palmitoylethanolamine by using phospholipase D, according to the procedure described by Comfurius and Zwaal (37). The 1-[3H]PG was prepared as described earlier (4). 1,2,3-[3H]Glycerolabeled PG was produced biosynthetically using Escherichia coli BB 26-36 strain (generously provided by Dr. D. Leuking, Michigan Technological University). Bacteria were first checked to verify growth in LB medium but were growth inhibited in glycero1-free minimal medium (E8). Bacteria from a colony showing growth were then used to seed 5 ml of minimal media E supplemented with 50 µM 1,2,3-[3H]glycerol (1 nCi). The bacteria were then grown for 8 h at which time the media with cells were extracted according to Bligh and Dyer (35), modified by the addition of 100 µl of acetic acid. The extracted lipids were separated by TLC using Silica H plates (Analtech) and chloroform/methanol/acetic acid (80:30:12, by volume) as the mobile phase. The separated lipids were located using the Bioscan radioactivity imaging scanner and the bands identified as phosphatidyl ethanolamine and PG were scraped and eluted from the silica by extraction as described above. The phosphatidylethanolamine was converted to PG using a 50% glycerol buffer and phospholipase D isolated from cabbage (39). The 1-[3H]alkyl-PG was synthesized from 1-[3H]alkylphosphatidylcholine as described previously (4); 1-[3H]
alkylphosphatidylcholine was the generous gift of Dr. Robert Wykle (Bowman Gray School of Medicine).

RESULTS

Macrophage-derived RAW 264.7 cells cultured with PGs labeled separately with [2-3H]glycerol and with 32P demonstrated that both glycerols and the phosphate of PG are incorporated as a unit into BMP in this cell line (Table I). As can be seen, the product BMP had a ³H/³²P ratio of 3.11, very close to that of the starting [³H]/[³²P]PG ratio of 3.5. These results with RAW 264.7 cells suggest that the synthetic pathway for BMP from PG is similar to or the same as that in alveolar macrophages (4). The PG derived from the cells had a slightly lower ratio, 1.9, that suggested some metabolism of that lipid had occurred, possibly through the exchange of the base glycerol moiety.

Fig. 1 shows that at the earliest time point taken, 3 h, the amount of cell-associated [³H]PG was twice the amount of [³H]BMP. Shortly thereafter, this relationship changed and [³H]BMP became the major labeled lipid indicative of a precursor-product relationship between PG and BMP. However, if the precursor PG was radiolabeled in either of the acyl moieties, little radiolabel was recovered in the product BMP (data not shown). In that case, the radiolabeled acyl groups were distributed among all other glycerides in the cells. Together, these results demonstrate that the bis(glycerophosphate) is incorporated into the product BMP, whereas the acyl chains are removed during this metabolic conversion.

We demonstrated that the removal of the acyl group at position-1 was essential to the synthesis of BMP, as we showed in the alveolar macrophage (4). This would provide additional evidence that the RAW 264.7 cells are a good model for the alveolar cells. Fig. 2 shows that 1-alkyl PG is metabolized at a much slower rate than the diacyl PG (cf. Fig. 1) and that very little is converted to BMP. It appears, therefore, that the removal of the acyl chain at position-1 of the PG is obligatory and that the presence of the 1-acyl group facilitates the metabolism of PG by the RAW 264.7 cells.

Since the extent of PG metabolism is quite different at early and later time points, we chose 3 and 20 h as times to study the localization of the precursor PG and product BMP in the RAW 264.7 cells using the discontinuous gradient flotation centrifugation (Scheme II). The system reported here gave the best separation of the radiolabeled BMP and the other cellular organelles (cf. Figs. 3 and 4) of the various gradient systems tested. The main fraction of interest, fraction 1 with the highest percentage of [³H]BMP, had measurable lysosomal marker but no endoplasmic reticulum present. Furthermore, the distribution of the marker enzymes did not change significantly from 3 to 20 h incubation of the cells with PG (data not shown). Markers for plasma membranes (ConA binding), Golgi (sialyl transferase), and mitochondria (cytochrome c oxidase) were examined in other gradient systems and all were found to be in fractions that would be as dense or denser than fraction 5 in the system described here.

As shown in Fig. 3, the radiolabeled BMP was found to a

![Fig. 1. RAW 264.7 cells were cultured with 4 μM [2-3H]glycerol-PC (1 × 10⁶ dpm/dish) for the indicated period of time in 35 mm dishes (1 ml, total volume). The cells were then extracted and the lipids separated as described under "Experimental Procedures." The total radioactivities in cellular lipids recovered at the different time points were: 3 h, 11,200 dpm; 6 h, 17,300 dpm; 9 h, 16,000 dpm; 21 h, 20,800 dpm; and 28 h, 21,100 dpm. These results are the average of duplicate experiments. PC, phosphatidylcholine.

