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Phosphatidylinositol Kinase and Diphosphoinositide Kinase of Rat Kidney Cortex

PROPERTIES AND SUBCELLULAR LOCALIZATION

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The properties of phosphatidylinositol kinase and diphosphoinositide kinase from rat kidney cortex were studied. The enzymes were completely Mg^{2+}-dependent. Cutscum detergent activated phosphatidylinositol kinase, but diphosphoinositide kinase was inhibited by all detergents tested. The pH optima were 7.7 for phosphatidylinositol kinase and 6.5 for diphosphoinositide kinase. On subcellular fractionation of kidney-cortex homogenates by differential centrifugation, the distribution of phosphatidylinositol kinase resembled that of the marker enzymes for brush-border, endoplasmic-reticulum and Golgi membranes. Diphosphoinositide kinase distribution resembled that of thiamin pyrophosphatase (assayed in the absence of ATP), diphosphoinositide phosphatase and triphosphoinositide phosphatase. Activities of both kinases were low in purified brush-border fragments. Diphosphoinositide kinase is probably localized in the Golgi complex.

Successive phosphorylation of phosphatidylinositol to diphosphoinositide (phosphatidyl-*myo*-inositol 4-phosphate)‡ (eqn. 1) and then to triphosphoinositide (phosphatidyl-*myo*-inositol 4,5-bisphosphate) (eqn. 2) was first indicated by incorporation studies *in vivo* (Brockerhoff & Ballou, 1961; Ellis & Hawthorne, 1961):

$$\text{Phosphatidylinositol} + \text{ATP} \rightarrow \text{diphosphoinositide} + \text{ADP} \quad (1)$$

$$\text{Diphosphoinositide} + \text{ATP} \rightarrow \text{triphosphoinositide} + \text{ADP} \quad (2)$$

Phosphatidylinositol kinase, the enzyme that catalyses reaction (1), was first demonstrated in erythrocyte membranes by Hokin & Hokin (1964) and has since been described in many tissues [reviewed by Hawthorne & White (1975)]. It often appears to be localized in the plasma membrane (Harwood & Hawthorne, 1969*a*,*b*). Diphosphoinositide kinase (eqn. 2) has been found in rat brain (Kai *et al.*, 1968) and kidney (Tou & Huggins, 1970), although its existence had been indicated by labelling experiments in a number of other tissues (Santiago-Calvo *et al.*, 1964). Like phosphatidylinositol kinase, it requires ATP and Mg^{2+}, and appears to be a supernatant enzyme in brain (Kai & Hawthorne, 1966). It was reported to be located in the plasma membrane of kidney (Tou *et al.*, 1970).

The present paper reports the characterization and subcellular localization of the two kinases in rat kidney cortex and evidence is presented that indicates the presence of the enzymes in the Golgi complex.

Materials and Methods

Materials

All substrates, cofactors and inhibitors were from Sigma (London) Chemical Co., London S.W.6, U.K., except for UDP-[U-^{14}C]galactose, which was from Atomic Energy of Canada, Ottawa, Ont., Canada. Cutscum (iso-octylphenoxypolyethoxyethanol) was from Kodak (Kirkby, Liverpool, U.K.), and Triton X-100 was from Lennig Chemicals (Jarrow, Co. Durham, U.K.). Silica gel H was from Camlab, Edmonton, Alberta, Canada.

Methods

Preparation of homogenates and subcellular fractions. The procedures used are described in Cooper & Hawthorne (1975).

Preparation of substrates. Phosphatidylinositol was prepared from ox brain and purified by using

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‡ The names diphosphoinositide and triphosphoinositide are retained in the text since they are widely known and there is as yet no internationally agreed systematic nomenclature.

alumina (Long & Owens, 1962) followed by silicic acid chromatography (Hanahan, 1960). Phosphatidylinositol was also prepared from maize as described by Carter & Weber (1966). It was then purified as above. Diphosphoinositide was prepared as described previously (Cooper & Hawthorne, 1975). Purity of the samples was shown by thin-layer and paper chromatography. Phosphatidylinositol was stored in chloroform at −20°C, and diphosphoinositide was stored as an emulsion in water at 4°C. Both lipids were in the form of sodium salts. Terminally labelled [^{32}P]ATP was prepared enzymically by the method of Glynn & Chappell (1964). The 0.25M-HCl eluate from the resin column was made to pH7.4 with solid Tris and stored at −20°C. Before use, non-radioactive ATP was added to give a final concentration of 20mM. The specific radioactivity was then approx. 7.5×10^4–2.5×10^5 c.p.s./μmol, and this solution was used directly in making up assay mixtures.

Chemical methods and enzyme assays

Except for phosphatidylinositol kinase and diphosphoinositide kinase, these are described in Cooper & Hawthorne (1975).

