Accumulation of phosphatidic acid mass and increased *de novo* synthesis of glycerolipids in platelet-activating-factor-activated human neutrophils

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Incubation of human neutrophils with 100 nM-platelet-activating factor (PAF) but without cytochalasin B resulted in a rapid (5 s) accumulation (1.6-fold) of phosphatidic acid (PtdOH) mass. The increased PtdOH mass reached a maximum (2.8-fold) at 1 min and remained elevated (1.7-fold) at 10 min. No methylamine-stable lyso-PtdOH was detectable in the total lipid extract from control or from PAF-activated cells, suggesting that diacyl-PtdOH was the predominant species. In PAF-activated cells, changes in 1,2-diacylglycerol (DG) mass were not detectable at 5 or 15 s. Increased DG mass (1.7-fold) was detected between 30 s and 2 min, but then it declined to basal levels by 10 min. PAF enhanced [³H]glycerol incorporation into PtdOH and DG by 2- and 3-fold respectively during 1–10 min incubations. PAF also increased the radioactivity but not the mass of phosphatidylinositol and of choline glycerophospholipid by 8-fold and 4-fold respectively at 10 min. In addition, PAF-activated cells showed increased (2-fold) glycerol incorporation into triacylglycerol. These results demonstrate that PAF enhances rapid accumulation of diacyl-PtdOH mass, and that increased *de novo* synthesis may contribute to PtdOH mass accumulation.

INTRODUCTION

Neutrophils synthesize platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in response to particulate or soluble stimuli (reviewed in [1]). PAF in turn amplifies neutrophil activation. It primes superoxide anion production [2,3] and elastase release [3] by chemotactic-peptide-activated neutrophils. It also acts directly to trigger neutrophil aggregation [4], actin polymerization [5], exocytosis of gelatinase [6], and cytochalasin B-dependent degranulation [7].

Although the biochemical mechanisms by which PAF activates neutrophils have not been fully explored, some of the biological effects of PAF appear to be mediated by its action on lipid metabolism. PAF stimulates arachidonic acid release from choline glycerophospholipid (CGPL) and PtdIns and subsequent production of leukotriene B₄ [8,9]. Leukotriene B₄ is a potent agonist for neutrophil chemotaxis and aggregation [10,11], though it has not been determined whether the amounts of leukotriene B₄ produced in response to PAF are sufficient to elicit neutrophil chemotaxis. Activation of PtdIns(4,5)P₂-specific phospholipase C also occurs during the neutrophil-PAF interaction [12,13]. In [2-³H]inositol-labelled human neutrophils, PAF enhances Ins(1,4,5)P₃ formation [12]. Ins(1,4,5)P₃ raises cytosolic Ca²⁺ levels by releasing intracellular stored Ca²⁺ [14], and mobilization of intracellular Ca²⁺ participates in neutrophil activation [15]. In ³²P-labelled rabbit peritoneal neutrophils PAF causes the loss of ³²P from PtdIns(4,5)P₂ and the production of ³²P-labelled phosphatidic acid (PtdOH) [13]. However, it is not known whether the increased radioactivity represents increased PtdOH mass or an increase in its specific radioactivity. Increased ³²P-labelled PtdOH formation in these cells could be secondary to increased diacylglycerol (DG) formation after phospholipase C activation. It could also arise from activation of phospholipase

D [16]. In addition, increased *de novo* synthesis may contribute to PtdOH formation in PAF-activated neutrophils. In the present study we have investigated the effects of PAF on PtdOH mass and on *de novo* synthesis. Our data demonstrate that PAF increases PtdOH mass and *de novo* synthesis of PtdOH and other glycerolipids in human neutrophils.

