Biosynthesis of 1-Alkyl-2-acetyl-sn-glycero-3-phosphocholine (Platelet-activating Factor) from 1-Alkyl-2-acyl-sn-glycero-3-phosphocholine by Rat Alveolar Macrophages

PHOSPHOLIPASE A₂ AND ACETYLTRANSFERASE ACTIVITIES DURING PHAGOCYTOSIS AND IONOPHORE STIMULATION

(Received for publication, May 24, 1982)

Daniel H. Albert‡ and Fred Snyder§

From the Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

1-Alkyl-2-acetyl-sn-glycero-3-phosphocholine (alkylacetyl-GPC) comprises 11% of the total phospholipids of rat alveolar macrophages. This endogenous pool of alkylacyl-GPC was prelabeled by incubating the macrophages with [1,2-³H]alkyllyso-GPC (54 Ci/mmol), which enters the cells and is acylated. The effect of various stimuli on the synthesis and release into the media of labeled alkylacyl-GPC (platelet-activating factor) from the cells was used to establish the role of inactive alkylacyl-GPC as a precursor of the biologically active derivative. A phagocytic agent (zymosan, 100 µg/ml) and an ionophore (A23187, 2 µM) stimulated the release of both alkylacyl-GPC and alkyllyso-GPC into the media at the expense of cellular alkylacyl-GPC. Phospholipase A₂ activity (at pH 4.5 and in 1 mM EDTA) was also increased in the media. The stimulatory effect of zymosan and the ionophore on alkylacyl-GPC release was prevented by mepacrine (0.1 mM), an agent that inhibits the release of fatty acids from phospholipids. These data indicate that phospholipase activity is required for the biosynthesis of alkylacyl-GPC. However, since the inhibitory effect of mepacrine was not apparent when acetate was present, it appears that the acetylation step is rate limiting. Exposure of alveolar macrophages in culture to zymosan or A23187 stimulated acetyltransferase activity 250-300%. In contrast, phorbol myristate acetate (1.6 µM), which stimulated the accumulation of lyso phospholipids but not the level of alkylacyl-GPC in the media, did not substantially increase acetyltransferase activity. We conclude that alkylacyl-GPC serves as a precursor of alkylacyl-GPC and that the production of this potent mediator by rat alveolar macrophages can be stimulated by agents that affect phospholipase A₂ and acetyltransferase activities. The latter enzyme appears to have a regulatory function in the biosynthesis of alkylacyl-GPC.

Alveolar macrophages appear to have a major responsibility in phospholipid metabolism in the lung (for reviews, see Refs. 1 and 2). One potentially important aspect of their role may be the biosynthesis and/or catabolism of inflammatory mediators derived from phospholipids. As part of our study of pulmonary phospholipid metabolism, we have investigated the biosynthesis and cellular release of alkylacyl-GPC, i.e. platelet-activating factor.

Alkylacyl-GPC is a biologically active phospholipid with potent platelet-activating and antihypertensive activities (for reviews, see Refs. 3-5). It has also been implicated as a mediator in immune responses including inflammation and anaphylaxis (6). A number of investigators have demonstrated that alkylacyl-GPC is released by a variety of tissues and cell types, e.g. basophils (7), platelets (8), neutrophils (9), and macrophages (10-12). Specific enzymatic reactions involved in the biosynthesis of alkylacyl-GPC have been documented in some cell types. These reactions include the formation of the ether bond by alkylidihydroxyacetone-P synthase and its acylation by acyltransferase.

Alkylacyl-GPC is a potential precursor of alkylacyl-GPC derived from alkylacyl-GPC by an acyltransferase (15-19). It is not known which of these reactions is quantitatively the most important in the biosynthetic pathway(s) leading to the synthesis of alkylacyl-GPC in macrophages or any other cell. However, it is clear that acyltransferase is markedly stimulated by agents shown to evoke platelet-activating factor responses (18, 19).

