Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 196-201

DIACYLGLYCEROL POTENTIATES PHOSPHOLIPASE ATTACK UPON PHOSPHOLIPID BILAYERS: POSSIBLE CONNECTION WITH CELL STIMULATION

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Received October 10, 1983

Many phospholipases of the A or C types show a very limited ability to hydrolyse phospholipids such as phosphatidylcholine or phosphatidylinositol which adopt a bilayer form when hydrated. The addition of unsaturated 1,2 or 1,3 diacylglycerols to such substances produces a marked stimulation of the attack. In contrast, the hydrolysis of phosphatidylethanolamine, which normally exists in the hexagonal II structure, is not stimulated by diacylglycerol. It is suggested that the liberation of diacylglycerol which often occurs during cell stimulation, may play a part in activating the associated cascade of phospholipid reactions.

When many cells are stimulated by agonists, there occurs a rapid breakdown of phosphoinositides which results in the immediate release and accumulation of diacylglycerol (1,2,3). Until recently it has been assumed that this diacylglycerol played little direct part in the receptor activation sequence, although it was known to be further metabolised. However, Nishisuka and his colleagues (3) have demonstrated that this lipid is a potent stimulator of the isolated protein kinase C phospholipid complex, which is known to be activated during cell stimulation attended by enhanced phosphoinositide catabolism. Here we report that diacylglycerol also produces a marked activation of many phospholipases when these are hydrolysing substrates such as phosphatidylcholine and phosphatidylinositol; these phospholipids are known to exist as closely packed bilayers when hydrated (4,5). We conclude that diacylglycerol causes a perturbation of the bilayer structure perhaps inducing a bilayer - non bilayer phase transition which is conducive to phospholipase activity. It is possible that the rapid formation of diacylglycerol which occurs after many types of cell stimulation, may thus control or amplify the associated cascade of phospholipid reactions which ultimately results in prostaglandin synthesis.

EXPERIMENTAL

Lipids. ^{32}P-labelled phospholipids were prepared biosynthetically (6). 1,2 diacylglycerol was prepared from egg phosphatidylcholine using Cl welchii

0006-291X/83 $1.50

α toxin and other samples of diacylglycerol were purchased from Sigma. Acyl migration of the diacylglycerol during storage was monitored by thin layer chromatography (silica gel Merck F254 plates; diethylether - petroleum ether - acetic acid, 50:50:1 parts by volume).

Phospholipase preparation and assay. In general the diacylglycerol was added to the substrate (^{32}P-labelled phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol; 0.34 μmol, approx. 10^5 d.p.m.) in chloroform solution and the mixed lipids taken to dryness before suspending in buffer etc. Human blood platelet supernatant was prepared and the phosphatidylinositol phosphodiesterase assayed by the procedure of Rittenhouse-Simmons (3) except that deoxycholate was not added as an activator. Purified phospholipase A_1 of liver was isoalted and measured by procedures previously described (7). A preparation of soluble rat intestinal mucosal phospholipase A_2 (8) was obtained by centrifuging (100,000g) a 0.25M sucrose homogenate of the mucosal cells. The ^{32}P-phosphatidylcholine substrate was incubated with the intestinal supernatant for 30 min at 33°C in 0.033M Tris-maleate buffer pH 7.4, containing 8 mM EDTA. After butanol and diethylether extraction of the incubate, water-soluble counts in glycerophosphocholine were assayed. Although lysophosphatidylcholine released was quantitatively deacylated because of an extremely active phospholipase B in the enzyme source, any residual counts in the lysophosphatidylcholine were also routinely assayed (7).