MATERIALS AND METHODS

Materials

PtdOH prepared from egg yolk lecithin, lyso-PtdOH (1-oleoyl-*sn*-glycero-3-phosphate), fatty-acid-free BSA and Malachite Green [colour index (CI) = 42000] were purchased from Sigma Chemical Co. Poly(vinyl alcohol) (98 % hydrolysed, average *M_r* 13000–23000) was from Aldrich Chemical Co. PAF, *sn*-1,2-dioleoylglycerol, bovine cardiolipin and other glycerolipids were obtained from Avanti Polar Lipids. Octyl β-D-glucoside (ULTROL Grade) was from Calbiochem. *Escherichia coli* DG kinase was from Lipidex. Silica gel H plates were from Analtech. Merck silica gel 60 F-254 glass and plastic plates were from VWR Scientific. [1,2,3-³H]Glycerol (40.4 Ci/mmol) was purchased from Du Pont–New England Nuclear, and [γ-³²P]ATP (650 Ci/mmol) was from ICN Biochemicals.

Preparations of human neutrophils

Neutrophils were isolated from heparinized peripheral blood from healthy volunteers as previously described [17]. Cells were suspended in Dulbecco's balanced salt solution [18] containing 5 mM-glucose at a concentration of 20 × 10⁶ cells per ml. Cell counts were made in a haemocytometer, and cell viability was measured by Trypan Blue exclusion. Cell preparations contained more than 95 % neutrophils.

Abbreviations used: PAF, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); CGPL, choline glycerophospholipid; PtdOH, phosphatidic acid; DG, diacylglycerol; PtdSer, phosphatidylserine; SM, sphingomyelin; EGPL, ethanolamine glycerophospholipid; TG, triacylglycerol.

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Incubation of neutrophils

PAF was suspended in 0.9% NaCl containing 2.5 mg of BSA/ml. Each incubation tube contained, in a final volume of 2 ml, 0.2 mg of BSA, 0–100 nM-PAF and 20×10^6 cells. Ethanol at a final concentration of 0.5% was included in some incubation tubes where indicated. Incubation tubes and cells were separately kept at 37 °C for 5 min, and then incubations were started by adding 1 ml of cells to each tube. After 5 s–10 min at 37 °C, incubations were terminated by adding 5 ml of methanol to each tube. Total lipids were extracted according to the method of Bligh & Dyer [19] and were dissolved in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene.

Measurement of phospholipid phosphorus

Phospholipids from 24×10^6 cells were resolved by two-dimensional t.l.c. [20] on silica gel 60 F-254 plastic plates and were stained with iodine vapour. The silica areas containing individual phospholipids were scraped. Phospholipid phosphorus was measured according to the procedure described by Veldhoven & Mannaerts [21].

Analysis of ether-linked PtdOH by methylamine deacylation of total lipid extract

Lipids for deacylation were extracted from 40×10^6 cells which had been incubated for 2 min in the absence or the presence of 100 nM-PAF. Lipids were deacylated with the methylamine reagent of Clarke & Dawson [22], as modified by Nolan & Lapetina [23]. The methylamine reagent consisted of 40% aqueous methylamine/water/butan-1-ol/methanol (36:8:9:47, by vol.). Lipids in chloroform were dried under vacuum and dissolved in 2 ml of methylamine reagent. The tightly stoppered tubes were incubated at 50 °C for 2 h. At the end of the incubation, tubes were cooled in ice and 5 ml of ethanol was added to each tube. The mixture was evaporated at 37 °C under vacuum by a rotary evaporator. The dried lipid residue was redissolved in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene. Methylamine-treated lipids from 24×10^6 cells were resolved by two-dimensional t.l.c. on silica gel 60 F-254 glass plates. Lipids were visualized by spraying the plates with 10% cupric sulfate in 8% phosphoric acid [24] and charred at 140 °C for 15 min.

DG mass estimation with *E. coli* DG kinase

DG levels in crude lipid extracts from neutrophils were measured by the method of Preiss *et al.* [25]. Neutrophils (20×10^6 ml) were incubated with or without 100 nM-PAF for 5 s–10 min. Each incubation tube contained lipid extract derived from 20×10^6 cells. After drying under vacuum, lipids were solubilized in 20 μ l of 7.5% octyl β -D-glucoside and 5 mM-cardiolipin in 1 mM-diethylenetriaminepenta-acetic acid. DG in the solubilized lipid mixture was converted to [32 P]PtdOH in the presence of *E. coli* DG kinase and [γ - 32 P]ATP. A linear quantitative conversion into PtdOH was obtained when known amounts (100–400 pmol) of *sn*-1,2-dioleoylglycerol were assayed in parallel. The amount of DG present in the original sample was calculated from the amount of [32 P]PtdOH produced, the sample volume and the specific radioactivity of the [γ - 32 P]ATP. DG mass is expressed as pmol/ 10^7 cells.