In theory, the stimulation of the release of alkylacyl-GPC could be due to a combined enzymatic process: 1) activation of phospholipase A₂, which causes an increase in the concentration of the precursor alkyllyso-GPC; and 2) stimulation of acyltransferase activity, which catalyzes the acetylation of the lyso precursor. Our approach to investigate this hypothesis for the biosynthesis of alkylacyl-GPC has been to prelabel the endogenous pool of alkylacyl-GPC by incubating macrophages with [1,2-³H]alkyllyso-GPC of high specific activity, and then to determine the effect of various stimuli or inhibitors on the synthesis and release of alkylacyl-GPC into the media. We demonstrate here that alkylacyl-GPC is an effective precursor of the acetylated bioactive phospholipid and that stimulation of rat alveolar macrophages by zymosan and the ionophore A23187 increases the release of alkylacyl-GPC and alkyllyso-GPC (derived from alkylacyl-GPC) from the alveolar macrophages. The release is correlated with an increase in intracellular acetyltransferase and an increase in...
extracellular phospholipase A2 activity. It is equally significant that mepacrine, a phospholipase A2 inhibitor, can effectively reduce these responses and that acetate can reverse this inhibition.

**EXPERIMENTAL PROCEDURES**

**Materials**—1-[1',2',3'-H]Alkyllyso-GPC (5 Ci/mmol) was prepared from 1-[1',2'-H]alkyllyso-GPC by deacylation with the mono-methylene reagent described by Clarke and Dawson (20). The preparation of [1',2'-H]alkyllyso-GPC from choline plasmalogens of beef heart has previously been described (21). [1-,3-14C]Acetyl-CoA (5.55 mCi/mmol) was purchased from Amersham Corp. Tris (hydroxymethyl)aminomethane (Tris), 1-methylamine reagent described by Clarke and Dawson (20). The preparation of [1',2',3'-H]alkyllyso-GPC from choline plasmalogens of beef heart has previously been described (21). 

**Preparation of Alveolar Macrophages and Culture Conditions**—The lungs of 4-month-old CDF rats were lavaged with 50 ml (per rat) of fluid (10 ml/wash at 37 °C) comprised of RPMI 1640 containing 10% fetal calf serum, 12 mM lidocaine by following the procedure described by Holt (22). The pooled lavage fluids were centrifuged at room temperature for 7 min at 180 × g and the resulting cell pellet was then resuspended in RPMI 1640. The suspension was then plated into 36 mm plastic Petri dishes and incubated at 37 °C in 5% CO2 for 2 h. We removed nonadherent cells from the adherent macrophages by washing the monolayer vigorously with RPMI. Generally, the wash fluid from one rat provided sufficient cells for four Petri dishes (50–100 × 10^6 protein/dish). Cells were maintained for 2–4 h in RPMI media containing 1 mg/ml BSA (BSA/RPMI media) and the indicated additions. To terminate the experiments, the media were collected, centrifuged at 4 °C to remove any cells, and then extracted by the method of Bligh and Dyer (23), except that the methanol contained 2% glacial acetic acid. In some instances, aliquots of the media were removed before extraction for assay of phospholipase A2 or acid phosphatase activity. The cells adherent to the Petri dish were washed thoroughly with phosphate-buffered saline at 4 °C and then collected with a rubber policeman in 1 ml of phosphate-buffered saline. Aliquots were taken for enzyme assays and protein determination (24), and the remaining suspension was extracted with lipids with chloroform/methanol (23).

**Analysis of Radioactive Lipids**—For routine analysis, aliquots of the lipid extracts obtained from the media and cells, along with appropriate standards, were applied on thin layer chromatography plates coated with 250 μm thick layers of Silica Gel G. The plates were then developed in chloroform/methanol/glacial acetic acid/water (50:25:8.4, v/v). The proportion of methanol and water in the developing solvent was often increased by as much as 29 and 50%, respectively, depending on relative humidity to ensure that alkylacyl-GPC, phosphatidylethanolamine glycerophospholipids was determined by the method described by Clarke and Dawson (20).

