Time-dependent inhibition of phospholipase C β -catalysed phosphoinositide hydrolysis: a comparison of different assays

Stephen R. JAMES^{*‡}, Sheila SMITH^{*}, Andrew PATERSON[†], T. Kendall HARDEN[†] and C. Peter DOWNES^{*}

^{*}Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee, DD1 4HN, U.K. and [†]Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, NC, U.S.A.

The properties of three different β -isoforms of phospholipase C (PLC) were analysed using substrate lipids dispersed in phospholipid vesicles, phospholipid–detergent mixed micelles and phospholipid monolayers spread at an air–water interface. Phosphatidylinositol 4,5-bisphosphate hydrolysis went virtually to completion in monolayers, but inositol trisphosphate production was curtailed prematurely in vesicular and micellar assays. Assays were linear for less than 2 min with vesicles; the linear portion could be significantly extended in micelles by increasing the ratio of micelles to enzyme molecules. However, onset of a second lower rate of substrate hydrolysis always

occurred when $\leq 10\%$ of PtdIns(4,5) P_2 had been utilized. This was not due to enzyme inactivation in the micellar interface, determined by addition of fresh substrate or fresh enzyme after the slow phase of activity had started, nor was it due to overt product inhibition of PLC or apparent entrapment of PLC at the micelle surface. These results are similar to those seen in assays using bacterial PLC and we suggest that the biphasic kinetics may be due to product-dependent changes in the presentation of substrate lipid to PLC in lamellar assays, leading to reduced activity.

INTRODUCTION

Extracellular signal-regulated phospholipases C (PLCs) form an extended family of enzymes which are responsible for the generation of inositol trisphosphate and diacylglycerol (DAG) second messengers from the membrane phospholipid PtdIns(4,5) P_2 [1]. A body of data concerning the manner in which these enzymes interact with lipid substrates is accumulating which shows general similarities and some subtle differences between different isoforms. Thus, PLC β and PLC δ are able to bind to interfaces with moderate affinities [2,3]; multiple interfacial contacts are made which facilitate processive behaviour of all three different PLC isoforms [3–5] and enzyme activities are differentially affected by characteristics of the lipid interface including composition and surface pressure [6–9].

A variety of assay procedures have been used to measure lipase activity, from which some of the above results concerning PLC have been derived. Most involve presentation of enzymes with pre-aggregated lipids, including phospholipid vesicles, phospholipid–detergent mixed micelles, pre-immobilized and cross-linked lipids and phospholipid monolayers. When comparing such experimental techniques, it is crucial to know whether and how the assay conditions influence kinetic parameters and enzyme regulation. We have previously developed a kinetic model of PLC β activity based upon initial velocity determinations of PtdIns(4,5) P_2 hydrolysis [3], using lipid–dodecylmaltoside mixed micelles. We showed that PLC β isoforms obey dual substrate-type kinetics, suggesting the enzyme makes multiple contacts with lipid interfaces which may be important for efficient activation by G-protein subunits and rapid lipid hydrolysis.

During kinetic analyses of PLC β activity using mixed micelles, we noticed that linear kinetics were observed only when $\leq 10\%$ of substrate was consumed. In this paper, we have compared three different assay techniques for PLC, including micelles, vesicles and monolayers and show that the characteristics of each

differs with the time of incubation. Hydrolysis of PtdIns(4,5) P_2 in monolayers can continue virtually to completion, but hydrolysis in vesicles and micelles is markedly biphasic, with linearity over hydrolysis of $\leq 10\%$ substrate, followed by a slower catalytic phase up to approx. 30% hydrolysis. This behaviour does not appear to be due to interfacial inactivation of the enzyme, but rather seems to be caused by a product-dependent alteration in the characteristics of the micelles such that enzyme and substrate are sequestered from each other.