Incorporation of [3 H]glycerol into lipids

Neutrophils (20×10^6 cells in 2 ml) were incubated with 10 μ Ci (1.1 μ M) of [1,2,3- 3 H]glycerol in the absence or the presence of 100 nM-PAF. After 1–10 min, incubations were terminated by adding 5 ml of methanol to each tube. Total lipids were extracted according to the method of Bligh & Dyer [19] and dissolved in

chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene. Individual phospholipids were resolved by two-dimensional t.l.c. and neutral glycerolipids were separated by one-dimensional t.l.c. on silica gel H as described previously [20]. The radioactivity of each lipid was counted by liquid scintillation spectrometry and expressed as d.p.m./ 6×10^6 cells.

RESULTS

PAF induces a rapid rise in PtdOH mass

Fig. 1 depicts the time course (5 s–10 min) of PAF (100 nM)-induced PtdOH mass increase. PtdOH mass elevation was detectable as early as 5 s (1.6-fold) during the neutrophil-PAF interaction. It reached a maximum at 1–2 min (2.8-fold) and remained elevated at 5–10 min (1.7-fold). However, changes in the mass of other phospholipids were undetectable by phosphate assay. In resting neutrophils, the average mass of each phospholipid expressed as nmol/ 10^7 cells was: PtdOH, 0.25; PtdIns, 5.91; phosphatidylserine (PtdSer), 10.5; sphingomyelin (SM), 14.8; CGPL, 36.8; ethanolamine glycerophospholipid (EGPL), 32.5.

The increased PtdOH mass in PAF-activated cells could arise from phospholipase D activation. To examine CGPL-specific phospholipase D activation, cells were incubated with 0.5% ethanol in the absence or the presence of 100 nM-PAF. PtdOH mass was measured after 1–10 min of incubation. If the increased PtdOH mass is secondary to CGPL-specific phospholipase D activation, it would be attenuated in the presence of ethanol due to the formation of phosphatidylethanol at the expense of PtdOH [26]. Under our experimental conditions ethanol did not affect PtdOH mass in the absence or the presence of PAF (results not shown). This suggests that CGPL-phospholipase D activation is not a major source of increased PtdOH mass in PAF-activated cells.

Diacyl-PtdOH is the predominant species in control and in PAF-activated cells

The diacyl- and ether-linked species in PtdOH were assessed by deacylation of lipid extracts from control and PAF-activated cells with methylamine reagent [22]. The methylamine-stable lyso-PtdOH was taken to represent ether-linked species of PtdOH.

Fig. 1. Time course of the effect of PAF on PtdOH mass

Human neutrophils (20×10^6 cells in 2 ml) were incubated at 37 °C for various periods of time (5 s–10 min) in the absence (○) or the presence (●) of 100 nM-PAF. A lipid extract from 24×10^6 cells was resolved by t.l.c. on silica gel 60 F-254 as described in the Materials and Methods section. PtdOH phosphorus was measured by using the Malachite-Green-based method [21]. Data are means $\pm$ S.E.M. from three separate experiments.

Fig. 2. Two-dimensional t.l.c. of neutrophil lipids before (a) and after (b) deacylation

(a) T.l.c. separation of a lipid extract from 24×10^6 neutrophils which had been incubated with 100 nM-PAF for 2 min at 37 °C. (b) T.l.c. separation of methylamine-stable lipids from 24×10^6 neutrophils which had been incubated with 100 nM-PAF for 2 min at 37 °C. The numbered lipids were identified as: 1, PtdOH; 2, PtdSer; 3, PtdIns; 4, SM; 5, CGPL; 6, lactosylceramide; 7, EGPL; 8, lyso-CGPL; 9, lyso-EGPL. O, Origin.