**Enzyme Assays**—Phospholipase A2 was assayed under the two sets of optimal conditions described by Wightman et al. (27), except that the radioactive metabolite which co-chromatographed with alkylacyl-GPC, pooled fractions of this product were treated with phospholipase C (26). The resulting labeled nonpolar lipid derived by phospholipase C treatment co-chromatographed with the expected product, alkylacyl-glycerol, on a silica gel layer developed with chloroform/methanol/98% ethyl alcohol (98:2, v/v). The other linked lipid content of choline and ethanolamine glycerophospholipids was determined by the method described by Clarke and Dawson (20).

**RESULTS**

**Phospholipid Composition**—The phospholipid composition of rat alveolar macrophages is given in Table I. The alkylacyl-GPC fraction represented a substantial proportion (>10%) of the total phospholipids of these cells and was readily labeled by incubating macrophages with [1,2-3H]alkyllyso-GPC (Fig. 1). After a 4-h incubation, greater than 80% of the radioactivity recovered in the cells was in the alkylacyl-GPC fraction, less than 5% was in the alkylacyl-GPC fraction, and only traces of radioactivity (<0.1%) were in the alkylacyl-
results demonstrate that alveolar macrophages have an efficient mechanism for the uptake and acylation of the alkyllysophospholipid for storage as an inactive precursor (alkylacyl-GPC). Cellular Metabolism of Alkylacyl-GPC—Exposure of macrophages labeled with [³H]alkylacyl-GPC to a phagocytic agent (zymosan) or a calcium ionophore (A23187) led to an increase in the appearance of labeled metabolites in the media and a commensurate loss of radioactivity from the cells (Fig. 2A). At the beginning, the rate of loss of radioactivity from the cells was highest in cells exposed to the ionophore. However, after an initial lag of approximately 1 h, the rate of loss of label from the cells exposed to zymosan also increased substantially, so that after a 1.5-h incubation, cells exposed to either stimuli had 8-15% less total radioactivity than did the control cells (Fig. 2A). This loss of cellular radioactivity was reflected primarily by a >20% reduction in the radioactivity in the alkylacyl-GPC fraction of phospholipids of cells exposed to the stimuli for 1.5 h (Fig. 2B). In contrast, the rate of loss of radioactivity from the intracellular alkylacyl-GPC fraction was approximately one-fourth that of the alkylacyl-GPC fraction and was not appreciably altered by exposures to zymosan or the ionophore. Thus, the radioactivity released from the stimulated macrophages into the media was derived from the cellular pool of alkylacyl-GPC.

Chromatographic analysis of the radioactive phospholipids released into the media demonstrated that [³H]alkylacyl-GPC accumulated in the media of cells exposed to ionophore and at a slower rate and to a lesser extent than in the media of cells exposed to zymosan (4.0 and 2.5%, respectively, of the radioactivity in the media at 60 min, Fig. 3A). Ionophore and zymosan also stimulated the release of alkyllyso-GPC, the predominant labeled phospholipid in the media (67 and 90%, respectively, of the total at 60 min, Fig. 3B). Phorbol myristate acetate, in contrast to the ionophore and zymosan, had no effect on the quantity of [³H]alkylacyl-GPC found in the media (0.5% of the total, Fig. 3A), although it did stimulate the accumulation of alkyllyso-GPC (93% of the total [H], Fig. 3B).

Significant quantities of alkylacyl-GPC (25% of the total at 60 min) were also detected in the media of cells exposed to the ionophore (Fig. 3C). The reason for the appearance of this labeled alkylacyl phospholipid in the media is not clear. It apparently is not due to cell lysis since exposure of macrophages to A23187 did not affect the release of lactate dehydrogenase or acetyltransferase into the media (data not shown).

