Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 59-65

THE METABOLISM OF INOSITOL 4-MONOPHOSPHATE IN RAT MAMMALIAN TISSUES

A. Delvaux, J.E. Dumont, and C. Erneux

Institut de Recherche Interdisciplinaire (IRIBHN), Université Libre de Bruxelles, Campus Erasme, B - 1070 Brussels, Belgium

Received April 3, 1987

SUMMARY: Rat brain soluble fraction contains an enzymatic activity that dephosphorylates inositol 1,4-bisphosphate ($Ins(1,4)P_2$). We have used anion exchange h.p.l.c. in order to identify the inositol monophosphate product of $Ins(1,4)P_2$ hydrolysis (i.e. $Ins(1)P_1$, $Ins(4)P_1$ or both). When $[^3H]Ins(1,4)P_2$ was used as substrate, we obtained an inositol monophosphate isomer that was separated from the co-injected standard $[^3H]Ins(1)P_1$. This suggested an $Ins(1,4)P_2$1-phosphatase pathway leading to the production of the inositol 4-monophosphate isomer. The dephosphorylation of $[^{32}P]Ins(4)P_1$ was measured in rat brain, liver and heart soluble fraction and was Li^+-sensitive. Chromatography of the soluble fraction of a rat brain homogenate on DEAE-cellulose resolved a monophosphate phosphatase activity that hydrolyzed both $[^3H]Ins(1)P_1$ and $[4\text{-}^{32}P]Ins(4)P_1$ isomers.

The newly discovered second messenger $Ins(1,4,5)P_3$ is responsible for mobilizing intracellular calcium from a pool associated to the endoplasmic reticulum (reviewed in refs 1 and 2). $Ins(4)P_1$ was initially reported in rat liver as one of the products of $Ins(1,4,5)P_3$ dephosphorylation (3). $Ins(4)P_1$ was also detected by gas chromatography-mass spectrometry in rat cerebral cortex (4). In human platelets prelabelled with $[^3H]$ inositol and stimulated by vasopressin lithium augmented the accumulation of $Ins(1)P_1$, $Ins(1,4)P_2$ but also $Ins(4)P_1$ (5). A rapid increase of $Ins(4)P_1$ potentiated by Li^+, in the absence of a significant change of $Ins(1)P_1$ was recently reported in rat adrenal glomerulosa cells stimulated by angiotensin (6). Those reports show that the formation of $Ins(4)P_1$ can be regulated by receptor activation and is potentiated by Li^+, an inhibitor of $Ins(1)P_1$ and $Ins(1,4)P_2$ dephosphorylation (3, 7-9). We have

Abbreviations: $Ins(1)P_1$, inositol 1-phosphate; $Ins(4)P_1$, inositol 4-phosphate; $Ins(1,4)P_2$ inositol 1,4-bisphosphate; $Ins(1,4,5)P_3$ inositol 1,4,5-trisphosphate.

0006-291X/87 $1.50

previously reported the presence in rat brain of two phosphatase activities that dephosphorylate Ins(1)P_1 and Ins(1,4)P_2 respectively. Those activities could be separated by DEAE-cellulose chromatography and were inhibited by Li^+ at millimolar concentration. Ins(1,4,5)P_3 phosphatase was not Li^+-sensitive (9,10). Here we show that in rat brain the product of Ins(1,4)P_2 dephosphorylation is essentially Ins(4)P_1. The latter inositol monophosphate is further dephosphorylated by a Li^+-sensitive phosphatase in rat brain, liver and heart soluble fraction. As Ins(4)P_1 can not be formed by phospholipase C cleavage of phosphatidylinositol, its identification in intact cell is suggestive of a specific Ins(1,4)P_2 1-phosphatase pathway.

MATERIALS AND METHODS

Materials

The sources of chemicals, materials and the preparation of [4-^{32}P] Ins(1,4)P_2 and [4,5-^{32}P] Ins(1,4,5)P_3 from ^{32}P-labelled erythrocyte membranes were as previously published (9-12). [2-^{3}H]Ins(1)P_1 (sp. radioactivity 8 Ci/mmol), [2-^{3}H]Ins(1,4)P_2 (sp. radioactivity 2Ci/mmol) and [2-^{3}H]Ins(1,4,5)P_3 (sp. radioactivity 3.6 Ci/mmol) were obtained from New England Nuclear Corp. [^{32}P]Pi (sp. radioactivity 10 mCi/ml, carrier free) was from Amersham. DEAE-cellulose (DE-52) was from Whatman and the h.p.l.c. column (μ Bondapak NH_2; 30 cm x 0.89 cm) was purchased from Waters. Sephadex G-10 was supplied by Pharmacia. Ammonium molybdate, perchloric acid and isobutanol/toluol (v/v) were from Merck.