MATERIALS AND METHODS

Materials

Di-oleoyl phosphatidylcholine (PtdCho) and phosphatidylethanolamine were purchased from Sigma and phosphatidylserine (PtdSer) was from Boehringer Mannheim. [³H]PtdIns(4,5) P_2 was purchased from Amersham; unlabelled PtdIns(4,5) P_2 was purified from Folch extract of brain lipid (Sigma) by amino-cyano HPLC as previously described [8]. Long-chain DAG (pig liver) was bought from Serdary Lipids. [³²P]ATP was from Amersham and was used to synthesize ³²P-labelled PtdIns(4,5) P_2 from PtdIns4P using partially purified rat brain PtdIns4P kinase, as previously described [8]. Dodecylmaltoside was from Boehringer Mannheim. PLC enzymes were expressed and purified as previously described [3].

Micellar and vesicular PLC assays

[³H]PtdIns(4,5) P_2 solutions were prepared at 4 times the final assay concentrations by mixing unlabelled and labelled lipids (sufficient for 15000–25000 d.p.m. per assay) from organic solutions which were dried to a film under vacuum. Lipids were resuspended by probe sonication (3 mm diameter probe, 30 s at

Abbreviations used: DAG, diacylglycerol; PLC β , β -isoforms of phospholipase C; TEPLC, β -isoform of PLC from turkey erythrocyte cytosol; PtdCho, phosphatidylcholine; PtdSer, phosphatidylserine; Ins P_3 , inositol 1,4,5-trisphosphate; c.m.c., critical micellar concentration.

‡ To whom correspondence should be addressed.

10 W power on ice) in 10 mM Hepes, pH 7.2. Final concentrations of PtdIns(4,5) P_2 in assays were either 50 or 100 μ M. For vesicle assays (100 μ l final volume), lipid was added to tubes containing 25 μ l of assay buffer (final concentrations: 10 mM Hepes, pH 7.2, 120 mM KCl, 10 mM NaCl, 2 mM EGTA, 1 mM MgCl₂, 1 μ M free Ca²⁺) and 25 μ l of water. Assays were performed at 37 °C and were started by addition of 25 μ l of PLC solution, containing between 1 and 100 ng of enzyme, as indicated in the Figure legends. Lipid hydrolysis was ended after the indicated times by addition of 750 μ l of chloroform/methanol/concentrated HCl mixture (40:80:1, by vol.), 100 μ l of water, 250 μ l of chloroform and 250 μ l of 0.1 M HCl and tritiated inositol 1,4,5-trisphosphate (InsP₃) release was measured by liquid scintillation spectrometry of 500 μ l of the upper phase. Micellar assays were performed identically except that dodecylmaltoside was added to dilute the surface concentration of PtdIns(4,5) P_2 and increase the total interfacial concentration in the tubes.

Monolayer assays

Monolayer PLC assays were performed as described previously [8] with some modifications. The monolayer trough was milled out of Teflon to a depth of 4 mm, with a surface area of 21.6 cm² (16 ml subphase volume sitting slightly proud of the rim of the trough), supplied by Nima Technology Ltd., Coventry, U.K. Monolayer surface pressure was measured continuously using filter paper Wilhelmy plates suspended from an electronic microbalance. Surface radioactivity was monitored continuously using a Bioscan FC-006 remote detector (from LabLogic, Sheffield, U.K.) suspended 0.5 cm above the monolayer, coupled to Labchrom computer software (LabLogic) which recorded the data and was used to integrate radioactivity traces. The subphase buffer comprised 10 mM Hepes, pH 7.2, 120 mM KCl, 10 mM NaCl, 2 mM EDTA, 1 mM MgCl₂ and 1 μ M free Ca²⁺, which was stirred with a Teflon-coated stirrer flea, and composite lipid monolayers {70 % PtdCho/27 % PtdSer/3 % [³²P]PtdIns(4,5) P_2 } were spread at the surface. An aliquot (1 μ g) of PLC was introduced after 5 min and assays were performed for up to 25 min. Time courses were determined from the continuous trace recordings of the loss of radioactivity from the monolayer due to PtdIns(4,5) P_2 hydrolysis, and the extent of hydrolysis was determined by sampling the radioactivity remaining in the monolayer and in 1 ml of subphase buffer at the end of the assay.