RESULTS

To illustrate the action of diacylglycerol on phospholipases we provide three examples of widely differing enzymes where the addition of this lipid to the phospholipid substrate has a major effect on enzyme activity. The soluble phosphodiesterase (phospholipase C type) in human blood platelets which hydrolyses phosphatidylinositol producing diacylglycerol as the lipoidal product (3) was markedly activated by adding either 1,2-diacyl-sn-glycerol or diolein (predominantly 1,3-isomer) (Fig. 1). Clearly, both types of

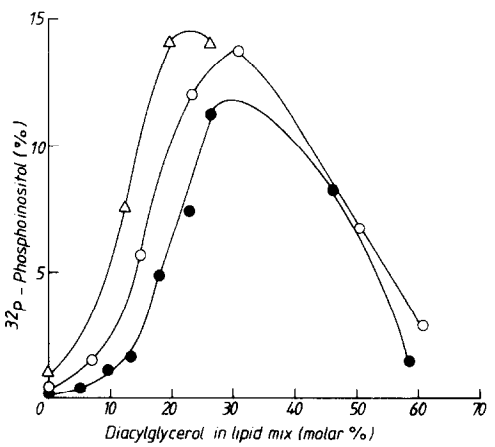

Fig. 1 Activation of human platelet phosphatidylinositol phosphodiesterase by diacylglycerol.
- o 1.2-diacyl-sn-glycerol prepared from egg phosphatidylcholine.
- Δ dioleoyl-glycerol (85% 1,3 isomer).
- • 1,2-diacyl-sn-glycerol (as above) and ^{32}P-phosphatidylinositol substrate (0.34 μmol) admixed with egg phosphatidylcholine (0.16 μmol).

diacylglycerol are thus functioning like deoxycholate which is added in the usual assay of this enzyme. The effect was still observed if egg phosphatidylcholine was admixed with the substrate; this phospholipid is known to have a marked inhibitory action on soluble phosphatidylinositol phosphodiesterase (9). However, somewhat more diacylglycerol was required to produce the equivalent activation.

The soluble phospholipase A_2 of rat intestinal mucosa (8) showed some ability to deacylate an exogenous phosphatidylethanolamine substrate but had negligible activity towards phosphatidylcholine. If, however, diacylglycerol was added to the latter substrate, by the time the diolein content had increased to 10% molar with the 1,3 isomer and 20% with the 1,2 isomer, there was a dramatic increase in the activity (Fig. 2) which then far exceeded the equivalent hydrolysis of phosphatidylethanolamine. The deacylation of the latter substrate was not significantly enhanced by diacylglycerol at any concentration (data not shown). At higher diacylglycerol concentrations (30-40% molar) the activity declined as observed previously with Ptd Ins phosphodiesterase (Fig. 1). A similar activation of the phospholipase A_2 hydrolysis was observed with diacylglycerols which had been prepared from egg phosphatidylcholine (mainly 1-stearoyl, 2 oleoyl-sn-glycerol) and from pig liver phosphatidylinositol (mainly 1-stearoyl, 2-arachidonyl-sn-glycerol). However, 1,2 or 1,3 isomers of dipalmitins and distearins showed little ability to activate.

A somewhat differing effect of diacylglycerol was seen with the liver alkaline phospholipase A_1, an intracellular enzyme that preferentially deacylates phosphatidylethanolamine provided that the negative zeta potential existing at the substrate's surface is discharged by soluble counter-ions or long chain cations introduced into the substrate (7). Phosphatidylcholine

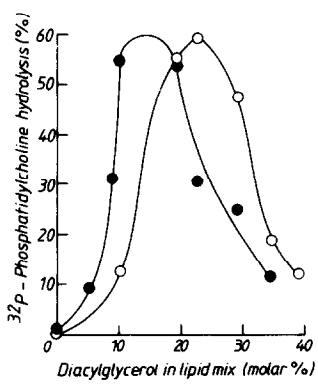

Fig. 2 Activation of rat intestinal mucosal phospholipase A_2 by diacylglycerol.
o 1,2-dioleoyl-glycerol
• 1,3-dioleoyl-glycerol

acts as a powerful inhibitor of phosphatidylethanolamine hydrolysis, a property which we have attributed to the ability of the choline-containing phospholipid to cause an hexagonal-to-bilayer conformation change in the hydrated substrate structure (7). We have now found that diacylglycerol has the ability to reverse this inhibition (Fig. 3). Thus, 8 to 12% molar 1,3- or 1,2-diolein very substantially restored the capacity of the enzyme to deacylate ^{32}P-phosphatidylethanolamine admixed with phosphatidylcholine.