Phospholipase A₂ Activity—The decrease in label from the intracellular alkylacyl-GPC pool from cells exposed to zymosan or ionophore and the accumulation of labeled alkyllyso-GPC in the media would appear to be the result of a stimulation of phospholipase A₂ by these stimuli. Addition of a phospholipase A₂ inhibitor, mepacrine, to the incubation media of cells exposed to zymosan or the ionophore prevented the loss of radioactivity from the cellular pool of alkylacyl-GPC (Table II). Furthermore, the inhibitor also caused a sharp reduction (60%) in the amount of alkylacyl-GPC accumulated in the media of cells exposed to the ionophore.

**Fig. 2.** Effect of zymosan and ionophore on the loss of intracellular radioactivity from alveolar macrophages. Cellular alkylacyl-GPC was labeled by incubating macrophages with [³H]alkyllyso-GPC for 4 h. After washing the cells with RPMI, the media was then replaced with BSA/RPMI media (control, □), or BSA/RPMI media containing either zymosan (300 µg/ml, △) or A23187 (2 µM, ○) for the indicated time. Cells were then harvested and radioactivity was determined: A, per cent of total radioactivity in cells (5092 ± 576 dpm/mg protein at time 0) and B, radioactivity in the alkyllyso-GPC (broken lines) and alkylacyl-GPC (solid lines) fractions of the cells. Plotted values are the means from two experiments with S.E. value indicated by the vertical line.

**Fig. 3.** Time course for the accumulation of radioactive metabolites in the media of zymosan- and ionophore-stimulated macrophages. Cellular alkylacyl-GPC was prelabeled as described in Fig. 2. The macrophages were then incubated with BSA RPMI media containing the following additions: none (□), zymosan (200 µg/ml, △), ionophore A23187 (2 µM, ○), or TPA (1.6 µM, □). After the indicated time, radioactive phospholipids in the media were analyzed as described under “Experimental Procedures.” Results are the means from three experiments with S.E. values indicated by the vertical line. Probabilities of significant differences compared to control were calculated for the 60-min interval values. p < 0.05 indicated by asterisk.
and to a lesser extent (50%) in cells exposed to zymosan (Table II). Bromophenacyl bromide (0.1 mM), another phospholipase inhibitor, also had a similar effect on the conversion of alkylacyl-GPC to alkylacyl-GPC (data not shown).

The agents that stimulated the release of alklyl-GPC from cells were also tested for their effect on phospholipase A₂ activity. The A₂ activity when assayed at pH 4.5 with 1 mM EDTA in the incubations was approximately 1.4- to 1.9-fold higher in the media from the cells exposed to zymosan, ionophore, or TPA than in the media from cells not exposed to the stimuli (Table III). However, the total activity of the cells and media combined was not significantly increased by the stimuli. We interpret these results to indicate that such agents increase the amount of phospholipase released from the cells into the media rather than directly stimulating phospholipase A₂ activities. The activity of a phospholipase A₂ (27) measured at neutral pH in the presence of calcium in the cells into the media rather than directly stimulating activity recovered was not significantly altered by the zymosan or ionophore treatment. Between 82 and 94% of the total phosphatase activity in both the cells and media, a significant increase occurred in the relatively small amount of phosphatase activity released into the media (Table IV). Thus, these agents appear to stimulate the release of lysosomal enzymes from the macrophages. TPA caused a moderate stimulation (35-40%) of phosphatase activity in both the cells and media, which suggests that TPA can activate acid phosphatase as well as affect its release from macrophages.