![Fig. 2. The experiments with [³H]1-alkyl PC were carried out as described in the legend to Fig. 1, except that 2 × 10⁶ dpm of labeled PG were used per dish. No differentiation between 1-alkyl and 1-acyl product is made here. The total radioactivities recovered at the different time points were: 1 h, 14,800 dpm; 8 h, 30,000 dpm; 14 h, 35,400 dpm; and 26 h, 40,800 dpm. These results are the average of triplicate experiments. PC, phosphatidylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.](http://www.jbc.org/)

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**Table I**

| Product  | ³H   | ³²P  | ³H/³²P |
|----------|------|------|--------|
| BMP      | 9514 | 3063 | 3.11   |
| PC       | 1353 | 718  | 1.90   |
| All others | 1226 | 422  | 2.91   |

*Initial ratio in PG = 3.5.*
FIG. 3. The lipids from cells cultured for 3 h with [3H]PG were extracted and separated as described under "Experimental Procedures." The total amount of [3H]BMP in each fraction was calculated as the percentage of [3H]BMP in that fraction times the total radioactivity. The percent distribution was then calculated by dividing the amount of [3H]BMP in that fraction by the total amount of [3H]BMP recovered times 100. The total amount of [3H]BMP was 1.72 × 10⁶ dpm in this experiment. These results are representative of six separate experiments.

large extent in the lighter fractions 1 and 3. Comparison of the precursor [3H]PG with the product [3H]BMP further substantiates the enrichment of BMP in the less dense fractions. In the cells cultured for 3 h with [3H]PG the ratio of [3H]BMP to [3H]PG increased from 0.4 in fractions 6-8 to 1.3 in fraction 1 (Fig. 5). Since degradation of ³H-lipids produces water-soluble ³H-products, radioactivity in the methanol-H₂O phase of the centrifugation fractions was also determined. The determination of radiolabel in the CHCl₃ and methanol-H₂O phases of the extracts showed that the majority of the non-lipid radioactivity was recovered in the more dense fractions 7 and 8 (Table II). In most cases the amount of radiolabel recovered in the methanol-H₂O phase of fractions 7 and 8 was higher when compared with the 1000 × g supernatant mixture which suggests that some catabolism may have occurred during the manipulation of the sample. More lipid was degraded in the samples from cells cultured for 3 h when compared with 20-h cultures (ratio of 2.7 versus 7.7). In determinations not shown, a significant shift of [3H]BMP from fractions 7 and 8 to the top of the gradient occurred with the longer culture period.

We next sought to find if BMP could be catabolized in cell homogenates and the isolated fractions. Such a finding would indicate that BMP is metabolically active, as we previously demonstrated in intact alveolar macrophages (23). In those studies with intact alveolar macrophages, no appreciable changes in the amount of BMP occurred even though arachidonic acid was released in response to phorbol 12-myristate 13-acetate challenge. Here we used conditions that should lead to net catabolism of lipids via deacylation. Since BMP is esterified at the primary hydroxyl of the glycerols, phospholipase A₁ might be responsible for the degradation of BMP. We (25, 36, 40) and others (41, 42) demonstrated the presence of an active phospholipase A₁ in the lysosomes of liver and macrophages, therefore, we first established here that RAW 264.7 cells had a similar phospholipase A₁. When assayed in the cell homogenates, the hydrolysis of PG was optimal at pH 4 if EDTA were present (data not shown). In these studies 1-[³H]PG or 1-[³H]DAGP were used as model substrates for BMP due to its limited availability in pure form.

The distribution of the phospholipase A₁ was similar to that of lysozyme with a low but measurable activity in fraction 1 and the bulk of activity in the more dense fractions (Fig. 6). The finding that some catabolism of [³H]lipid to water-soluble products derived from [³H]glycerol-labeled PG occurred in the more dense fractions during cell fractionation (Table II) is consistent with the distribution of the phospholipase A₁ in fractions 7 and 8. Even though the phospholipase A₁ is optimally active at pH 4, 25% of maximal activity was found at pH values close to that used for cell fractionation.

FIG. 4. The cell fractions were isolated and assayed for their NADPH-cytochrome c reductase (A, endoplasmic reticulum) and lysozyme (B, lysosome) activities as described under "Experimental Procedures." The reduction activity was measured with 50-, 100-, 150-μl aliquots of each sample and the data are expressed as the total change in absorbance at 550 nm. These results were obtained from the same gradient fractions used for Fig. 3.
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In the experiment described in Fig. 3 from the TLC analysis of the products in each fraction. The difference between the sum of $[^{3}H]$BMP and $[^{3}H]$PG and 100% is the result of small amounts of other lipids becoming labeled (cf. Fig. 1). These results are representative of four separate determinations.