Phosphatidylinositol kinase (*EC* 2.7.1.67). The basic assay of this enzyme is described by Kai *et al.* (1966); extraction method B was used. The assay medium had the following composition: 5mM-phosphatidylinositol emulsified in water; 40mM-Tris/HCl buffer, pH7.4; 1% (v/v) Cutscum; 4mM-[^{32}P]ATP; 30mM-$MgCl_2$; and approx. 1mg of protein of enzyme sample in a final volume of 1.0ml. The reaction was started by the addition of enzyme after a 2min preincubation at 37°C, and the reaction allowed to proceed for 5min. After extraction of the lipids by the method of Garbus *et al.* (1963) the lower chloroform phase was taken and Folch diphosphoinositide fraction (60μg of P) added as carrier. The extracts were concentrated to a small volume under N_2 and the lipids separated either by paper chromatography (Kai *et al.*, 1966) or by the thin-layer separation of Gonzalez-Sastre & Folch-Pi (1968). Potassium oxalate-impregnated silica gel H of 0.5mm thickness was used. To prevent the streaking caused by the presence of detergent, the plates were developed in chloroform first followed by chloroform/methanol/4M-NH_3 (9:7:2, by vol.). The lipid areas were made visible in an iodine tank and the spots corresponding to diphosphoinositide scraped into 15ml scintillation vials, and 10ml of toluene phosphor [1 litre of toluene containing 5g of 2,4-diphenyloxazole and 500mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene] was added. The samples were then gelled by the method of Bollinger *et al.* (1967), and radioactivity was measured with a Nuclear-Chicago Mark II automatic scintillation counter. As a standard, a known amount of [^{32}P]ATP was counted for radioactivity at the same time.

Diphosphoinositide kinase (*EC* 2.7.1.68). Diphosphoinositide kinase has been assayed by a method almost identical with that of phosphatidylinositol kinase, by using paper chromatography (Kai *et al.*, 1968). The method was here adapted to t.l.c. The assay mixture had a total volume of 1ml and was of the following composition: 50mM-imidazole/HCl buffer, pH6.5; 4mM-[^{32}P]ATP; 40mM-$MgCl_2$; 0.5mM-diphosphoinositide emulsified in water; and approx. 1mg of enzyme protein. The reaction was started by addition of diphosphoinositide after preincubation for 2min at 37°C and the incubation was for 5min at 37°C. The reaction was stopped by the addition of 4.5ml of chloroform/methanol (1:2, v/v) containing Folch diphosphoinositide fraction (60μg of P) and the lipids were extracted by the method of Hajra *et al.* (1968). The final chloroform extracts were concentrated to dryness and the residues dissolved in 250μl of chloroform. The samples were kept in ice, and 200μl was applied to the oxalate-impregnated thin-layer plates as described for the phosphatidylinositol kinase assay. The plates were developed and the radioactivity of the triphosphoinositide was determined as described above for diphosphoinositide.

Results and Discussion

Properties of phosphatidylinositol kinase

ATP requirement (*Fig.* 1*a*). Maximum activity occurred at 2mM-ATP, but at higher concentrations diphosphoinositide labelling decreased markedly until at 12mM the activity was almost zero. There are two possible explanations for this. (i) Anomalous kinetics of ATPases.* To test this, ATP hydrolysis was measured under the conditions of assay of the kinase. The rate was proportional to ATP concentration over the range 1–10mM. Further, Cutscum markedly inhibits ATPase, and significant amounts of ATP were shown to be available to the kinase throughout the incubation period. (ii) Substrate inhibition by the ATP.

Phosphatidylinositol requirement. Maximum activity was reached at a phosphatidylinositol concentration of 5mM, with inhibition at higher concentrations (Fig. 1*b*).

Mg^{2+} requirement (*Fig.* 1*c*). The reaction was completely dependent on the presence of Mg^{2+} and a maximum of activity was observed at 30mM-$MgCl_2$.

Effect of Cutscum (*Fig.* 1*d*). The non-ionic detergent Cutscum gave greatest activation of phosphatidylinositol kinase at a concentration of 2% (v/v) with inhibition at higher concentrations.

Reaction rate (*Fig.* 2). The rate was constant for the first 5min. Diphosphoinositide synthesis reached a maximum at 15min, after which labelling of diphosphoinositide decreased rapidly. If the reaction rate

* Abbreviation: ATPase, adenosine triphosphatase.