Fig. 3. Time course of the effect of PAF on DG mass

The incubation conditions were the same as those in Fig. 1. Total DG mass was measured by using *E. coli* DG kinase as described in the Materials and methods section. Results are means $\pm$ S.E.M. from three separate experiments. $\circ$, Control; $\bullet$, +100 nM-PAF.

Fig. 2(a) shows the resolution of PtdOH and other polar lipids extracted from PAF-activated cells (24×10^6), and Fig. 2(b) reveals the separation of methylamine-stable lipids derived from the same number of PAF-activated cells. The R_f values of standard lyso-PtdOH in the first and second dimensions were respectively 0 and 0.44. Lyso-PtdOH was not detectable in methylamine-treated lipid extracts from control (results not shown) or PAF-activated cells (Fig. 2b), indicating that diacyl-PtdOH is the predominant species. The identities of lipids 1–9 were assessed by comparing their t.l.c. mobilities with those of authentic standards and by spraying with specific reagents [27]. Deacylation of EGPL appears to be incomplete under our experimental conditions (Fig. 2b). SM (lipid 4), lactosylceramide (lipid 5), ether-linked lyso-CGPL (lipid 8) and ether-linked lyso-EGPL (lipid 9) are methylamine-stable.

PAF promotes an increase in DG mass

A time course of the effects of PAF on DG mass changes was examined. If the early rise in PtdOH mass is partly derived from increased DG mass, an increase in DG mass is expected to occur after 5 s exposure of cells to PAF. By using *E. coli* DG kinase [25],

Fig. 4. Time course of stimulation of [3 H]glycerol incorporation into glycerolipids by PAF

Neutrophils (20×10^6 in 2 ml) were incubated with 10 μ Ci of [3 H]glycerol in the absence ($\circ$) and the presence ($\bullet$) of 100 nM-PAF. After 1–10 min at 37 °C lipids were separated as described in the Materials and methods section. Results are means $\pm$ S.E.M. from three separate experiments. (a) PtdOH, (b) CGPL, (c) DG, (d) PtdIns, (e) EGPL, (f) TG.

DG mass was measured after cells had been incubated with PAF (100 nM) for 5 s–10 min. However, no measurable increase in DG mass was detected at 5 or 15 s (Fig. 3), a time period in which a 1.6-fold increase in PtdOH mass was observed (Fig. 1). A 1.7-fold increase in DG mass (170 pmol/ 10^7 cells) was detected during 30 s–2 min of incubation, and it diminished to control values by 10 min. The results suggest that increased DG mass may contribute to PtdOH mass accumulation after 30 s or more exposure of cells to PAF.

PAF promotes *de novo* synthesis of PtdOH

In order to examine whether *de novo* synthesis of PtdOH contributes to the increased PtdOH mass in PAF-activated cells, the effect of PAF on [3 H]glycerol incorporation into neutrophil glycerolipids was examined. Fig. 4 illustrates the time course of the PAF (100 nM) effect on labelled glycerol incorporation into glycerolipids. PtdOH radioactivity increased in PAF-activated cells. The extent of the increase did not appear to change significantly with incubation time. A 2-fold increase was demonstrated with 1 and 10 min incubations, and a 2.5-fold increase was shown with 2 and 5 min incubations. PtdIns radioactivity in PAF-activated cells increased with incubation time, and was 10 times that in control cells after a 10 min

Fig. 5. Concentration-response curves of PAF-stimulated [^3H]glycerol incorporation into glycerolipids

Neutrophils (20×10^6 cells in 2 ml) were incubated with 0.1–100 nM-PAF for 5 min. Lipids were extracted and the radioactivity of each glycerolipid was measured as described in the Materials and methods section. Results are means $\pm$ S.E.M. from three separate experiments.