In additional experiments we have observed that diacylglycerol enhanced triphosphoinositide phosphodiesterase attacking its substrate (phosphatidylinositol 4,5-^{32}P bisphosphate) contained as a minor constituent of a phospholipid-cholesterol mixture designed to mimic the plasma membrane bilayer. Activity occurred at low Ca^{2+} concentrations (10^{-6}M Ca^{2+} at pH 7.25) and in the presence of Mg^{2+} (1 mM) and KCl (80 mM). Under certain incubation conditions diacylglycerol could also substantially enhance the activities of the phospholipase A_2 of pancreas and the phospholipase C of Cl welchii when these enzymes were attacking an egg phosphatidylcholine substrate.

DISCUSSION

It is apparent that the ability of diacylglycerol to influence enzymes attacking phospholipid/water interfaces is of wide occurrence. Although the mechanism of activation is not certain all of the results are consistent with the hypothesis that it is in some way perturbing the tightly-packed bilayer structure which pure phosphatidylcholine and phosphatidylinositol adopt in an

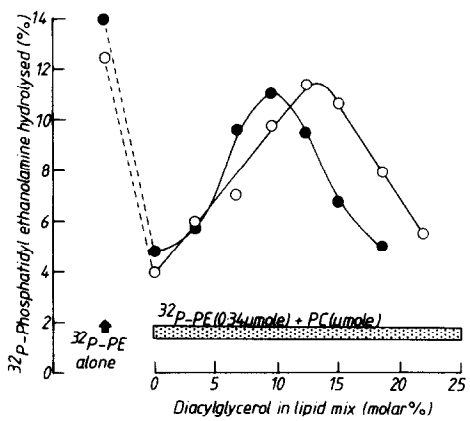

Fig. 3 Reversal of the phosphatidylcholine inhibition of ^{32}P-phosphatidylethanolamine deacylation by rat liver phospholipase A_1 with diacylglycerol.

The substrate 0.34 μmol ^{32}P-phosphatidylethanolamine (PE) was used alone or with 0.23 μmol egg phosphatidylcholine (PC) added as inhibitor. Diacylglycerol was added to the inhibited system.

o 1,2-diacylglycerol (prepared from PC)

• 1,3-dioleoyl-glycerol.

aqueous environment (4,5). Thus, although diacylglycerol has been shown to influence the attack of intestinal phospholipase A_2 on phosphatidylcholine, it does not do so with phosphatidylethanolamine which adopts a non-bilayer hexagonal-type structure (4). It is possible that insertion of the smaller hydrophilic head group of diacylglycerol into the phospholipid bilayer allows better access of the active centre of the enzyme to the susceptible bonds of the phospholipid, although it is perhaps significant that cholesterol with an even smaller head group causes no equivalent activation. Furthermore, the invariable observation that there is a lag before sequential additions of diacylglycerol produce a significant activation could be interpreted as indicating that it causes a more profound change in the bilayer structure, such as a bilayer-non bilayer phase transformation. A change of this type could also be responsible for the marked enhancement of protein phosphorylation on adding diacylglycerol to the phospholipid-protein kinase C complex (3) since phospholipid is an essential part of the enzyme and its activation.

It has been known for many years that the physico-chemical conditions, such as charge, packing density and order of a phospholipid/water interface can have a pronounced influence on the specificity and rate of phospholipase attack (11). The present results supplement our previous suggestions that the phospholipids in the normal membrane of a cell are protected from the action of the potent intracellular phospholipases by the bilayer structure, and activity only takes place when this structure is perturbed (7,12,13).