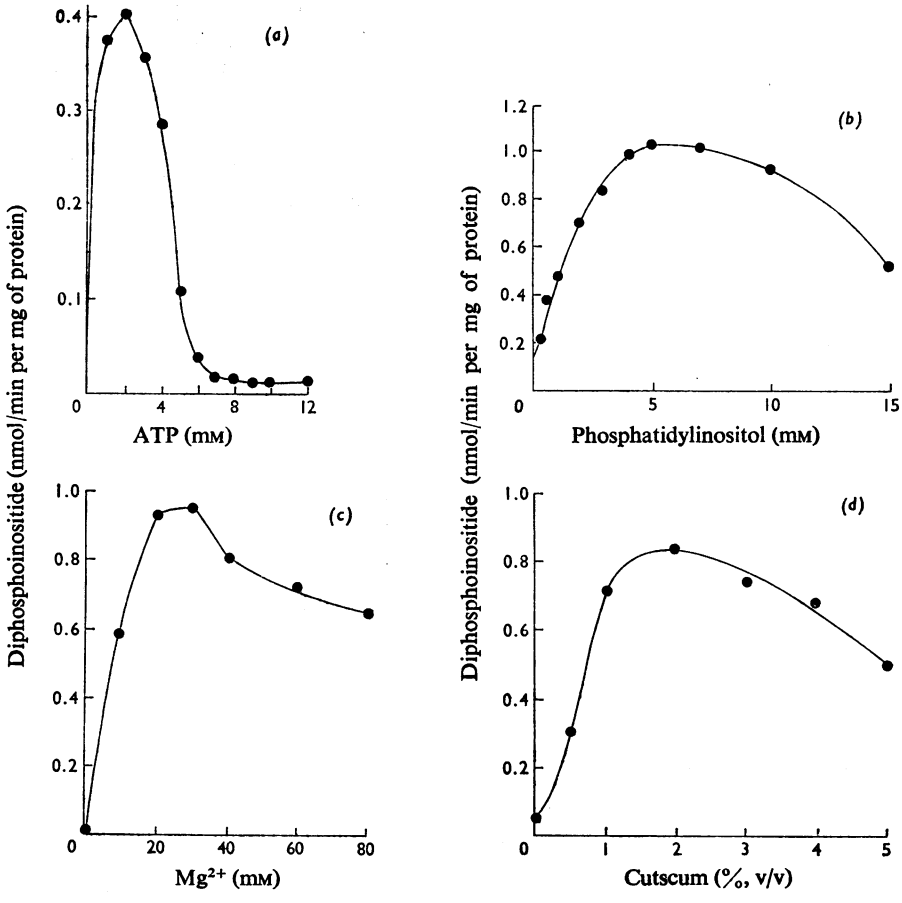


Fig. 1. *Conditions for phosphatidylinositol kinase*

(*a*) Shows the ATP requirement. Incubation was for 5min at 37°C in a total volume of 1ml using kidney microsomal fraction (1.8mg of protein). The mixture contained 1mM-phosphatidylinositol, 1% (v/v) Cutscum, 40mM-$MgCl_2$ and 50mM-Tris/HCl buffer, pH 7.4. (*b*) Shows the phosphatidylinositol requirement. The conditions were the same except that 3mM-ATP was present. (*c*) Shows the Mg^{2+} requirement. The conditions followed those for (*b*), except that 1mM-phosphatidylinositol was present, with 1.1mg of microsomal protein. (*d*) Shows the effect of Cutscum. The conditions were exactly as in (*c*) with the addition of the detergent and 40mM-$MgCl_2$.

was decreasing simply because of lack of ATP, a plateau in the time-curve would be reached, but there would be no subsequent loss of diphosphoinositide. ATP breakdown was studied under the conditions of the kinase assay. After 15min under these conditions, only 33% of the ATP was hydrolysed. Thus diphosphoinositide synthesis could still continue under almost optimal conditions. The probable explanation for the loss of diphosphoinositide is that when we measure phosphatidylinositol kinase we are only measuring net synthesis of diphosphoinositide in the presence of other inositide-metabolizing enzymes in the microsomal fraction. Diphosphoinositide phosphatase is present in this fraction (Cooper & Hawthorne, 1975) and under optimal conditions is about 200 times as active as phosphatidylinositol kinase. The peak in diphosphoinositide formation and the subsequent plateau at a low rate would both be expected if this lipid is acting as substrate for a second enzyme.

When the plateau is reached, diphosphoinositide concentration is less than 1 μM. Work with purified diphosphoinositide phosphatase from brain (M. S. Nijjar & J. N. Hawthorne, unpublished work) showed a K_m of 25 μM. If the kidney enzyme is similar its rate will be very much below $V_{max.}$ under these equilibrium conditions, simply on the basis of substrate concentration. In addition, the phosphatase is