incubation. The extent of stimulation of PtdIns synthesis was greater than that of PtdOH synthesis, suggesting an activation of PtdIns synthesis from PtdOH. A 2-fold increase of CGPL radioactivity was demonstrated at 5–10 min. Although EGPL radioactivity was increased in PAF-activated cells, glycerol was poorly incorporated into EGPL during the incubation periods (1–10 min). It should be noted that [^3H]glycerol incorporation into PtdOH and PtdIns but not into CGPL and EGPL represents *de novo* synthesis, because [^3H]glycerol incorporation into CGPL and EGPL may not correlate with the synthesis of CDP-choline and CDP-ethanolamine in neutrophils. Tysnes *et al.* [28] showed that in platelets both [^{32}P]P₁ and [^3H]glycerol were incorporated into PtdIns with a $^{32}\text{P}/^3\text{H}$ ratio similar to that in glycerol 3-phosphate. However, CGPL and EGPL exhibited a much lower $^{32}\text{P}/^3\text{H}$ ratio. These studies clearly demonstrated that the labelling of CGPL and EGPL by [^3H]glycerol does not indicate *de novo* synthesis of these phospholipids.

Fig. 4 also shows that PAF stimulated glycerol incorporation into DG, and that the stimulation reached a maximum (3.5-fold) at 5 min. Triacylglycerol (TG) radioactivity also increased in the presence of PAF. In both control and PAF-activated cells TG exhibited the highest radioactivity among glycerolipids after 5–10 min of incubation, indicating rapid metabolism of DG into TG.

PAF stimulation of glycerol incorporation into glycerolipids was concentration-dependent. PAF at concentrations below 1 nM showed no detectable effect on glycerolipid labelling by glycerol. Fig. 5 illustrates increased radioactivity of all glycerolipids in neutrophils incubated with PAF at 1–1000 nM for 5 min. PAF stimulation of glycerol incorporation into DG, TG, CGPL and

EGPL reached a maximum at 100 nM, but the radioactivity in PtdOH and PtdIns continued to increase up to 1000 nM.

DISCUSSION

The present study presents evidence that PAF increases PtdOH mass and *de novo* synthesis of PtdOH and other glycerolipids in human neutrophils. Chemotactic peptide has also been shown to enhance PtdOH mass in neutrophils primed with cytochalasin B [29,30]. The PtdOH formed in cytochalasin B-primed cells has been shown to arise mainly from the activation of CGPL-specific phospholipase D [26,31]. In the present study, PtdOH mass accumulation was demonstrated in PAF-activated neutrophils, but in the absence of cytochalasin B. The accumulated PtdOH in PAF-activated cells is primarily of the diacyl species, whereas PtdOH derived from CGPL has a high content (40%) of ether-linked species [26]. These findings suggest that the action of CGPL-phospholipase D may not be a major source of the elevated PtdOH mass in PAF-activated cells in the absence of cytochalasin B. It is likely that the activity of CGPL-phospholipase D would be expressed in PAF-activated cells primed by cytochalasin B. During the preparation of this paper, Kanaho *et al.* [32] showed PAF stimulation of [^3H]alkyl-PtdOH formation in the presence of cytochalasin B in neutrophils which were prelabelled with 1-O- ^3H octadecyl-lyso-PAF. However, [^3H]alkyl-PtdOH formation was not accompanied by decreased radioactivity in alkyl-phosphatidylcholine.

The increased PtdOH mass in PAF-activated cells could partly arise from increased DG mass, since a transient (30 s–2 min) rise in DG mass was detected. However, PAF-activated neutrophils did not demonstrate an early (5–15 s) change in DG mass, whereas these cells showed a 1.6-fold increase in PtdOH mass at 5 s. It is possible that the early small changes in DG mass cannot be detected by the enzymic method used. The possibility also exists that part of the early increase in PtdOH mass may arise from the action of phospholipase D on phospholipids other than CGPL, in view of the finding that PtdOH is composed of primarily diacyl species. A PtdIns-specific phospholipase D has been demonstrated in human neutrophils activated by chemotactic peptide [29] and by Ca^{2+} ionophore A23187 [29,33]. Stimulation by PAF of glycerol incorporation into PtdOH suggests that increased *de novo* synthesis may contribute to the increase in PtdOH mass during 1–10 min incubation. However, it is difficult to assess the quantity of PtdOH that is actually derived from *de novo* synthesis, since glycerol incorporation into PtdOH does not reflect the total amount of PtdOH synthesized *de novo*. Undoubtedly, acylation of endogenous *sn*-glycerol 3-phosphate and dihydroxyacetone phosphate would also occur during the PAF-stimulated neutrophil activation. Increased glycerol incorporation into PtdOH has also been shown in neutrophils activated by chemotactic peptide [34] and by ionophore A23187 [34,35]. However, PAF-activated but not ionophore-A23187-activated cells [35] showed increased glycerol incorporation into TG. The rapid metabolism of DG into TG could attenuate DG mass, thereby preventing a delayed and sustained rise in DG mass in PAF-activated cells.