Data presentation and analysis

All experiments shown here have been repeated with three PLC β isoforms: recombinant mammalian PLC β 1 and PLC β 2, and PLC β purified from the cytosol of turkey erythrocytes (TEPLC). A detailed kinetic analysis of the micellar system has been reported previously [3] and will not be reiterated here. PtdIns(4,5) P_2 concentrations were generally chosen such that added dodecylmaltoside concentrations were greater than the critical micellar concentration (c.m.c.) of 100 μ M at 0.1 M salt [10]. Only in Figure 2 was the added detergent concentration less than 100 μ M (75 μ M was added) and the actual c.m.c. in the presence of phospholipid and bivalent ions is unclear. First-order rate constants (k) were obtained by transforming time-course data into expressions of substrate remaining at each time point, and fitting the results to the first-order rate equations:

$$[S] = [S]_0 \cdot e^{-kt} \quad (1)$$

and its logarithmic derivative

$$\log[S] = -k \cdot t/2.3 + \log[S]_0 \quad (2)$$

RESULTS AND DISCUSSION

Kinetics of PtdIns(4,5) P_2 hydrolysis in different assays

Although the use of phospholipid vesicles is arguably the simplest way to present lipid-metabolizing enzymes with substrate and avoids using non-physiological components, they are relatively difficult to manipulate systematically. Thus, suspensions of vesicles in aqueous solution are heterogeneous in size unless extruded through membranes with pores of a fixed diameter, and vesicles comprising different phospholipids are not necessarily homogeneously mixed. Furthermore, we have shown that vesicles of mixed phospholipid composition are not suitable for measurements of PLC β activity, but rather appear to be inhibitory [3]. The development of a mixed micellar assay for PLC β in which dodecylmaltoside is added as an inert diluent of substrate lipid has allowed characterization of the dependence of this enzyme activity on bulk lipid concentration and mole fraction. However, systematic variation of certain interfacial qualities is not possible, such as curvature and surface pressure, and lamellar structure is not necessarily preserved in this system. Phospholipid monolayers spread at the air–water interface can be used to vary surface pressure, surface potential and lipid composition while maintaining the monomolecular structure, in a manner that can be thought of as a simple model of the inner leaflet of biological membranes. Thus, each assay technique for measuring lipase activity can be used to investigate different features of enzyme action and are complementary to each other, enabling the construction of an overall picture of the enzymic mechanism.

PLC β activity as a function of time was measured in micellar, vesicular and monolayer assays (Figures 1a and 1b). The rate of substrate hydrolysis was followed for up to 1 h and Figure 1(a) shows a comparison between hydrolysis of 100 % PtdIns(4,5) P_2 vesicles and dodecylmaltoside–PtdIns(4,5) P_2 mixed micelles in parallel assays. Micellar assays were linear for between 5 and 10 min at the chosen substrate mole fraction of 0.4, after which a second, slower phase of hydrolysis was observed. By contrast, two phases of activity were more marked in vesicular assays, which comprised an apparent burst of activity followed by a pronounced slower phase. When transformed into first-order plots of the concentration of PtdIns(4,5) P_2 remaining against time, a similar biphasic relationship was seen (inset Figure 1a). The first-order rate constant of the second phase was similar regardless of the mole fraction of PtdIns(4,5) P_2 in the assay at the chosen enzyme concentration and was $(1.47 \pm 0.2) \times 10^{-4}$ per min (mean $\pm$ S.D., $n = 6$). Thus, the catalytic rate became particularly low after an initial burst of lipid hydrolysis. The initial linear phase generally subsided before 10 % of the lipid was hydrolysed, and in prolonged time courses (up to 3 h; S. R. James, unpublished work), no more than 30 % of substrate was utilized.