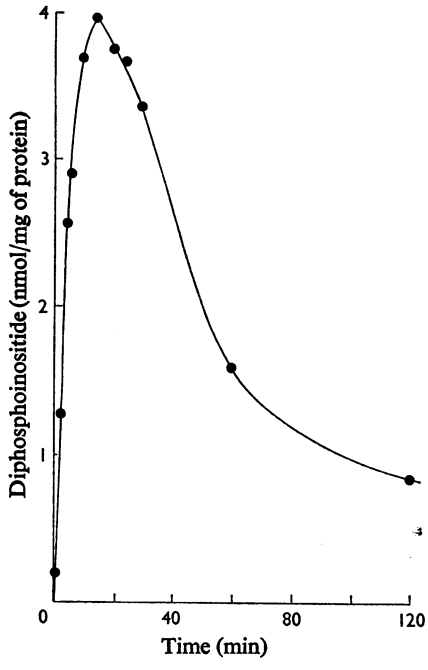


Fig. 2. *Time-course of the phosphatidylinositol kinase reaction*

The reaction mixture (total volume 30ml) contained 5mM-phosphatidylinositol, 1% (v/v) Cutscum, 3mM-ATP, 40mM-$MgCl_2$ and 50mM-Tris/HCl buffer, pH7.4. After preincubation for 2min at 37°C the reaction was started by adding microsomal fraction (30mg of protein). Duplicate 1ml samples were removed for analysis at various times.

inhibited by Cutscum (Cooper & Hawthorne, 1975).

Diphosphoinositide kinase is also present in the microsomal fraction (see Table 2) and could use the synthesized diphosphoinositide as substrate. However, since this kinase is inhibited by Cutscum, its presence is unlikely to affect net synthesis of diphosphoinositide.

Effect of enzyme concentration (*Fig.* 3). The reaction rate is constant over the range 0–2mg of protein and decreases with higher enzyme concentrations. A possible explanation is that ATPases in the enzyme preparation have a higher affinity for ATP than the kinase. At higher concentrations of microsomal fraction such ATPases would lower the concentration of ATP available to the kinase. A similar explanation could apply to the fall in rate of diphosphoinositide kinase at high concentrations of homogenate.

Effect of pH (*Fig.* 4). Since Harwood & Hawthorne (1969*a*) had observed two pH optima for phosphatidylinositol kinase in the liver microsomal fraction, experiments were performed to establish pH curves for kidney. Representative results are shown in Fig. 4. Considerable variation was observed between experiments, although maximum activity was always obtained at pH7.6–7.8. A second pH optimum at about pH8.0 was observed in some experiments. Generally, however, only the single peak was observed, indicating the presence of one enzyme only, although activity remained high up to pH9.0.

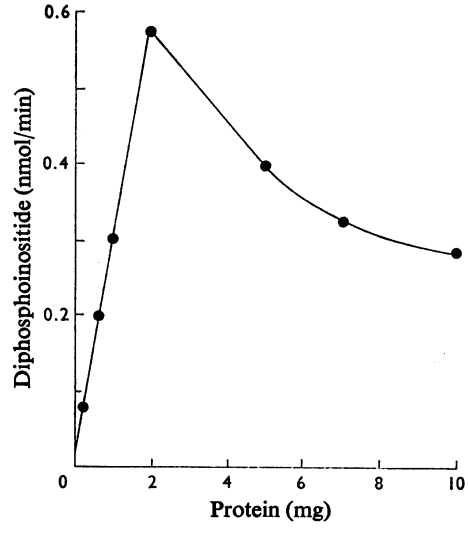


Fig. 3. *Effect of enzyme concentration on diphosphoinositide synthesis*

Conditions were those of Fig. 1(*b*), with 5mM-phosphatidylinositol and various amounts of microsomal fraction.