The effects of increased diacyl-PtdOH mass on neutrophil activation deserve further studies. In chemotactic-peptide-activated neutrophils primed by cytochalasin B, PtdOH generation through phospholipase D shows kinetic correlation with the release of azurophilic granules [29] and activation of NADPH oxidase [36]. However, it is not known whether the diacyl- or ether-linked PtdOH is the active species. In cultured A431 cells, exogenous PtdOH activates PtdIns(4,5) P_2 -specific phospholipase C [37], and an experiment *in vitro* has also shown activation of PtdIns(4,5) P_2 -phospholipase C by PtdOH [38]. It remains to be

investigated whether or not PtdOH plays a role in agonist-stimulated PtdIns(4,5) P_2 hydrolysis. In PAF-activated neutrophils the increased PtdOH mass may serve as the precursor for PtdIns synthesis. Increased PtdIns synthesis would not only maintain PtdIns mass after increased deacylation in PAF-activated cells [8], it would also serve as a precursor to replenish PtdIns(4,5) P_2 following hydrolysis by phospholipase C [12,13]. In addition, increased synthesis of PtdOH and PtdIns may also be needed for the synthesis of PtdIns(3,4,5) P_3 in activated neutrophils. A recent study has demonstrated that chemotactic peptide and leukotriene B_4 cause a transient increase in PtdIns(3,4,5) P_3 formation in human neutrophils [39], though PtdIns(3,4,5) P_3 formation has not been examined in PAF-activated cells. It is interesting to note that PtdIns(3,4,5) P_3 has recently been proposed to modulate actin polymerization in intact neutrophils [40].