**Influence of Acetate on Mepacrine Inhibition of the Zymosan- and Ionophore-stimulated Release of Alkylacyl-GPC**—The effect of acetate on the accumulation of alkylacyl-GPC in the cells exposed to stimuli and mepacrine (Table V) provides indirect evidence that the important role of the acetyltranferase-catalyzed step in the

| cells were incubated for 2 h in BSA/RPMI media containing zymosan, A23187, or TPA as described in Table III. Acid phosphatase was assayed in the media and cells as described under "Experimental Procedures." Values in parentheses refer to per cent control. Results represent the mean ± S.E. of four assays. |
|---|
| Stimulus | Media | Cells | Total |
|---|---|---|---|
| None (control) | 8.9 ± 0.8 (100) | 137 ± 21 (100) | 146 |
| Zymosan | 11.9 ± 1.0 (133) | 146 ± 12 (106) | 158 |
| A23187 | 27.6 ± 1.3* (308) | 124 ± 16 (90) | 152 |
| TPA | 11.6 ± 1.3* (133) | 188 ± 7* (136) | 200 |

* p < 0.001 when compared to control value.
* p < 0.05 when compared to control value.

**Table V**

Effect of acetate on alkylacyl-GPC release from alveolar macrophages

Cells were prelabeled and incubated as described in Table II except sodium acetate (0.2 mM) was included in the incubation media as indicated. Results are the mean ± S.E. Values in parentheses refer to per cent control.

| Stimulus | No additions | Plus acetate | Plus acetate and mepacrine * |
|---|---|---|---|
| Alkylacyl-GPC in media | | | |
| None (n = 3) | 178 ± 60 (100) | 204 ± 62 (100) | 255 ± 132 (100) |
| Zymosan (n = 2) | 258 ± 93 (100) | 321 ± 98 (100) | 366 ± 182 (100) |
| A23187 (n = 3) | 1121 ± 58 (100) | 1616 ± 517 (100) | 1784 ± 901 (100) |
| TPA (n = 2) | 194 ± 7 (100) | 188 ± 12 (100) | 123 ± 28 (100) |

* See Table II for the inhibitory effects of mepacrine on stimulus.

**Table II**

Effect of zymosan, ionophore A23187, and mepacrine on intracellular alkylacyl-GPC and the release of alkylacyl-GPC from alveolar macrophages

Cells prelabeled as described in Fig. 2 were incubated for 2 h with BSA/RPMI media or BSA/RPMI media containing either zymosan (200 μg/ml) or A23187 (2 μM), with or without mepacrine (0.1 mM). Radioactive alkylacyl-GPC in the media and intracellular alkylacyl-GPC was determined as described under "Experimental Procedures." Results are expressed as the mean ± S.E. Control values: alkylacyl-GPC (n = 4), 2868 ± 116 dpm/μg of protein; alkylacyl-GPC (n = 6), 2848 ± 1013 dpm/μg of protein.

| Stimulus | Cellular alkylacyl-GPC | Alkylacyl-GPC in media |
|---|---|---|
| | Minus mepacrine | Plus mepacrine | Minus mepacrine | Plus mepacrine | % control |
| None (control) | 100 | 104 ± 21 (100) | 102 ± 36 (100) | 102 ± 36 (100) | 100 |
| Zymosan | 75 ± 7 | 99 ± 3* | 167 ± 35 | 85 ± 52* | 98 |
| A23187 | 75 ± 5 | 107 ± 18* | 505 ± 224 | 206 ± 105* | 100 |

* p < 0.05 when compared to the values obtained for the zymosan or A23187 samples minus mepacrine.

**Table III**

Effect of various stimuli on phospholipase A₂ activity in alveolar macrophages

Cells were incubated for 2 h in BSA/RPMI media containing no additions, zymosan (200 μg/ml), A23187 (2 μM), or TPA (1.6 μM). The media and cells were then collected and phospholipase A₂ activity was assayed as described under "Experimental Procedures." Values in parentheses are activities of control (picomoles/flash/h). Results are expressed as the mean ± S.E. of two experiments.