Table II

| Culture time | Fraction | CHCl$_3$/dpm methanol-H$_2$O | dpm CHCl$_3$/dpm methanol-H$_2$O | g sol.
|--------------|----------|------------------------------|---------------------------------|------|
| 3 h          | 1        | 20.0                         | 10.0                            | 7.3  |
|              | 2        | 7.7                          | 7.1                             | 8.0  |
|              | 3        | 8.0                          | 0.91                            | 8.6  |
|              | 4        | 2.7                          | 1.08                            | 7.7  |

We also cultured the cells with $[^{3}H]$PG for 3 h so that the metabolism of PG and BMP could be monitored. To determine if the $[^{3}H]$lipids in the isolated fractions could be catabolized under the conditions optimal for the phospholipase A$_1$, the fractions were acidified to pH 4.5 and incubated in the presence of EDTA. Fractions 1, 3, and 5 were capable of degrading $[^{3}H]$lipid to water-soluble radiolabeled product, whereas fractions 7 and 8 had undergone partial degradation during isolation (Table II) and little further degradation was noted here (data not shown). The hydrolysis observed in fraction 1 was more rapid at pH 4.5 than when the pH was not adjusted from 7.4 and EDTA omitted (Fig. 7, A and B). Chromatographic analysis of the lipid fraction following incubation at pH 4.5 showed that a compound was formed that co-chromatographed with $[^{3}H]$APG in addition to the increase in radioactivity in the methanol-water fraction (Fig. 7A). The identity of $[^{3}H]$products has been verified by two-dimensional TLC (43). This metabolism was both time and pH dependent (cf. Fig. 7, A and B). Furthermore, APG appeared to have PG as the primary precursor rather than BMP. Here a slight decrease in BMP was seen although with longer incubations (2-3 h), up to a 20% increase in BMP was seen (data not shown). Similar results were obtained with fraction 3. It appears, therefore, as if PG undergoes a transacylation that forms the APG, in addition to being deacylated.

FIG. 5. The amount of $[^{3}H]$PG and $[^{3}H]$BMP was determined in the experiment described in Fig. 3 from the TLC analysis of the products in each fraction. The difference between the sum of $[^{3}H]$BMP and $[^{3}H]$PG and 100% is the result of small amounts of other lipids becoming labeled (cf. Fig. 1). These results are representative of four separate determinations.

DISCUSSION

BMP is known to be synthesized from exogenous sources of lipid even though it is not synthesized de novo in macrophages. Other cell systems were shown to synthesize $[^{32}P]$BMP from inorganic $^{32}$P although the pathway was not established (44). The various studies cited in the Introduction strongly suggest that BMP accumulation in inherited or drug-induced lipid storage diseases is derived from endogenous membrane sources. While direct evidence for the lipid precursor in BMP synthesis is lacking in situ, PG and related lipids appear to be the most likely candidates. Indeed, exogenously added lyso-PG can be converted to BMP by isolated cell fractions (21, 22).

Here we report that exogenous PG and its product BMP are incorporated into light density membranes with properties similar to those reported for the BMP containing membranes derived from endogenous lipids. A similar fraction obtained by differential centrifugation of cultured baby hamster kidney cells also yielded a "floating" fraction rich in BMP (13). One characteristic in common is the low level of total lysosomal marker enzyme activity in these light membranes, even though there was an enrichment in lysosomal enzyme specific activity in the studies with baby hamster kidney cells. However, as we show here, these membranes are metabolically active since metabolism of the lipid takes place under the appropriate conditions. While it is tempting to speculate that the phospholipase A$_1$ we previously described in the lysosomes of macrophages is responsible for hydrolysis of PG, direct evidence is lacking. Both Huterer and Wherrett (45) and Matsuzawa and Hostetler (46) demonstrated that lysosomes are capable of degrading BMP, although the pathway is not.
Our results suggest that the extracellular [3H]PG is first taken into the dense membrane fraction. This suggestion is based on the shift in 3H-labeled lipid from dense to light membrane fractions with time and the greater proportion of the precursor [3H]PG in the dense fractions, relative to the product [3H]BMP. Our attempts to further fractionate the more dense membrane fractions has not shed additional light on the type of organelle that initially metabolizes the [3H] PG. However, the most reasonable interpretation of our results is that the initiation of BMP synthesis originates in the dense, primary lysosomes that become lipid filled and less dense. The low hydrolysis rate of BMP under conditions known to activate lysosomal phospholipases would lead to this lipid accumulation. The lipid-filled lysosomes, however, still retain phospholipase activity and the capacity to degrade substrates, such as PG. These light, lipid-filled lysosomes also retain anabolic activity that leads to the formation of APG that has an undetermined stereochemistry. Our observation that significant hydrolysis of lipid occurs in the dense fractions is consistent with the conclusion that the precursor [3H] PG rather than the product [3H]BMP is attacked by phospholipase(s). This suggestion is based on the observations that water-soluble products are found in the [3H]PG-rich dense fractions and more hydrolysis occurs at the shorter culture time, 3 h, when more precursor [3H]PG is present (Table II).