Incubation and assay of inositol polyphosphate phosphatases.

Soluble rat brain, liver and heart fractions and DEAE-cellulose purified Ins(1,4)P_2 phosphatase activity from rat brain were prepared as previously described (9). Incubation and assay of inositol polyphosphate phosphatases by the use of Dowex (formate) anion exchange chromatography except for [^{32}P]Ins(4)P_1 dephosphorylation (see below) were as previously (Erneux et al., 1986). We have identified the product(s) of [^{3}H] or [^{32}P]Ins(1,4)P_2 dephosphorylation by h.p.l.c. on a μ bondapak NH_2 column eluted as described in refs 5 and 13 (see also legend of Fig. 1).

Measurement of [^{32}P]Pi released during enzymatic dephosphorylation of [^{32}P]Ins(4)P_1.

The method is based on previous published procedures (11,14). Samples of [^{32}P]Ins(4)P_1 were incubated in 0.1 ml with the enzyme preparation and in standard incubation medium used for inositol polyphosphate phosphatase assay i.e. 50 mM Na-Hepes pH 7.4, 2 mM $MgCl_2$, 5 mM C_2H_6OS, 1 mg bovine serum albumin/ml. The reaction was terminated by the addition of 0.1 ml perchloric acid to a final concentration of 1 M. After centrifugation (3000 x g, 30 min) to remove the proteins, the supernatant (0.170 ml) was taken and ammonium molybdate was added to a final concentration of 1% (weight/volume). Two volumes (0.380 ml) of a isobutanol/toluol (1:1 v/v) solution were then mixed to the sample. After two hours, the upper phase containing the [^{32}P] phosphomolybdate complex was taken and measured for radioactivity by cerenkow counting. About 97% of Pi is extracted by this

procedure. [^{32}P]Ins(4)P_1, [^{32}P]Ins(1,4)P_2 and [^{32}P]Ins(1,4,5)P_3 were not extracted by the method and thus remained in the lower aqueous phase. The blank value is determined by adding the perchloric acid to the enzyme preparation before the [^{32}P]-labelled substrate.

Dephosphorylation of [^{32}P]Ins(1,4)P_2 by DEAE-cellulose purified Ins(1,4)P_2 phosphatase activity.

[4-^{32}P]Ins(4)P_1 was prepared by dephosphorylation of [4-^{32}P]Ins(1,4)P_2. About 40000 cpm of [^{32}P]Ins(1,4)P_2 was incubated for 10 min at 37°C in the presence of 0.02 mg/ml DEAE-cellulose purified Ins(1,4)P_2 phosphatase. The incubation was made in 0.1 ml in the incubation medium used for assay of inositol phosphate phosphatases (10). The reaction was terminated by adding 0.1 ml of water. After filtration on a Centricon TM microconcentrator to remove proteins, the sample was loaded onto a μ Bondapak NH_2 anion exchange h.p.l.c. column eluted as described earlier (5,13). The product of [4-^{32}P]Ins(1,4)P_2 dephosphorylation was desalted, lyophylised and resuspended in 0.6 ml of water.

RESULTS AND DISCUSSION

Both [4-^{32}P]Ins(1,4)P_2 prepared from [^{32}P]Pi-labelled erythrocytes or [^{3}H]Ins(1,4)P_2 were rapidly dephosphorylated by rat brain soluble fraction. A single peak of phosphatase activity, shown to be lithium-sensitive was further fractionated by DEAE-cellulose chromatography (9). h.p.l.c. analysis of the product(s) of [^{3}H]Ins(1,4)P_2 hydrolysis was used to identify the inositol monophosphate isomer produced (i.e. Ins(1)P_1 or Ins(4)P_1). A single peak of an inositol monophosphate was separated from Ins(1,4)P_2 (Fig. 1a, Ins(1,4)P_2 is eluted after 40-45 min). If the product of [^{3}H]Ins(1,4)P_2 was co-injected with [^{3}H]Ins(1)P_1 (used as standard), two peaks (B and C) were obtained (Fig. 1b). Such data suggested that in our experiments, [^{3}H]Ins (1,4)P_2 was dephosphorylated by an Ins(1,4)P_2 1-phosphatase and that Ins(4)P_1 was specifically produced. The latter enzyme was Li^+-sensitive (9). Those data obtained in rat brain, contrasted with a model of Ins(1,4)P_2 dephosphorylation proposed by Storey et al. (3) and Michell (2) in rat liver. They suggested that degradation of Ins(1,4)P_2 via Ins(4)P_1 was Li^+-insensitive whereas degradation via Ins(1)P_1 was Li^+-sensitive. The reasons of discrepancy which may be related to tissue/cell specificity are not yet understood.