| Stimulus | pH 4.5, 1 mM EDTA | pH 8.5, 2 mM CaCl² |
|---|---|---|
| | Media | Cells | Media | Cells | Media | Cells |
|---|---|---|---|---|---|---|
| None | 100 | 100 | 100 | 100 | 100 | 100 |
| (13.4 ± 1.1) | (44.3 ± 1.0) | (17.8 ± 1.0) | (16.4 ± 0.3) | (16.4 ± 0.3) | (16.4 ± 0.3) | (16.4 ± 0.3) |
| Zymosan | 144 ± 7 | 72 ± 2 | 114 | 118 ± 1 | 108 ± 6 | 108 ± 6 |
| A23187 | 187 ± 6 | 93 ± 1 | 114 | 118 ± 1 | 108 ± 6 | 108 ± 6 |
| TPA | 196 ± 6 | 89 ± 2 | 110 | 106 ± 3 | 111 ± 5 | 111 ± 5 |

**Table IV**

Effect of zymosan, ionophore A23187, and TPA on acid phosphatase activity in alveolar macrophages

Cells were incubated for 2 h in BSA/RPMI media containing zymosan, A23187, or TPA as described in Table III. Acid phosphatase was assayed in the media and cells as described under "Experimental Procedures." Values in parentheses refer to per cent control. Results represent the mean ± S.E. of four assays.

| Stimulus | Activity |
|---|---|
| | Media | Cells | Total |
|---|---|---|---|
| None (control) | 8.9 ± 0.8 (100) | 137 ± 21 (100) | 146 |
| Zymosan | 11.9 ± 1.0 (133) | 146 ± 12 (106) | 158 |
| A23187 | 27.6 ± 1.3* (308) | 124 ± 16 (90) | 152 |
| TPA | 11.6 ± 1.3* (133) | 188 ± 7* (136) | 200 |

* p < 0.001 when compared to control value.
* p < 0.05 when compared to control value.

**Biosynthesis of Alkylacyl-GPC**
**Biosynthesis of Alkylacyl-GPC**

**Table VI**

| Additions          | Specific activity | % control |
|--------------------|-------------------|-----------|
| None (control)     | 186 ± 18          | 100       |
| Zymosan (200 μg/ml)| 442 ± 121         | 238       |
| Ionophore A23187   | 465 ± 124         | 252       |
| TPA (1.6 μM)       | 231 ± 29          | 1.32      |

*p < 0.02 when compared to control value.

**Table VII**

| Additions          | Specific activity | % control |
|--------------------|-------------------|-----------|
| None (control)     | 238 ± 84          | 580 ± 163*|
| 1 mM EDTA          | 18 ± 10*          | 153 ± 49  |
| 1 mM CaCl₂         | 309 ± 94          | 275 ± 94  |

*p < 0.05 when compared to control value minus the A23187.

The biosynthesis of the bioactive lipid, Quantitative assessment of the effect by acetate on the accumulation of alkylacyl-GPC was hampered by a large variation in the magnitude of the responses in different cell populations. However, certain consistent features of these responses to acetate were apparent. Addition of acetate alone was ineffective in stimulating alkylacyl-GPC accumulation and had only slight effect on the responses in the ionophore- or zymosan-treated cells (Table V). However, the presence of acetate in the media prevented the inhibitory effects of mepacrine on the release of alkylacyl-GPC from macrophages after stimulation by zymosan and A23187 (Table V). The extent that alkylacyl-GPC accumulated in the media of cells exposed to TPA (with and without mepacrine) was not increased by acetate (Table V).

**Acetyltransferase Activity**—Homogenates prepared from cells that had been incubated for 1 h in media containing zymosan or the calcium ionophore contain a higher level of acetyltransferase activity than homogenates from cells not exposed to the stimuli (Table VI); TPA was relatively ineffective in affecting acetyltransferase activity. In addition to the stimulatory effect of ionophore A23187 on intact cells, the ionophore also directly stimulated acetyltransferase activity in cell homogenates when the assay was done in the absence of added calcium (Table VII). The activity in the homogenate was inhibited by EDTA, whereas the activity was not stimulated above control values by the addition of calcium (Table VII).