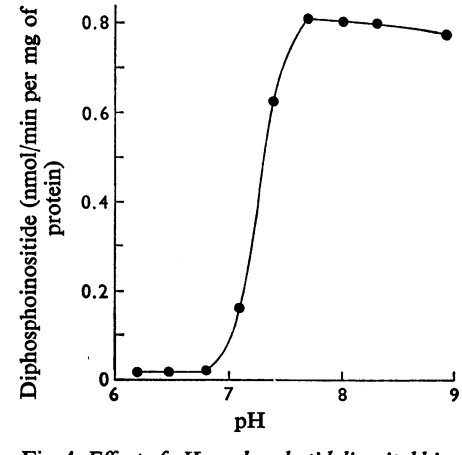
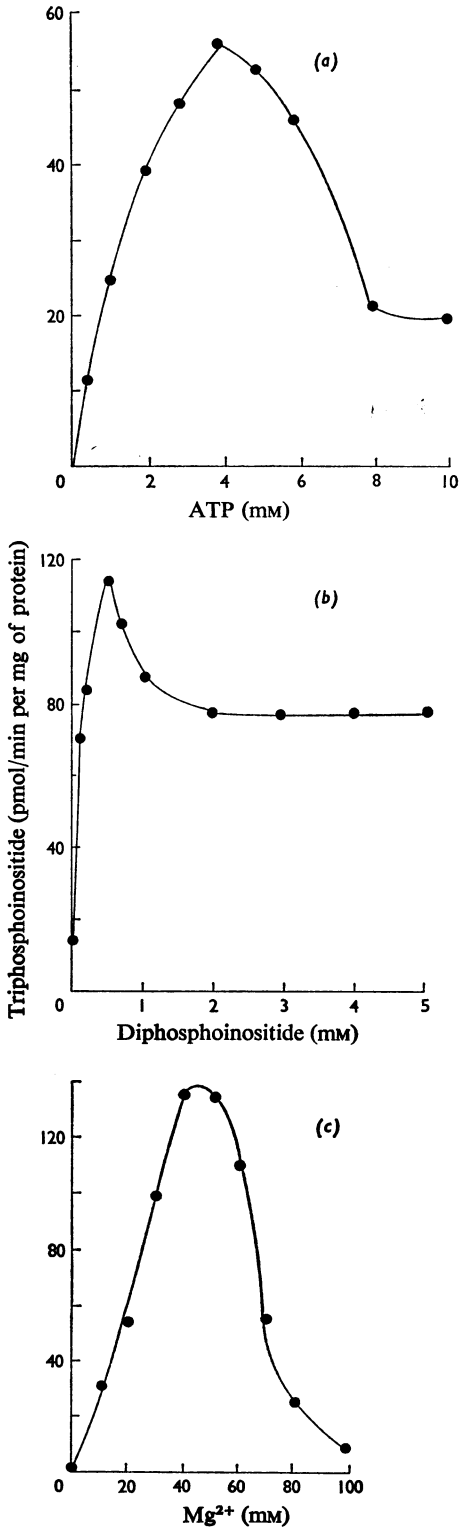


Fig. 4. *Effect of pH on phosphatidylinositol kinase*

Conditions are those of Fig. 3 and Tris/HCl buffers were used.



Presence in kidney cortex and medulla. Phosphatidylinositol kinase activity in cortex was more than twice that in medulla. Measured in the presence of Cutscum, activities (pmol of diphosphoinositide/min per mg of protein) were 81 and 33 respectively.

Properties of diphosphoinositide kinase

ATP requirement (Fig. 5a). Optimal activity was reached at a concentration of 4mM, above which marked inhibition occurred. In this respect diphosphoinositide kinase was similar to phosphatidylinositol kinase.

Effect of EDTA. EDTA stimulates brain diphosphoinositide kinase (Kai *et al.*, 1968), but the kidney enzyme was slightly inhibited at all concentrations between 0.5mM and 5mM.

Diphosphoinositide requirement (Fig. 5b). A rather unusual curve was obtained in which the activity rose to a sharp peak at 0.5mM and then fell to a constant value at concentrations greater than 2mM. The initial fall in activity may be due to substrate inhibition. Diphosphoinositide will be in the Mg^{2+} salt form as a precipitate at higher concentrations and so not available to the enzyme, either as substrate or inhibitor. Diphosphoinositide will also be removed by phosphatase action.

Table 1. *Metal ion activation of diphosphoinositide kinase*

Conditions followed those of Fig. 5(*c*). Specific activities are presented as pmol/min per mg of protein.

Metal ion (2mM)	Specific activity
—	1.3
Mg^{2+}	12.8
Fe^{2+}	3.7
Ba^{2+}	1.5
Co^{2+}	11.2
Hg^{2+}	0.8
Ca^{2+}	1.3
Cd^{2+}	1.1
Cu^{2+}	0.9
Mn^{2+}	8.2
Zn^{2+}	3.4

Fig. 5. *Conditions for diphosphoinositide kinase*

(*a*) Shows the ATP requirement. Incubation was for 5min at 37°C in a volume of 1ml with kidney homogenate (1.5mg of protein). The medium contained 0.9mM-diphosphoinositide, 30mM-$MgCl_2$, 4mM-EDTA (sodium salt) and 40mM-Tris/HCl buffer, pH7.4. (*b*) Shows the diphosphoinositide requirement. The conditions were similar except that EDTA was omitted and 4mM-ATP was present. (*c*) Shows the Mg^{2+} requirement. The same conditions were used except that diphosphoinositide concentration was 0.5mM.

Metal ion activation. Diphosphoinositide kinase was only activated appreciably by Mg^{2+}, Mn^{2+} and Co^{2+} (Table 1). The presence of Mg^{2+} appeared to be essential for activity and in excess exerted a considerable inhibitory effect on the enzyme (Fig. 5*c*).

Effect of detergents. Three different classes of detergent were used: (i) non-ionic (Cutscum and Triton X-100); (ii) anionic (sodium deoxycholate); (iii) cationic [Cetavlon (cetyltrimethylammonium bromide)]. The kidney enzyme was inhibited by all types of detergent, as has been found for brain (Kai *et al.*, 1968). Further, it would appear that unlike polyphosphoinositide phosphatase (Cooper & Hawthorne, 1975) activity was not enhanced when the reaction was started with a diphosphoinositide/Cetavlon mixture.