In comparison, PtdIns(4,5) P_2 hydrolysis was more extended in monolayer assays. Figure 1(b) shows PLC β 1-catalysed hydrolysis of PtdCho/PtdSer/PtdIns(4,5) P_2 monolayers at an initial pressure of 15 mN/m. Mixed composition monolayers were used because we have previously observed that PLC β is not able to hydrolyse 100 % PtdIns(4,5) P_2 monolayers, which we attribute to the high negative surface charge contributed by this lipid (S. R. James, R. A. Demel and C. P. Downes, unpublished work). When vesicular assays of similar mixed lipid composition were performed, only a little InsP₃ production was detected with no measurable linear phase (S. R. James and S. Smith, unpublished work), due to the inhibitory effects of these lipids in lamellar assays [3]. Enzyme was added to the calcium-containing subphase at 5 min, and after an initial lag time, hydrolysis of > 80 % of the phosphoinositide present (seen as a decrease in monolayer radioactivity) was observed which was apparently linear with

Figure 1 Time course of (a) PtdIns(4,5) P_2 hydrolysis by PLC β 2 in vesicles and micelles and (b) PLC β 1-catalysed loss of radioactivity from [^{33}P]PtdIns(4,5) P_2 -containing phospholipid monolayers

(a) Micelles (substrate mole fraction was 0.4 containing 150 μM dodecylmaltoside, shown by circles) and vesicles (squares) containing 100 μM PtdIns(4,5) P_2 were incubated with 15 ng of enzyme for the indicated times and InsP₃ production was measured as described in the Materials and methods section. Error bars which are larger than the symbol indicate the range of duplicates. Inset: first-order transformation of the time-course data fitted to eqn (2). Results are representative of several experiments which have been repeated with PLC β 1 and TEPLC. (b) Monolayers were formed at an initial surface pressure of 15 mN/m and 1 μg of PLC β 1 was added to the subphase after 5 min. The free calcium concentration was 1 μM . Results were taken as described in the Materials and methods section and show a smoothed trace, representative of three experiments performed at that pressure.

time. Although vesicle assays are more properly compared with monolayer assays at higher pressure (> 25 mN/m), we have previously published similarly extensive phosphoinositide hydrolysis in identical monolayers by TEPLC at 30 mN/m [8]. Thus, InsP₃ formation in composite monolayers occurs with different characteristics from bilayer assays.

When the mole fraction of PtdIns(4,5) P_2 was reduced by increasing the concentration of dodecylmaltoside in micellar assays without altering the bulk lipid concentration, the linear phase (initial velocity) of PLC-catalysed lipid hydrolysis was prolonged (Figure 2a). Using the concentration of PLC β 2 shown in Figure 2(a), reduction of the mole fraction of PtdIns(4,5) P_2 from 0.4 to 0.15 caused the linear phase to continue for 10 min, albeit at a reduced rate. This indicated that a factor relevant to establishing linear kinetics in PLC β assays was the relative saturation of micelles with enzyme molecules. In Figure 2(a), the reduction in substrate mole fraction from 0.4 to 0.15 increased the ratio of micelles to PLC molecules from 675 to over 2500.

The ratio of micelles to enzyme molecules was varied in a

Figure 2 (a) Time courses of PtdIns(4,5) P_2 hydrolysis by PLC β 2 in mixed micelles at different mole fractions and (b) measurements of the linear phase of PtdIns(4,5) P_2 hydrolysis by PLC β 1 using increasing concentrations of enzyme

(a) Assays contained 15 ng of enzyme and 50 μM PtdIns(4,5) P_2 . Mole fractions were 0.4 (squares) and 0.15 (circles), obtained by adding 75 μM and 283 μM dodecylmaltoside respectively. Ratios of the number of micelles to the number of PLC molecules were 675 and 2600 respectively. Data presentation is as in Figure 1 and are representative of several experiments which gave similar results with PLC β 1 and TEPLC. (b) Assays were performed using 50 μM PtdIns(4,5) P_2 at a mole fraction of 0.4 (75 μM dodecylmaltoside) and 1 ng (○), 5 ng (□), 10 ng (●), 50 ng (×) and 100 ng of PLC β 1 (+). Ratios of micelles to PLC molecules were 11500, 2250, 1000, 200 and 100, respectively. Solid lines are fits of the data to eqn. (1) using the computer program Ultrafit.