**Discussion**

Alveolar macrophages form and release alkylacyl-GPC and alkyllyso-GPC when exposed to phagocytic or ionophoretic stimuli (Ref. 29 and present study). We interpret that the accumulation of the labeled alkyl lysoospholipid found in the media at the expense of labeled cellular alkylacyl-GPC is due to an increase in the activity of a phospholipase A₂. This enzyme appears to be closely coupled with the acetyltransferase activity that is responsible for the formation of alkylacyl-

**Figure 4** Proposed pathway for the biosynthesis of alkylacyl-GPC by rat alveolar macrophages.

1. **ALKYLACYL-GPC**
2. **PHOSPHOLIPASE A₂**
3. **ALKYLLYSO-GPC**
4. **ACETYL-CoA**
5. **ACETYLTRANSFERASE**
6. **ALKYLACYL-GPC**
capable of catalyzing the acetylation of alkyllyso-GPC. This activity has also been recently demonstrated in murine peritoneal macrophages (18), and in polymorphonuclear leucocytes (15, 19) and eosinophils from humans (19). Moreover, acetyltransferase activity has been shown to be modulated by calcium ionophore A23187 (19) and zymosan (15, 19). The mechanism for the in vivo regulation of acetyltransferase activities in rat alveolar macrophages, as well as other cells, has yet to be established. The stimulation of acetyltransferase by a calcium ionophore in intact cells and the inhibition of its activity in homogenates by a calcium chelating agent as observed in the present study suggests a regulatory function of calcium. On the other hand, our observation that the ionophore and not calcium per se stimulated acetyltransferase activity in homogenates suggests that the ionophore can assert a direct effect on the enzyme rather than just on calcium availability.

The regulatory significance of the acetylation reaction in alveolar macrophages is illustrated by the effect of acetate upon the stimulation of the formation and cellular release of alkylacyl-GPC by ionophore A23187 under conditions in which deacylation is partially inhibited by mepracine. The fact that, when acetate is added to the incubation media, the inhibitory effect of mepracine on the formation of alkylacyl-GPC is prevented suggests that the rate-limiting step in the deacylation of alkylacyl-GPC. Indeed, an increased formation of alkylacyl-GPC without a parallel increase in acetyltransferase activity may not be sufficient to stimulate alkylacyl-GPC formation. This is apparent with TPA, a membrane perturbant that increases the level of the lysophospholipid precursor, but unlike the zymosan or ionophore does not appreciably stimulate acetyltransferase and, therefore, does not induce an accumulation of alkylacyl-GPC in the media even in the presence of added acetate.

We conclude that in rat alveolar macrophages both phospholipase A and acetyltransferase behave as synergistic enzymes in the synthesis of alkylacyl-GPC from alkylacyl-GPC, with alkyllyso-GPC as an intermediate (Fig. 4). The acetyltransferase activity would appear to be an important regulatory enzyme in the biosynthesis of platelet-activating factor.