Effect of enzyme concentration. The reaction rate was proportional to enzyme concentration over the range 0–1.25 mg of protein. At higher enzyme concentrations the rate declined.

Reaction rate. The reaction rate was constant for only 5 min (Fig. 6). With phosphatidylinositol kinase (Fig. 2) there was a rapid loss of product after 15 min. This was not shown by diphosphoinositide kinase, though there was little net synthesis of triphosphoinositide after 40 min. The loss of product is again likely to be due to phosphatase action. Since the diphosphoinositide substrate is also attacked by the phosphatase (Cooper & Hawthorne, 1975), it will compete with the relatively small amount of triphosphoinositide product, thus partially protecting the latter from hydrolysis.

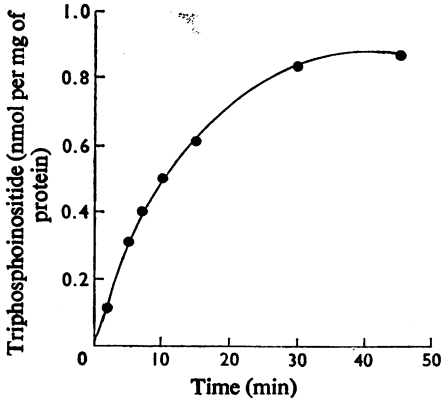


Fig. 6. *Time-course of the diphosphoinositide kinase reaction*

Conditions followed those of Fig. 5(*c*), except that 40 mM-$MgCl_2$ was present. After 5 min preincubation at 37°C, the reaction was started by adding diphosphoinositide. Duplicate 1 ml samples were removed for analysis at the times indicated.

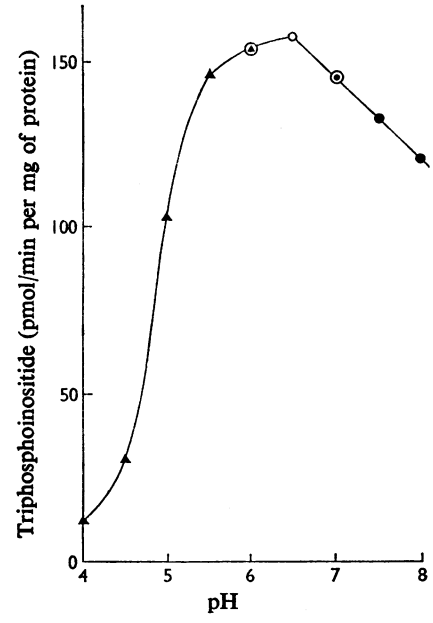


Fig. 7. *Effect of pH on diphosphoinositide kinase*

Assay conditions are given with Fig. 6. ▲, Sodium acetate buffer; ○, imidazole/HCl buffer; ●, Tris/HCl buffer.

Effect of pH. The optimum was observed at pH 6.5 (Fig. 7) and it is noteworthy that at this pH, phosphatidylinositol kinase is almost inactive.

Subcellular distribution of the kinases. Kidney cortex was homogenized by using a Potter–Elvehjem pestle and fractionated as described in the Materials and Methods section. The results of the distribution of the marker enzymes have been published previously in connexion with the studies on polyphosphoinositide phosphatase (Cooper & Hawthorne, 1975) and only the relevant results are repeated here (Tables 2 and 3, Fig. 8). In experiments on rat liver, the inclusion of Cutscum detergent caused an increase of phosphatidylinositol kinase activity in microsomal and lysosomal fractions without appreciably affecting that in the nuclear fraction (Harwood & Hawthorne, 1969*a*). This and other results suggested that there were two kinases in liver, one associated with the plasma membrane and one with the endoplasmic reticulum. This effect was not observed with kidney, however (Table 2), distribution of phosphatidylinositol kinase being the same in the presence or absence of Cutscum.

The subcellular distribution of kidney phosphatidylinositol kinase resembled that of alkaline phosphatase (marker for brush border), arylesterase, glucose 6-phosphatase (microsomal), Ca^{2+}-stimulated ATPase (plasma membrane), galactosyltrans-