In order to check our conclusion, we have used a different approach and attempted to directly produce Ins(4)P_1 by enzymatic conversion. We thus prepared [^{32}P]Ins(4)P_1 by dephosphorylation of [^{32}P]Ins(1,4)P_2 using

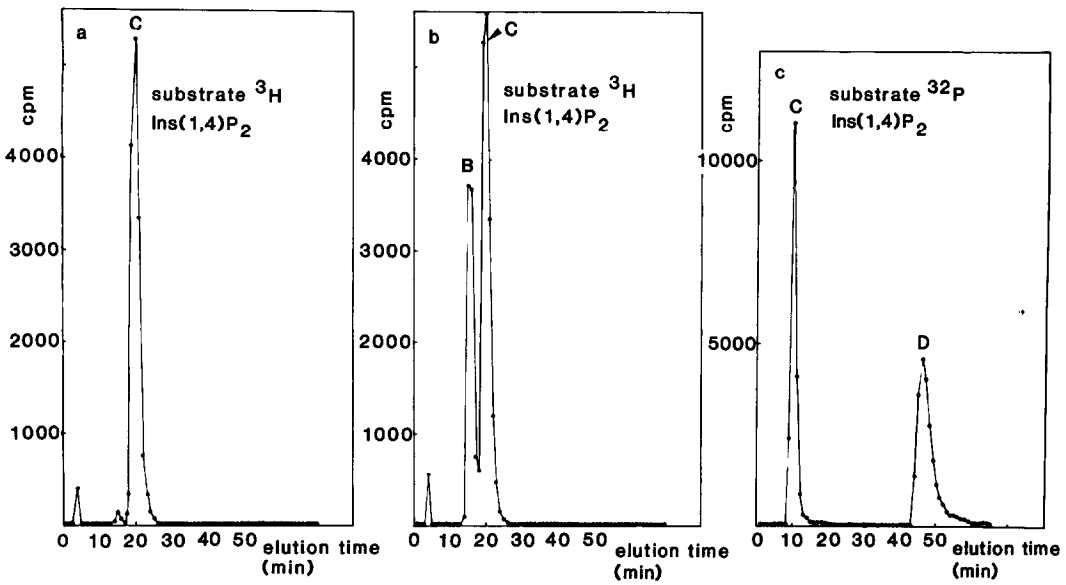

Figure 1. h.p.l.c. analysis of the products of Ins(1,4)P_2 dephosphorylation. [^{3}H]Ins(1,4)P_2 (20000 cpm) was incubated for 5 min in the presence of DEAE-cellulose purified Ins(1,4)P_2 phosphatase activity under condition of total [^{3}H]Ins(1,4)P_2 dephosphorylation. After incubation, filtration on a Centricon TM microconcentrator, a sample (50 µl) was loaded onto a µ Bondapak NH_2 anion exchange column in the absence (1a), and presence (1b) of [^{3}H]Ins(1)P_1 as standard. The dephosphorylation of [^{32}P]Ins(1,4)P_2 by the same enzyme preparation is shown in Fig. 1c (see Methods). Separation was carried out using a 10 min isocratic elution with a 20 mM ammonium acetate/acetic acid pH 4.0 followed by a 60 min linear gradient to 1M ammonium acetate/acetic acid pH 4.0. Fractions were collected every min (flow rate 1 ml/min). Note that the dead time was 4 min and that an older column was used for the separation shown in Fig. 1c.

DEAE-cellulose purified Ins(1,4)P_2 phosphatase activity (Methods). A single peak of radioactivity (C) was purified by h.p.l.c. (Fig. 1c). As only the 4-phosphate of [^{32}P]Ins(1,4)P_2 is ^{32}P-labelled (11,14) the peak was tentatively identified as Ins(4)P_1. Provided that the [^{32}P]-labelled product can be further hydrolysed, it could only correspond to [^{32}P]Ins(4)P_1. We have used the technique of solvent partition of the molybdate complex of P_i to assay for Ins(4)P_1 phosphatase (11, 14 and Methods). A blank value of [^{32}P]Ins(4)P_1 versus [^{32}P]P_i and inositol polyphosphates is shown in Table 1.