different manner in an attempt to semi-quantify the proportion of micelles to PLC molecules associated with a given period of linearity (Figure 2b). Thus, assays containing 50 μM PtdIns(4,5) P_2 at a fixed mole fraction of 0.4 (in the presence of 75 μM dodecylmaltoside) were performed with 1 ng, 5 ng, 10 ng, 50 ng and 100 ng of PLC. The results showed that at the lowest enzyme concentration, the assay was linear for 15 min. As the amount of added PLC increased, linearity was proportionally reduced and substrate utilization was increased. The ratio of micelles to PLC was calculated using an aggregation number for

dodecylmaltoside of 98 molecules per micelle [10], from which general relationships were established. At a ratio of 100, assays were not measurably linear; when ≥ 1000 , assays were linear for 5 min and when greater than 10000, linearity was maintained for up to 15 min. Thus, reducing the enzyme concentration in the assay results in a predictable decrease in the rate of InsP_3 formation, but a feature of this lower rate is that, while lipid hydrolysis remains below 10%, linear kinetics are maintained. Taken together, these results show that the ratio of micelles to PLC molecules is of critical importance to PLC micellar assays, which is key to retaining linear assays and high reaction rates must be sacrificed for accurate kinetics based upon initial enzyme rates.

Curtailed activity is not due to enzyme inactivation

The onset of a slower phase of catalysis before significant depletion of substrate concentrations has been reported for several lipid-metabolizing enzymes including membrane-bound phospholipase A_2 [11], high-molecular-mass phospholipase A_2 [12,13] and bacterial PLC [14]. In the case of membrane-bound phospholipase A_2 , the effect was due to arachidonic acid-mediated product-inhibition of the enzyme, while for the high-molecular-mass enzyme, re-addition of substrate or enzyme showed that the loss in catalytic rate was due to enzyme inactivation during the reaction [12]. We examined these possibilities for PLC β by adding either fresh enzyme or fresh substrate to PLC-catalysed reactions after the slower phase of activity had been reached (Figures 3a and 3b). Lipid and enzyme were added in 20 μl volumes, and subsequent assays were therefore diluted by one-fifth. Addition of fresh equivalent amounts of enzyme to time courses after 15 min had little effect on the slow phase of lipid hydrolysis (Figure 3a), and InsP_3 production continued at a slightly enhanced rate. In contrast, addition of fresh $\text{PtdIns}(4,5)P_2$ at the same mole fraction (0.4) as in the original assay resulted in a second similar time-course profile, comprising an initial burst of activity followed by a lower catalytic rate (Figure 3b). These results suggest that loss of the fast initial PLC catalysed-reaction velocity was not due to inactivation of the enzyme.

Addition of fresh substrate mixed micelles would not be expected to result in complete fusion with product-containing micelles already present under the assay conditions employed, and suggests that there is some intermicellar movement of PLC molecules. Thus, it appears that the reduced catalytic rate was not due to irreversible entrapment of all the enzyme molecules present at the micellar interface in the assay in some time- or product-dependent fashion. Indeed, pre-incubation of PLC with unlabelled $\text{PtdIns}(4,5)P_2$ substrate for 15 min before addition of labelled lipid resulted in a time course of InsP_3 production similar to that shown in Figures 1–3, indicating intermicellar movement of PLC from unlabelled to labelled lipid (S. R. James and S. Smith, unpublished work). Furthermore, the observation that re-addition of fresh enzyme after 15 min to assay tubes had only a moderate effect on the slower catalysis indicated that the effects were not due to total inhibition of original enzyme and are more likely due to some alteration in the form of the substrate micelles present.

Reductions in the rate of enzyme activity in lamellar assays could have been interpreted as evidence for product-inhibition of PLC β . Although PLC δ has been shown to bind InsP_3 specifically, which inhibits lipid hydrolysis by preventing association of the enzyme with substrate-containing interfaces [15], we have not been able to demonstrate this for PLC β (S. R. James and S. Smith, unpublished work). We therefore do not feel that the

Figure 3 Addition of (a) fresh enzyme to the slow phase of PLC activity has little effect on the rate of InsP_3 formation and (b) fresh lipid substrate to the slow phase of PLC β activity causes a second burst in enzyme activity

(a) Assays containing 100 μM $\text{PtdIns}(4,5)P_2$ and 150 μM dodecylmaltoside with 50 ng of PLC β 2 continued for 15 min and 50 ng of fresh enzyme in 20 μl volume were added at the arrows. Data presentation is as in Figure 1. (b) Conditions were exactly the same as in (a), and fresh lipid at a mole fraction of 0.4 in 20 μl volume was added at the arrow.

inositol trisphosphate head group product of PLC catalysis inhibits PLC β activity.