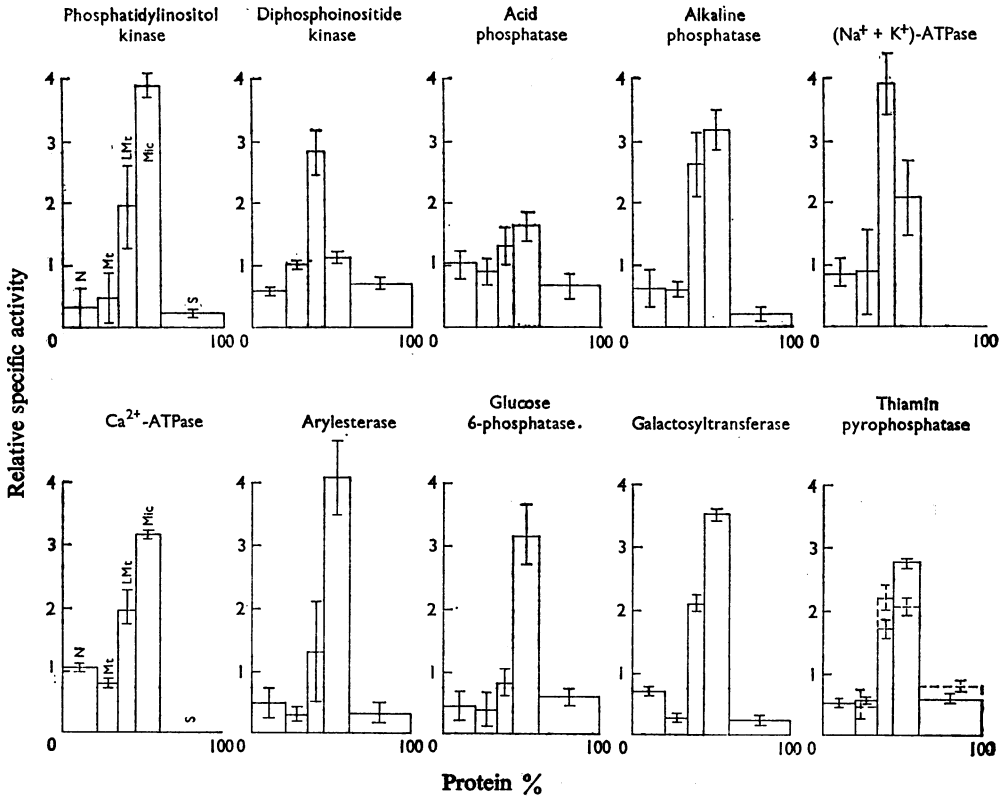


Fig. 8. *Subcellular distribution of polyphosphoinositide kinases and marker enzymes in kidney cortex*

Relative specific activity represents the percentage of total enzyme activity recovered in a fraction/percentage of total protein recovered in that fraction. There was no significant difference between activities of phosphatidylinositol kinase with and without Cutscum. The dashed lines for thiamin pyrophosphatase represent activities without added ATP. N, nuclear fraction; Mt, mitochondrial fraction; LMt, light mitochondrial fraction; Mic, microsomal fraction; S, supernatant. Means of three experiments are shown. Vertical bars represent S.D.

Table 2. *Subcellular distribution of phosphatidylinositol kinase and diphosphoinositide kinase in kidney cortex*

Specific activities are given as nmol/min per mg of protein. The means ± S.D. of three experiments are shown. Marker enzyme activities and distributions are given in Table 4 (Potter–Elvehjem homogenizer) of the previous paper (Cooper & Hawthorne, 1975).

	Percentage distribution in fractions						
Assay	Nuclear	Mitochondrial	Light mitochondrial	Microsomal	Supernatant	Recovery (%)	Homogenate sp. activity
Protein	20.7±2.2	14.7±2.9	10.1±1.0	14.8±0.9	39.7±1.9	98.6±1.9	—
Phosphatidylinositol kinase (+Cutscum)	6.5±6.5	6.6±6.3	20.0±6.7	57.5±2.7	9.4±3.4	109.6±1.9	0.120±0.042
Phosphatidylinositol kinase (no Cutscum)	6.9±1.1	6.6±0.4	22.2±3.6	56.8±6.0	7.6±0.9	107.0±1.2	0.015±0.001
Diphosphoinositide kinase	12.3±0.8	14.7±0.3	28.4±3.7	16.6±1.1	28.0±3.3	95.6±4.0	0.101±0.007

Table 3. *Correlation of marker histograms with those of the polyphosphoinositide kinases*

The calculations are based on Fig. 8 and previous results (Cooper & Hawthorne, 1975). Histograms of the distribution of the different markers are compared with those for each kinase. Areas that overlap are expressed as the percentage of overlapping plus non-overlapping areas.