[^{32}P]Ins(4)P_1 produced by enzymatic source was used as a substrate for further characterization. It was dephosphorylated by rat brain, heart or liver soluble fraction but was not by human red cell membranes (a source of Ins(1,4,5)P_3 5-phosphatase, 1 and 11). At 10 mM, LiCl but not

Table 1.

Distribution of P_i and inositol polyphosphates by solvent partition of the molybdate complex of P_i

[^{32}P]labelled molecules	Upper organic phase	Lower aqueous phase
[^{32}P]Ins(4)P_1	94 ± 0.03	471 ± 17
[^{32}P]P_i	1292 ± 40	91 ± 18
[^{32}P]Ins(1,4)P_2	25 ± 0.5	451 ± 17
[^{32}P]Ins(1,4,5)P_3	21 ± 0.75	1438 ± 8

A sample of [^{32}P]Ins(4)P_1 (about 500 cpm) produced by enzymatic conversion of [^{32}P]Ins(1,4)P_2 was analyzed by solvent partition of the molybdate complex of P_i as described in Materials and Methods. [^{32}P]P_i, [^{32}P]Ins-(1,4)P_2 and [^{32}P]Ins(1,4,5)P_3 are shown for comparison. cpm ± SEM

NH_4Cl, KCl or NaCl was inhibitor of Ins(4)P_1 dephosphorylation in all rat tissues we tested. DEAE-cellulose chromatography of rat brain soluble fraction resolved a single peak of activity that was lithium-sensitive and was co-eluted with Ins(1)P_1 phosphatase (Fig. 2).

Those data are compatible with the very recent report of Ackermann et al. (15) suggesting that purified inositol 1-phosphatase from bovine brain also hydrolyses other inositol monophosphates, in particular Ins(4)P_1. However, these authors used a mixture of the two D and L-enantiomers of Ins(4)P_1 as substrate and mentioned the difference in kinetics with separated D and L-Ins(1)P_1.

In conclusion, although it is still possible that a Ins(1,4)P_2 ⟶ Ins(1)P_1 phosphatase pathway exists in rat brain as suggested in rat liver (2,3), we have not been able to identify Ins(1)P_1 production. On the other hand, our data clearly show the Ins(1,4)P_2 ⟶ Ins(4)P_1 pathway as a result of a soluble Li^+-sensitive Ins(1,4)P_2 1-phosphatase activity. The identification of Ins(4)P_1 in stimulated cells can obviously be considered as an index or "marker molecule" (5) of a specific phosphatase pathway. In contrast to Ins(1)P_1, Ins(4)P_1 cannot be produced by phospholipase C-provoked hydrolysis of either phosphatidylinositol or the phospho-