The effect of long-chain DAGs on PLC β activity was investigated by preparing $\text{PtdIns}(4,5)P_2$ micellar substrates co-sonicated with increasing concentrations of DAG. Figure 4 shows that time courses were not overtly affected by the presence of DAG in the assays. DAG and $\text{PtdIns}(4,5)P_2$, however, are likely to behave differently in aqueous media due to the different characteristics of the polar head groups [16]. Thus, DAG is more likely to form non-lamellar phases rather than reside completely in bilayer structures and we cannot be certain that co-sonication of phosphoinositide with DAG results in homogeneously mixed preparations of DAG- $\text{PtdIns}(4,5)P_2$ -dodecylmaltoside micelles in the assay. When lipids remaining after treatment with PLC were recovered from organic lower phases, dried to a film, sonicated into buffer and re-assayed to test their suitability as PLC β substrates, results showed that the presence of DAG produced endogenously by the action of enzyme did not markedly affect subsequent InsP_3 production (S. Smith and S. R. James, unpublished work). This experiment is also subject to the above discussion of the behaviour of DAG and from these data, we conclude that at the crude level DAG does not act as a product-inhibitor of PLC β .

Figure 4 The effects of increasing DAG concentrations on time courses of InsP_3 production by $\text{PLC}\beta 1$

100 μM $\text{PtdIns}(4,5)\text{P}_2$ was at an initial mole fraction of 0.4 relative to added dodecylmaltoide, which does not take into account the contribution of DAG to the interface. DAG additions were 0% relative to $\text{PtdIns}(4,5)\text{P}_2$ concentration (□), 1% (○), 5% (△), 10% (▽) and 15% (★). Assays used 50 ng of $\text{PLC}\beta 1$, and have been repeated several times with $\text{PLC}\beta 2$ and TEPLC .

DAG molecules produced endogenously in assays of $\text{PLC}\beta$ -catalysed cleavage of $\text{PtdIns}(4,5)\text{P}_2$ would be expected to remain in the micellar or vesicular interface. The effects of long-chain DAGs on interfacial characteristics have been well-documented [17,18] and, depending on the lipid composition, result in lateral phase separations forming phases with different fluidities and non-lamellar, hexagonal phases. The resultant 'defects' in bilayer integrity have been shown to promote the activity of several isoforms of phospholipase A_2 , a result attributed to the increased ease with which the enzyme can extract phospholipids and hydrolyse the *sn*-2 fatty acid. The catalytic action of PLC may not be dependent on similar factors. Thus, the use of polymerizable lipids, whose lateral and vertical motions are restricted, has been demonstrated not to affect bacterial PLC activity, suggesting mobility of phospholipid molecules is not critical to the hydrolysis of the head group [19]. However, this enzyme exhibits similar curtailed catalytic activity to that described here [14].

We suggest that in lamellar assays of $\text{PLC}\beta$ -catalysed hydrolysis of $\text{PtdIns}(4,5)\text{P}_2$, a substrate phase may develop which is not accessible to PLC and reduces enzyme activity. Although we have no evidence for this, there may be a precedent for product-induced changes in lipid phases from work with phospholipase A_2 . Thus, the lyso- PtdCho product of phospholipase A_2 action is thought to facilitate association of the enzyme with the interface, leading to the burst activity [13], possibly by a detergent effect on lamellar structure. ^1H - and ^{31}P -NMR analysis of vesicles before and after $\text{PLC}\beta$ treatment will help to discern whether such

alterations in the lipid interface occur. Monolayer assays of $\text{PLC}\beta$ activity are not prone to such alterations in lipid phases; DAG forms monolayers at an air-water interface readily, and while fluid phase separations may occur as the DAG concentration grows throughout assays, these are not seen to affect the extent of substrate utilization. These results have implications for experiments designed to test the influence of G-protein α - and $\beta\gamma$ -subunits on $\text{PLC}\beta$, and assay conditions must be carefully designed to ensure that linearity is maintained throughout the duration of the assay.

