Vol. 115, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1983 Pages 94-99

PHOSPHOLIPASE A_2 MODULATION BY CALMODULIN, PROSTAGLANDINS AND CYCLIC NUCLEOTIDES

Nathan Moskowitz, Lawrence Shapiro,[1]
William Schook, and Saul Puszkin[2]

Laboratory of Molecular Pathology, Department of Pathology
Mount Sinai School of Medicine of the City University of New York
New York, New York 10029

Received July 12, 1983

Phospholipase A_2 in the presence of Ca^{2+} was stimulated by calmodulin and by prostaglandin $F_{2\alpha}$. Prostaglandin E_2, cyclic-AMP and cyclic-GMP inhibited phospholipase A_2 in the presence or absence of calmodulin. Dimethylsuberimidate cross-linking of phospholipase A_2 with calmodulin was found to be Ca^{2+} dependent. These results indicate that phospholipase A_2 is directly regulated by a host of key intracellular regulators and is one of the calmodulin-regulated enzymes.

Phospholipase A_2 (PLA_2), which catalyzes the fatty ester hydrolysis of 1,2 sn-phosphoglycerides in the 2-position, is a Ca^{2+}-dependent enzyme ubiquitously distributed in eukaryotic and prokaryotic membranes (1). Membrane PLA_2 functions in lipid metabolism, prostaglandin (PG) production, membrane fusion, chemotaxis, neurotransmitter release and coated vesicle endocytosis (2-7). Calmodulin is an ubiquitous cellular Ca^{2+}-binding protein which activates a host of enzymes (8). Prostaglandins are oxygenated and unsaturated carbon fatty acids containing a cyclopentane ring performing many diverse cellular functions (9). We previously reported that PLA_2 activity was present in synaptic vesicles and was stimulated by calmodulin (CaM) and $PGF_{2\alpha}$ and inhibited by PGE_2 (6). To ascertain whether CaM, $PGF_{2\alpha}$ and PGE_2 had direct effects on PLA_2, we studied a highly purified PLA_2 which has an amino acid sequence homologous to mammalian PLA_2

[1] Present address: Department of Biophysiology, California Institute of Technology, Pasadena, CA 91125

[2] To whom correspondence should be addressed.

0006-291X/83 $1.50

(10). Because the actions of PGE_2 and $PGF_{2\alpha}$ may be mediated via interaction with adenylate and guanylate cyclases which generate cAMP and cGMP respectively (11), we tested the effects of cAMP and cGMP on this enzyme.

MATERIALS AND METHODS

Reaction mixtures containing 1 pgm PLA_2 purified from *Naja naja* snake venom (Sigma, St. Louis, Missouri), 1 mM $CaCl_2$ and 1.8 nmol [^{14}C]-β-arachidonylphosphatidylcholine (S.A. 54 mCi/mmol, Amersham, Arlington Heights, Illinois) were incubated for 10 minutes at pH 8.9 at 25°C under the following two conditions: 1) with increasing concentrations of CaM (10^{-9}-10^{-3} M), PGE_2, $PGF_{2\alpha}$, cAMP or cGMP (10^{-9}-10^{-3} M), or 2) with CaM (10^{-7} M) and increasing concentrations of PGE_2, $PGF_{2\alpha}$, cAMP or cGMP (10^{-9}-10^{-3} M). Reactions were stopped, processed and PLA_2 activity assayed as described previously (6, 13). Phospholipase cross-linking was performed using dimethylsuberimidate (DMS [Aldrich, Milwaukee, Wisconsin), an alkylimidate ester which joins proteins that form a complex within 11 Å from each other under a variety of conditions (14). Phospholipase A_2 (20 µg) was incubated in a total volume of 60 µl with 0.42 µg/ml DMS dissolved in 20 µM triethanolamine buffer, pH 8.5, for 30 minutes in the absence or presence of either PGE_2 (10^{-5} M), $PGF_{2\alpha}$ (10^{-5} M), cAMP (10^{-3} M) or cGMP (10^{-3} M). For each of these four conditions there were the following subconditions: incubation in the presence of 1) 1 mM $CaCl_2$; 2) 1 mM EGTA; 3) 1 mM $CaCl_2$ and 7 µg CaM; or 4) 1 mM EGTA and 7 µg CaM. The reactions were stopped with 14 µl phenol red solution containing 1 mM β-mercaptoethanol and 10% sodium-dodecylsulfate (SDS). Protein was measured as described by Lowry *et al.* (15) and protein composition was analyzed by 5-15% SDS polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie blue (16)