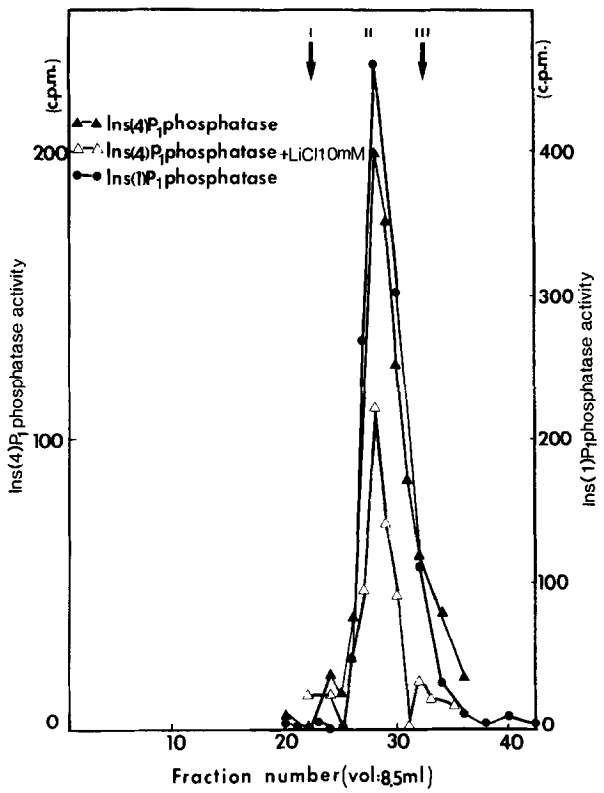

Figure 2. Separation of inositol phosphate activities by DEAE-cellulose chromatography of crude supernatant of rat brain homogenate. DEAE-cellulose was performed by eluting with a linear gradient of 0-0.25 M NaCl (total vol. 400 ml) made in 20 mM Tris/HCl (pH 7.5), 0.1 mM EDTA, 0.4 mM phenylmethanesulfonyl fluoride, 5 mM 2-mercaptoethanol, 10 % (v/v) glycerol (more details are given in ref. 9). Fractions (8.5 ml) were assayed for $Ins(1)P_1$, $Ins(4)P_1$ phosphatase activities (as shown), for $Ins(1,4,5)P_3$ phosphatase (maximum of activity, fraction 22) and for $Ins(1,4)P_2$ phosphatase (maximum of activity, fraction 32). The profile illustrates one representative experiment out of three.

inositides. Consequently, its accumulation in stimulated human platelets (5), rat brain (4) or angiotensin-stimulated rat adrenal glomerulosa cells (6) would suggest an increase in $Ins(1,4)P_2$ followed by dephosphorylation by an $Ins(1,4)P_2$ 1-phosphatase to $Ins(4)P_1$. The formation of $Ins(1)P_1$ is often measured by Dowex columns with no distinction between inositol monophosphate isomers (i.e. $Ins(1)P_1$ or $Ins(4)P_1$). It would be interesting to systematically compare the production of $Ins(4)P_1$ versus $Ins(1)P_1$ in stimulated cells, as $Ins(4)P_1$ can only result from polyphosphoinositide hydrolysis whereas $Ins(1)P_1$ would derive also [and in some cases such as the brain mainly, (15)] from monophosphatidylinositol hydrolysis. Finally,

the sensitivity of Ins(4)P_1 phosphatase activity to Li^+ easily explains the observation that Li^+ potentiates the agonist-induced Ins(4)P_1 accumulation in human platelets (5) and other cells (4,6). At least in rat brain, the dephosphorylation of both Ins(1)P_1 and Ins(4)P_1 is thus Li^+-sensitive.

ACKNOWLEDGMENTS

This work was supported by a grant from Duphar (Holland) and under contract of the Ministère de la Politique Scientifique (Belgium). We are grateful to Dr J.M. Boeynaems for criticism, Mrs C. Moreau for excellent technical assistance and Mrs D. Leemans for typing the manuscript.
C. Erneux is Chargé de Recherches au Fonds National de la Recherche Scientifique (Belgium).

REFERENCES

1. Irvine, R.F. (1986a) Br. Med. Bull. 42, 369-374.
2. Michell, B. (1986) Nature 319, 176-177.
3. Storey, D.J., Shears, S.B., Kirk, C.J., and Michell, R.H. (1984) Nature 312, 374-376.
4. Sherman, R.W., Munsell, L.Y., Gish, B.G., and Honchar, M.P. (1985) J. Neurochem. 44, 798-807.
5. Siess, W. (1985) FEBS Lett. 185, 151-156.
6. Balla, T., Baukal, A.J., Guillemette, G., Morgan, R.O., and Catt, K.J. (1986) Proc. Natl. Acad. Sci. USA 83, 9323-9327.
7. Hallcher, L.M., and Sherman, W.L. (1980) J. Biol. Chem. 255, 10891-10901.
8. Berridge, M.J., Downes, C.P., and Hanley, M.R. (1982) Biochem. J. 206, 587-595.
9. Delvaux, A., Erneux, C., Moreau, C., and Dumont, J.E. (1987) Biochem. J. 242, 193-198.
10. Erneux, C., Delvaux, A., Moreau, C., and Dumont, J.E. (1986) Biochem. Biophys. Res. Commun. 134, 351-358.
11. Downes, C.P., Mussat, M.C., and Michell, R.H. (1982) Biochem. J. 203, 169-177.
12. Irvine, R.F., Letcher, A.J., and Dawson, R.M.C. (1984) Biochem. J. 218, 177-185.
13. Binder, H., Weber, P.C., and Siess, W. (1985) Analyt. Biochem. 148, 220-227.
14. Hawkins, P.T., Michell, R.M., and Kirk, C.J. (1984) Biochem. J. 218, 785-793.
15. Ackermann, K.E., Gish, B.G., Honchar, M.P., and Sherman, W.R. (1987) Biochem. J. 242, 517-524.