In this paper, we have shown that lamellar assays of $\text{PLC}\beta$ activity result in significantly curtailed lipid hydrolysis, an observation not made when using monolayers. The effect is not due to enzyme inactivation, nor substrate restriction, nor classical product inhibition, but we believe may be due to product-dependent alterations in interfacial characteristics which are dependent on the presence of a bilayer and serve to sequester substrate from the enzyme. Further analysis will include detailed investigation of the substrate vesicles before and during catalysis, and coupled assays (using DAG kinase or triacylglycerol lipase) to remove product DAG, to see if restrictions in enzyme activity can be relieved.

We are grateful to Mary Roberts and Rudy Demel for helpful discussions of our data. This work was supported by a grant from the B.B.S.R.C. (to S.J. and C.P.D.). A.P. is the recipient of a Wellcome Trust Travel Grant.

REFERENCES

- Rhee, S. G. and Choi, K. D. (1992) *J. Biol. Chem.* **267**, 12393–12396
- Rebecchi, M., Peterson, A. and McLaughlin, S. (1992) *Biochemistry* **31**, 12742–12747
- James, S. R., Paterson, A., Harden, T. K. and Downes, C. P. (1995) *J. Biol. Chem.* **270**, 11872–11881
- Wahl, M. I., Jones, G. A., Nishibe, S., Rhee, S. G. and Carpenter, G. (1992) *J. Biol. Chem.* **267**, 10447–10456
- Cifuentes, M. E., Honkanen, L. and Rebecchi, M. J. (1993) *J. Biol. Chem.* **268**, 11586–11593
- Hirasawa, K., Irvine, R. F. and Dawson, R. M. C. (1981) *Biochem. J.* **193**, 607–614
- Rebecchi, M., Boguslavsky, V., Boguslavsky, L. and McLaughlin, S. (1992) *Biochemistry* **31**, 12748–12753
- James, S. R., Demel, R. A. and Downes, C. P. (1994) *Biochem. J.* **298**, 499–506
- Boguslavsky, V., Rebecchi, M., Morris, A. J., Jhon, D.-Y., Rhee, S. G. and McLaughlin, S. (1994) *Biochemistry* **33**, 3032–3037
- Neugebauer, J. (1988) in *A Guide to the Properties and Uses of Detergents in Biology and Biochemistry*, Calciochem Corporation
- Lister, M. D., Deems, R. A., Watanabe, Y., Ulevitch, R. J. and Dennis, E. A. (1988) *J. Biol. Chem.* **263**, 7506–7513
- Leslie, C. C. (1991) *J. Biol. Chem.* **266**, 11366–11371
- Creaney, A., Masters, D. J., Needham, M. B., Gordon, R. D., Mott, R. and Wilton, D. C. (1995) *Biochem. J.* **306**, 857–864
- Lewis, K. A., Garigapati, V. R., Zhou, C. and Roberts, M. F. (1993) *Biochemistry* **32**, 8836–8841
- Cifuentes, M. E., Delaney, T. and Rebecchi, M. J. (1994) *J. Biol. Chem.* **269**, 1945–1948
- Seddon, J. M. and Cevc, G. (1993) in *Phospholipids Handbook* (Cevc, G., ed.), pp. 403–454, Marcel Dekker Inc., New York
- De Boeck, H. and Zidovetzki, R. (1992) *Biochemistry* **31**, 623–630
- Zidovetzki, R., Laptalo, L. and Crawford, J. (1992) *Biochemistry* **31**, 7683–7691
- Soltys, C. E., Bian, J. and Roberts, M. F. (1993) *Biochemistry* **32**, 9545–9552