RESULTS AND DISCUSSION

Calmodulin induced a dose-dependent stimulation of PLA_2 activity reaching maximum stimulation at 1.0×10^{-6} M (Fig. 1.).

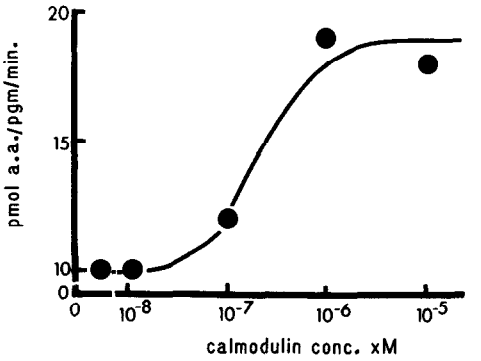

Fig. 1. *Naja naja* PLA_2 activity plotted as a function of CaM concentration. PLA_2 was incubated with $CaCl_2$ (1 µmole) and 1.85 nmol [^{14}C]-arachidonylphosphatidylcholine and incubated for 10 minutes at 25°C in 100 mM Tris-HCl buffer, pH 8.9.

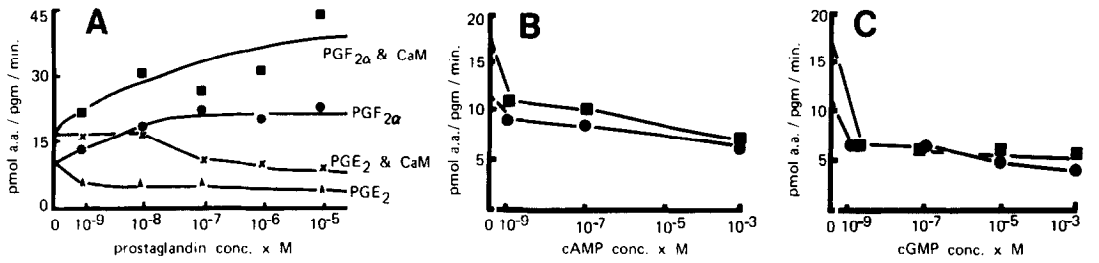

Fig. 2. A: PLA_2 was incubated with $CaCl_2$ (1 μmole) and 1.85 nmol [^{14}C]-arachidonylphosphatidylcholine and incubated for 10 minutes at 25°C in 100 mM Tris-HCl buffer, pH 8.0. PLA_2 activity is plotted as a function of PGE_2 (▲) or $PGF_{2\alpha}$ (●) in the presence of $CaCl_2$ or $CaCl_2$ and 1 μM CaM (X) and (■) respectively. The origin of the curves represents the baseline values of enzymatic activity in the presence or absence of CaM, *viz.*, 11.0 pmol of a.a./pgm/min in the absence of CaM and 17.0 pmol of a.a./pgm/min in the presence of CaM; B & C: PLA_2 activity plotted as a function of cAMP or cGMP concentrations in the absence (●) or presence (■) of CaM. The origin of the curves represents the baseline values of enzymatic activity in the presence or absence of CaM shown in Fig. 1.