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REFERENCES

1. Pinkard, R. N., Ludwig, J. C. & McManus, L. M. (1988) in *Inflammation Principle and Clinical Correlates: Platelet-Activating Factors* (Gallin, J. I., Goldstein, I. M. & Snyderman, R., eds.), pp. 139–167, Raven Press, New York
2. Gay, J. C., Beckmann, J. K., Zaboy, K. A. & Lukens, J. N. (1986) *Blood* **67**, 931–936
3. Vercellotti, G. M., Yin, H. Q., Gustafson, K. S., Nelson, R. D. & Jacob, H. S. (1988) *Blood* **71**, 1100–1107
4. Shaw, J. O., Pinkard, R. N., Ferrigni, K. S., McManus, L. M. & Hanahan, D. J. (1981) *J. Immunol.* **127**, 1250–1255
5. Shalit, M., Dabiri, G. A. & Southwick, F. S. (1987) *Blood* **70**, 1921–1927
6. Beatrice, D. & Baggiolini, M. (1986) *Biochim. Biophys. Acta* **888**, 42–48
7. Ingraham, L. M., Coates, T. D., Allen, J. M., Higgins, C. P., Bachner, R. L. & Boxer, L. A. (1982) *Blood* **59**, 1259–1266
8. Chilton, F. H., O'Flaherty, J. T., Walsh, C. E., Thomas, M. J., Wykle, R. L., DeChatelet, L. R. & White, B. M. (1982) *J. Biol. Chem.* **257**, 5402–5407
9. Lin, A. H., Morton, D. R. & Gorman, R. R. (1982) *J. Clin. Invest.* **70**, 1058–1065
10. Klikstein, L. B., Shapleigh, C. & Geotzl, E. J. (1980) *J. Clin. Invest.* **66**, 1166–1170
11. Palmblad, J., Malmsten, C. L., Uden, A., Radmark, O., Engstedt, L. & Samuelsson, B. (1981) *Blood* **58**, 658–661
12. Verghese, M. W., Charles, L., Jakoi, L., Dillon, S. B. & Snyderman, R. (1987) *J. Immunol.* **138**, 4378–4380
13. Naccache, P. H., Molski, M. M., Volpi, M., Shefcyk, J., Molski, T. F. P., Loew, L., Becker, E. L. & Sha'afi, R. I. (1986) *J. Leukocyte Biol.* **40**, 533–548
14. Berridge, M. J. (1987) *Annu. Rev. Biochem.* **56**, 159–193
15. Serhan, C. N., Broekman, M. J., Korchak, H. M., Smolen, J. E., Marcus, A. J. & Weissman, G. (1983) *Biochim. Biophys. Acta* **762**, 420–428
16. Exton, J. H. (1990) *J. Biol. Chem.* **265**, 1–4
17. Tou, J.-S. (1989) *Lipids* **24**, 812–817
18. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1986) *Data for Biochemical Research*, 3rd edn., p. 447, Clarendon Press, Oxford
19. Blish, E. C. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
20. Tou, J.-S. (1987) *Lipids* **22**, 333–337
21. Van Veldhoven, P. P. & Mannaerts, G. P. (1987) *Anal. Biochem.* **161**, 45–48
22. Clarke, N. G. & Dawson, R. M. C. (1981) *Biochem. J.* **195**, 301–306
23. Nolan, R. S. & Lapetina, E. G. (1990) *J. Biol. Chem.* **265**, 2441–2445
24. Baron, C. B. & Coburn, R. F. (1984) *J. Liq. Chromatogr.* **7**, 2793–2801
25. Preiss, J. E., Loomis, C. R., Bell, R. M. & Nidel, J. E. (1987) *Methods Enzymol.* **141**, 294–300
26. Billah, M. M., Eckel, S., Mullmann, T. J., Egan, R. W. & Siegel, M. I. (1989) *J. Biol. Chem.* **264**, 17069–17077
27. Skipski, V. P. & Barclay, M. (1969) *Methods Enzymol.* **14**, 544–547
28. Tynes, O.-B., Verhoeven, A. J. M. & Holmsen, H. (1987) *Biochim. Biophys. Acta* **930**, 338–345
29. Cockcroft, S. (1984) *Biochim. Biophys. Acta* **795**, 37–46
30. Agwu, D. E., McPhail, L. C., Wykle, R. L. & McCall, C. E. (1989) *Biochem. Biophys. Res. Commun.* **159**, 79–86
31. Thompson, N. T., Tateson, J. E., Randall, R. W., Spacey, G. D., Bonser, R. W. & Garland, L. G. (1990) *Biochem. J.* **271**, 209–213
32. Kanaho, Y., Kanoh, H., Saitoh, K. & Nozawa, Y. (1991) *J. Immunol.* **146**, 3536–3541
33. Balsinde, J., Diez, E. & Mollinedo, F. (1988) *Biochem. Biophys. Res. Commun.* **154**, 502–508
34. Reinhold, S. L., Zimmerman, G. A., Prescott, S. M. & McIntyre, T. M. (1989) *J. Biol. Chem.* **264**, 21652–21659
35. Tou, J.-S. (1979) *Biochim. Biophys. Acta* **572**, 307–313
36. Rossi, F., Grzeskowiak, M. K., Della Bianca, V., Calzetti, F. & Giorgia, G. (1990) *Biochem. Biophys. Res. Commun.* **168**, 320–327
37. Moolenaar, W. H., Kruijer, W., Tilly, B. C., Verlaan, L., Bierman, A. J. & de Laart, S. W. (1986) *Nature (London)* **323**, 171–173
38. Jackowski, S. & Rock, C. O. (1989) *Arch. Biochem. Biophys.* **268**, 516–524
39. Traynor-Kaplan, A. E., Thompson, B. L., Harris, A. L., Taylor, P., Omann, G. M. & Sklar, L. A. (1989) *J. Biol. Chem.* **264**, 15668–15673
40. Eberle, M., Traynor-Kaplan, A. E., Sklar, L. A. & Norgauer, J. (1990) *J. Biol. Chem.* **265**, 16725–16728

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