REFERENCES

1. Hocking, W. G., and Golde, D. W. (1979) N. Engl. J. Med. 301, 580–587
2. Van Golde, L. M. J. (1976) Am. Rev. Respir. Dis. 114, 977–1000
3. Pinckard, R. H., McManus, L. M., Demopoulos, C. A., Halonen, M., Clark, P. O., Shaw, J. O., Kniker, W. T., and Hanahan, D. J. (1980) J. Reticuloendothel. Soc. 28, 85s–103s
4. Vargaftig, B. B., Chignard, M., Benveniste, J., Lefort, J., and Wal, F. (1991) Annu. N. Y. Acad. Sci. 620, 119–137
5. Snyder, F. (1982) Ann. Rep. Med. Chem. 17, 243–252
6. McManus, L. M., Morley, C. A., Levine, S. P., and Pinckard, R. N. (1979) J. Immunol. 123, 2835–2841
7. Hanahan, D. J., Demopoulos, C. A., Liehr, J., and Pinckard, R. N. (1980) J. Biol. Chem. 255, 5514–5516
8. Chignard, M., Le Couedic, J. P., Vargaftig, B. B., and Benveniste, J. (1980) Br. J. Haematol. 46, 45–464
9. Lynch, J. M., Lotner, G. Z., Betz, S. J., and Henson, P. M. (1979) J. Immunol. 123, 1219–1226
10. Mencia-Huerta, J. M., and Benveniste, J. (1981) Cell. Immunol. 57, 281–292
11. Camussi, G., Tetta, C., Bussolino, F., Masera, C., Emanuelli, G., Ragni, R., and Porcellini, G. (1980) Pammunmera Med. 23, 117–124
12. Mencia-Huerta, J. M., and Benveniste, J. (1979) Eur. J. Immunol. 9, 409–415
13. Wykle, R. L., and Snyder, F. (1975) in The Enzymes of Biological Membranes (Martonsi, A., ed) Vol. 2, pp. 87–117, Plenum Press, New York
14. Renooj, W., and Snyder, F. (1981) Biochim. Biophys. Acta 663, 545–556
15. Alonso, F., Gil, M. G., Sánchez-Crespo, M., and Mato, J. M. (1982) J. Biol. Chem. 257, 3376–3378
16. Wykle, R. L., Malone, B., and Snyder, F. (1980) J. Biol. Chem. 255, 10256–10260
17. Mueller, H. W., O’Flaherty, J. T., and Wykle, R. L. (1982) Lipids 17, 72–77
18. Ninio, E., Mencia-Huerta, J. M., Heyranns, F., and Benveniste, J. (1982) Biochim. Biophys. Acta 710, 23–37
19. Lee, T., Malone, B., Wasserman, S. I., Fitzgerald, V., and Snyder, F. (1982) Biochim. Biophys. Res. Commun. 105, 1303–1308
20. Clarke, N. G., and Dawson, R. M. C. (1981) Biochem. J. 195, 301–306
21. Blane, M. L., Lee, T., Fitzgerald, V., and Snyder, F. (1981) J. Biol. Chem. 256, 175–178
22. Holt, P. (1979) J. Immunol. Methods 27, 189–198
23. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
24. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
25. Snyder, P., and Smith, D. (1966) Sep. Sci. 1, 709–722
26. Mavis, R. D., Bell, R. M., and Vagelos, P. R. (1972) Biochem. Biophys. Res. Commun. 45, 911–917
27. Wightman, P. D., Dahlgren, M. E., Davies, P., and Bonney, R. J. (1981) Biochem. J. 200, 441–444
28. Schuverd, J., and Baffigolin, M. (1978) J. Exp. Med. 148, 455–459
29. Arnoux, B., Duval, D., and Benveniste, J. (1980) Eur. J. Clin. Invest. 10, 437–441
30. Hopmann, S. L., Prescott, S. M., and Majerus, P. W. (1982) Arch. Biochem. Biophys. 215, 237–244
31. Disc, C. A., Burch, J. W., and Goodman, D. B. (1982) J. Biol. Chem. 257, 4701–4704
32. Lapetina, E. P., Billah, M. M., and Cuatrecasas, P. (1981) J. Biol. Chem. 256, 5037–5040
33. Vargaftig, B. B. (1977) J. Pharm. Pharmacol. 29, 227–228
34. Franson, R. C., and Waite, M. (1975) J. Cell Biol. 66, 621–627
35. Wightman, P. D., Humes, J. L., Davies, P., and Bonney, R. J. (1981) Biochem. J. 195, 427–433
36. Hsueh, W., Kuhn, C., and Needleman, P. (1979) J. Biol. Chem. 254, 184, 354–354
37. Franson, R., Becker, D. S., Wang, P., Waite, M., and Elsbach, P. (1979) Biochim. Biophys. Acta 296, 365–373