In the absence or presence of CaM, $PGF_{2\alpha}$ stimulated PLA_2 in a dose-dependent manner achieving maximum activation of 63.6% and 106% increases in enzymatic activity respectively (Fig. 2a). Prostaglandin E_2 in the absence or presence of CaM induced 50% and 35% inhibitions of PLA_2 at 1 nM and 10 μM, respectively (Fig. 2a). Thus $PGF_{2\alpha}$ and PGE_2 had opposite effects on PLA_2 in the presence and absence of CaM. Cyclic-AMP and cGMP inhibited PLA_2 activity in the presence or absence of CaM (Figs. 2b and 2c). Upon DMS cross-linking in the presence of Ca^{2+}, polypeptides of M_r 14K and 21K were observed (Fig. 3a). They constituted 77% and 23% of the total protein, respectively. When DMS cross-linking was performed with Ca^{2+} in the presence of cAMP, cGMP or PGE_2, the number of polypeptides remained essentially unchanged (data not shown). Cross-linking using DMS in the presence of $PGF_{2\alpha}$ and Ca^{2+} showed new polypeptides with M_rs 24K, 28K, 31K and 33K corresponding to polymers of PLA_2 (Figs. 3b). In the presence of $PGF_{2\alpha}$ and 1 mM EGTA, cross-linked polymers were not present (Fig. 3b) indicating the Ca^{2+} dependence of $PGF_{2\alpha}$-induced polymeriza-

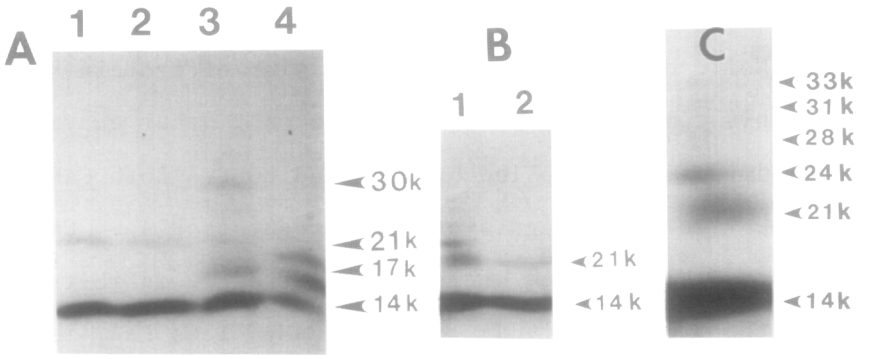

Fig. 3. SDS-PAGE 5-15% gel analysis and Coomassie blue staining. A: polypeptide composition of PLA_2 with DMS cross-linker in the presence of Ca^{2+} (Lane 1), 1 mM EGTA (Lane 2), 1 mM Ca^{2+} and 7 µg CaM (Lane 3), or 1 mM EGTA and 7 µg CaM (Lane 4); B: polyeptide composition of PLA_2 cross-linked with DMS in the presence of Ca^{2+} and 10 µg $PGF_{2\alpha}$ (Lane 1) or 1 mM EGTA and 10 µg $PGF_{2\alpha}$ (Lane 2). C: Larger magnification showing details of bands of gel B.

tion. Prostaglandin E_2, cAMP or cGMP did not alter the extent of PLA_2 polymerization (data not shown). Upon DMS cross-linking in the presence of Ca^{2+} and CaM, additional cross-linked polypeptides were observed (Fig. 3a). The 14K and 21K polypeptides corresponded to those observed with cross-linker and Ca^{2+} alone. The new PLA_2-CaM complex appeared as a 30K polypeptide (Fig. 3a). The presence of CaM-CaM polymers was not suspected since CaM does not cross link with itself (17).

Cross-linking of PLA_2 with DMS in the presence of $PGF_{2\alpha}$, PGE_2, cAMP or cGMP in the presence of Ca^{2+} and CaM did not reveal any significant changes in the level of PLA_2-CaM complex formation (data not shown). The inhibitory effects of cAMP, cGMP and PGE_2 in the presence of Ca^{2+} alone or Ca^{2+} and CaM may be due to the direct interaction of these modulators with monomers or polymers of PLA_2. The induced conformational changes may be such that substrate/enzyme interactions are impaired or, alternatively, these modulators may inhibit substrate binding to the enzyme and thereby inhibit substrate hydrolysis.