It has been realized recently that probably the first event in enhanced phosphoinositide metabolism after cell stimulation, and often occurring within seconds, is an hydrolysis of phosphatidylinositol 4,5 bis phosphate producing diacylglycerol (14,15). This is followed by a substantial breakdown of phosphatidylinositol within the cell either directly through phosphodiesterase action or by phosphorylation to produce triphosphoinositide (14) which is subsequently catabolised. Furthermore, there often occurs a deacylation of other phospholipids probably by phospholipase A action with the liberated arachidonic acid resulting in prostaglandin formation (16,17).

Whatever the route of breakdown, the immediate lipoidal product formed from the phosphoinositides is diacylglycerol, and where it has been measured, a substantial but transient increase in diacylglycerol levels occurs in cells on stimulation (1,2,3). It remains an open question as to what function, if any, this diacylglycerol accumulation fulfils. It is becoming increasingly apparent that diacylglycerol and analogous structures can have a dramatic direct effect on cell behaviour (3,18,19) and also, after lipase action the arachidonic acid content of diacylglycerol may partly be responsible for prostaglandin synthesis (16,17). The present results suggest that, as well as activating protein kinase C (3), a localised accumulation of diglyceride

in a cell membrane could play a specific role in activating the intracellular phospholipases that are stimulated following receptor occupation.

REFERENCES

1. Rittenhouse-Simmons, S. (1979). J. Clin. Invest. 63, 580-587.
2. Igrashi, Y. and Kondo, Y. (1980). Biochem. Biophys. Res. Commun. 97, 759-765.
3. Nishizuka, Y. (1983). Phil. Trans. R. Soc. Lond. B 302, 101-112.
4. Hui, S.W., Stewart, T.P., Yeagle, P.L. and Albert, A.D. (1981). Arch. Biochem. Biophys. 207, 227-240.
5. Nayer, R., Schmid, S.L., Hope, M.J. and Cullis, P.R. (1982). Biochim. Biophys. Acta 688, 169-176.
6. Irvine, R.F., Letcher, A.J. and Dawson, R.M.C. (1978). Biochem. J. 192, 279-283.
7. Dawson, R.M.C., Irvine, R.F., Hemington, N.L. and Hirasawa, K. (1983). Biochem. J. 209, 865-872.
8. Subbaiah, P.V., and Ganguly, J. (1970). Biochem. J. 118, 233-239.
9. Irvine, R.F., Hemington, N. and Dawson, R.M.C. (1979). Eur. J. Biochem. 99, 525-530.
10. Irvine, R.F., Letcher, A.J. and Dawson, R.M.C. (1980). Biochem. J. 192, 279-283.
11. Dawson, R.M.C. (1973). In "Form and Function of Phospholipids", edited by G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson, pp. 97-114. Elsevier, Amsterdam.
12. Dawson, R.M.C. (1982). J. Amer. Oil Chem. Soc. 59, 401-406.
13. Irvine, R.F., Letcher, A.J. and Dawson, R.M.C. (1984). Biochem. J. (in press).
14. Michell, R.H., Kirk, C.J., Jones, L.M., Downes, C.P. and Creba, J.A. (1981). Phil. Trans. R Soc. Lond. B 296, 123-137.
15. Berridge, M.J., Dawson, R.M.C., Downes, C.P., Heslop, J.P., and Irvine, R.F. (1983). Biochem. J. 212, 473-482.
16. Irvine, R.F., Dawson, R.M.C. and Freinkel, N. (1982). Contemporary Metabolism (N. Freinkel, editor) 2, 301-342. Plenum Press, New York.
17. Irvine, R.F. (1982). Biochem. J. 204, 3-16.
18. Sha'afi, R.I., White, J.R., Molski, T.F.P., Shefcyk, J., Volpi, M., Naccache, P.H. and Feinstein, M.B. (1983). Biochem. Biophys. Res. Commun. 114, 638-645.
19. Rink, T.J., Sanchez, A. and Hallam, T.J. (1983). Nature 305, 317-319.