Similar conclusions on the distribution of BMP have been reached by others who studied the synthesis of BMP from endogenous sources. The work of Hostetler’s group (1, 50) who used drugs targeted for lysosomes demonstrated that BMP accumulates in secondary lysosomes, although the origin of the secondary lysosomes was not established in these studies on lipid storage diseases. It was demonstrated, however, that PG of microsomal origin is the likely precursor for de novo synthesis of BMP in rat liver (50). It appears, therefore, that the metabolism and distribution of BMP in situ from an exogenous source of PG, as shown here and by Somerharju and Renkonen (1), is similar to induced synthesis from endogenous PG, as shown by others (50).

The formation of APG in large quantities was unexpected since we (23) and others (1) find that relatively small amounts of this compound are synthesized in intact cells. Presumably, the conversion of PG to APG occurs via transacylation since the factors required for lipid acylation, ATP and CoA remain in the original supernatant fractions. In transacylation reactions an acyl chain is transferred from one glyceride directly to an acceptor molecule. Both Huterer and Wherrett (22) and Matsuzawa et al. (21) have demonstrated that BMP can be formed via a transacylation in which lyso-PG was the acceptor molecule. While those studies are of considerable importance to our understanding of possible metabolic routes for the production of BMP, its quantitative significance is unknown since we find little lyso-PG accumulation. However, assuming that PG is the ultimate precursor for BMP, a synthetic route via lyso-PG would be initiated by a phospholipase A2 followed by transacylation. We found phospholipase A2 in lysosomes of macrophages active at pH 4.5 (40). Alternatively, transacylation of PG to yield APG would be followed by hydrolysis of an acyl chain by phospholipase A2. Huterer and Wherrett (22) suggested that a phospholipase A2 might be responsible for transacylase activity since the acyl donor phospholipid used in their studies was labeled in the acyl group at position-2 of the glycerol moiety. The latter pathway, we believe, is compatible with the results reported here since phospholipases A2 have been shown to degrade DAPG to APG and BMP (47). A third alternative pathway that we currently

![Figure 7](http://www.jbc.org/)

**FIG. 7.** The lipids in fraction 1 of cells radiolabeled for 3 h with 40 μM PG (4.3 x 10^6 dpm) and incubated at pH 4.5 (A) for the designated times were extracted into CHCl3. The radioactivity of the lipids was determined by counting the silicic acid that contained those lipids following their separation by TLC. A boiled sample was served at the zero time control. The experimental conditions in B were the same as those described for panel A except the incubation pH was 7.4. These results are representative of four separate experiments.

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favor could proceed by phospholipase A₁ hydrolysis of APG (formed from PG by transacylation) with subsequent acyl migration of the acyl group(s) from position-2 (2') to positions 1 or 3 of the glycerols. The attractive feature of this pathway is that it takes into account the observation that the acyl group at position 1 of the glycerol backbone must be removed in the synthesis of BMP (Fig. 2 and Ref. 4). This third pathway is also consistent with the higher amount of unsaturated acyl groups we found in BMP from alveolar macrophages (5). At present we cannot distinguish between these alternatives or if more than one pathway occurs. For a full understanding of the conversion of PG to BMP, it will be necessary to determine the stereochemistry of each compound formed since the stereoconversion of the glycerol moiety should have a major role in determining the metabolic pathway followed.

A recent study in our laboratory has defined the stereochemical conversion of 1,2-diacyl-sn-glycerol-3-phosphate (PG) to 3-acyl-sn-glycerol-1-phosphate-1',3' [3'acylglycerol] (BMP). PG, labeled with 14C in the number one carbon of the glycerol backbone of PG, underwent a flip of the glycerol upon conversion to BMP such that the sn-3 carbon now was labeled. This accounts for the retention of both glycerol moieties (Table I) and the failure of 1-alkyl PG to be converted to BMP (Fig. 2).

In summary, we have isolated a light density membrane fraction from a culture macrophage cell line grown in the presence of exogenous PG that is rich in BMP. This fraction contains low levels of lysosomal marker enzymes, yet actively metabolizes PG, APG, and BMP. We consider this membrane fraction to provide a useful cell-free system for the study of synthesis and turnover of BMP. This membrane fraction will be of considerable interest in studies of BMP turnover stimulated by the phorbol ester 12-O-tetradecanoylphorbol 13-acetate since we previously demonstrated that 12-O-tetradecanoylphorbol 13-acetate caused the liberation of large quantities of arachidonic acid from BMP in alveolar macrophages.

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