Classically, both cAMP and cGMP have been demonstrated to exert metabolic effects only via stimulation of protein kinases (18); in this communication, PLA_2 is the only other enzyme reported to be directly controlled by cAMP and cGMP. Both CaM and cAMP, two key cellular regulators, may regulate PLA_2. Calcium/CaM activates this enzyme, whereas cAMP directly inhibits the enzyme in the presence of Ca^{2+} or Ca^{2+}/CaM. Thus PLA_2 is a common focal point for both CaM and cyclic nucleotides having opposite effects. Prostaglandin E_2 and $PGF_{2\alpha}$, in addition to regulating adenylate and guanylate cyclases, respectively (11), also may exert antagonistic effects by the inhibition and stimulation of a common enzyme, PLA_2.

Calcium has been hypothesized to activate PLA_2 by binding to the enzyme thus inducing conformational changes and by facilitating nucleophile deprotonation (19, 20). Membrane PLA_2 is stimulated by CaM and hormones (21, 22) and inhibited by anesthetics, antibiotics, and lipomodulin (19, 20, 23-25). Our findings, that PLA_2 can be directly regulated by the modulators herein described, imply that PLA_2 is an important enzyme with a finely regulated mechanism.

ACKNOWLEDGMENTS

This work was supported in part by N.I.H. grants #NS12467 and #HL27928 to S.P. The authors thank Elaine Hua for experimental assistance and John Morgan for editing and preparing our manuscript.

REFERENCES

1. Van den Bosh, M. (1974) Ann. Rev. Biochem. 43, 243-277.
2. Mosoro, E.J. (1977) Ann. Rev. Physiol. 39, 301-329.
3. Flowers, R.J. and Blackwell, G.J. (1976) Biochem. Pharmacol. 25, 285-291.
4. Heilbrunn, E. in Psychopharmacology, Sexual Disorders and Drug Abuse, T.A. Ban, Ed., pp. 551-554, North Holland Publ. Co., Amsterdam, 1973.
5. Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Shiffmann, E. and Axelrod, J. (1979) Proc. Natl. Acad. Sci. USA 76, 2640-2643.
6. Moskowitz, N., Schook, W. and Puszkin, S. (1982) Science, 216, 305-307.
7. Moskowitz, N., Schook, W. and Puszkin, S. (1982) J. Cell Biol. 85, 442a.

8. Cheung, W.Y. (1980) Science 207, 19-27.
9. Samuelsson, B., Granstrom, E., Green, K., Hamberg, M. and Hammarstrom, S. (1975) Ann. Rev. Biochem. 44, 669-695.
10. Yang, C.C. and King, K. (1980) Toxicon 18, 543-547.
11. O'Dea, R.B. and Haddox, M.K. (1973) Adv. Cyclic Nucleotide Res. 3, 155-223.
12. Jamieson, Jr., G.A. and Vanaman, T.C. (1979) Biochem. Biophys. Comm. 90 1048-1051.
13. LaGarde, M., Menashi, S. and Crawford, N. (1981) FEBS Lett. 124, 23-26.
14. Peters, K. and Richards, F.M. (1977) Ann. Rev. Biochem. 46, 523-551.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193 265-275.
16. Laemmli, U.K. (1970) Nature 227, 680-685.
17. Lisanti, M.P., Shapiro, L.S., Moskowitz, N., Hua, E.L., Puszkin, S. and Schook, W. (1982) Eur. J. Biochem 125, 617-622.
18. Greengard, P. (1978) Science 199, 146-152.
19. Volwerk, J.J., Pieterson, W.A. and deHaas, G.H. (1974) Biochemistry 13, 1446-1454.
20. Wells, M.A. (1973) Biochemistry 12, 1086-1093.
21. Haye, B., Champion, S. and Jacquemin, C. (1973) FEBS Lett. 30, 253-255.
22. Wong, P.Y.K. and Cheung, W.Y. (1979) Biochem. Biophys. Res. Comm. 90, 473-480.
23. Scherphof, G.L., Scarpa, A. and Toorenenbergen, A.V. (1972) Biochim. Biophys. Acta 270, 226-240.
24. Sugatini, J., Saiton, K., and Honjo, I. (1979) Antibiot. (Tokyo) 32, 734-739.
25. Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D. and Axelrod, J. (1980) Proc. Natl. Acad. Sci. USA 77, 2533-2536.