	Correlation of area (%)		
	Phosphatidylinositol kinase		
	With Cutscum	Without Cutscum	Diphosphoinositide kinase
Arylesterase	90.2	88.1	55.9
Glucose 6-phosphatase	84.5	83.1	61.6
Galactosyltransferase	91.6	91.4	62.7
Thiamin pyrophosphatase (+ATP)	72.6	73.4	83.7
Thiamin pyrophosphatase (−ATP)	80.4	79.0	75.7
Alkaline phosphatase	86.3	88.9	70.0
K^+-stimulated nitrophenylphosphatase	70.9	71.9	79.2
Ca^{2+}-stimulated ATPase	79.8	80.2	60.5
$(Na^+ + K^+)$-stimulated ATPase	63.6	66.3	57.6
Triphosphoinositide phosphatase	64.0	62.6	80.6
Diphosphoinositide phosphatase	63.1	59.1	80.9
Acid phosphatase	60.5	59.1	82.8
Cytochrome oxidase	36.7	37.1	50.6
Catalase	33.1	31.7	52.5
β-Galactosidase	31.5	30.1	55.9
Lactate dehydrogenase	19.8	18.0	38.4
DNA	15.8	16.2	21.6

Table 4. *Kidney brush-border preparation*

Specific activities are given as nmol/min per mg of protein. Relative specific activity represents specific activity of fraction/specific activity of homogenate. N.D., No activity detectable.

		Brush border	
	Homogenate specific activity	Specific activity	Relative specific activity
Alkaline phosphatase	244	2057	8.4
$(Na^+ + K^+)$-ATPase	106	471	4.4
Glucose 6-phosphatase	74	N.D.	—
Acid phosphatase	49	N.D.	—
Thiamin pyrophosphatase (−ATP)	27	10	0.4
Thiamin pyrophosphatase (+ATP)	62	81	1.3
Phosphatidylinositol kinase	0.10	0.03	0.3
Diphosphoinositide kinase	0.08	0.09	1.1

ferase and thiamin pyrophosphatase (Golgi apparatus). Thus the enzyme is certainly membrane bound, but it is difficult to decide which type of membrane is involved. Activity of the enzyme was low in purified brush-border fragments (Table 4). Tou *et al.* (1969) concluded that phosphatidylinositol kinase of kidney was largely microsomal, but did not attempt to distinguish between the various membranes contributing to this fraction.

Diphosphoinositide kinase showed both supernatant and particulate activity as has been reported for brain (Kai *et al.*, 1968) and appeared to resemble acid phosphatase (lysosomal) and thiamin pyrophosphatase. It is unlikely that the enzyme is lysosomal, because it did not exhibit latency, as typical lysosomal enzymes do. Activity was also low in purified brush-border fragments.

Though it is difficult to localize the kinases precisely, it is noteworthy that the phosphatase attacking their products is also found in the Golgi fraction of kidney (Cooper & Hawthorne, 1975), as judged by thiamin pyrophosphatase as a marker. Phosphatidylinositol kinase appears to be present in several types of cell membrane, though not in brush border or mitochondria. In the adrenal medulla (Lefebvre *et al.*, 1976) it was found in microsomal membranes, but

also in the membranes of the chromaffin granules. Since the latter may well be derived from the Golgi membranes, it is significant that the kinase is a Golgi enzyme.

Tou *et al.* (1970) studied the properties and distribution of diphosphoinositide kinase in kidney. The enzyme appeared chiefly in nuclear and microsomal fractions. Its distribution resembled that of $(Na^+ + K^+)$-stimulated ATPase, except that there was more kinase in the soluble fraction. A plasma-membrane fraction was prepared and shown to have a 15-fold enrichment of diphosphoinositide kinase, compared with homogenate. The present study does not exclude the presence of the kinase in plasma membrane, except for the brush border, but Table 3 shows a better correlation with the Golgi marker thiamin pyrophosphatase than with $(Na^+ + K^+)$-stimulated ATPase. The properties of the plasma-membrane kinase of Tou *et al.* (1970) were similar to those of diphosphoinositide kinase in the present study, except for our higher Mg^{2+} optimum and lower pH optimum. The use of homogenate in the present work may account for the differences.

Tou *et al.* (1970) suggest that the polyphosphoinositides may be involved in cation transport in kidney. The absence of the kinases from brush-border membranes, however, seems to preclude a direct role in tubular reabsorption. It is more likely that triphosphoinositide plays some part in the control of Ca^{2+} concentrations within cells. Garrett *et al.* (1975) have shown, for instance, that entry of Ca^{2+} into erythrocytes leads to rapid hydrolysis of this lipid. Mitochondrial accumulation of Ca^{2+} could be related in some way to phosphatidylinositol, which is required for the ATP-induced contraction of liver mitochondria caused to swell by Ca^{2+} (Vignais *et al.*, 1964). It remains to be seen whether these observations might be relevant to the kidney.

References

Bollinger, J. N., Mallow, W. A., Register, J. W. & Johnson, D. E. (1967) *Anal. Chem.* **39**, 1508–1509

Brockerhoff, H. & Ballou, C. E. (1961) *J. Biol. Chem.* **236**, 1907–1911

Carter, H. E. & Weber, E. J. (1966) *Lipids* **1**, 16–20

Cooper, P. H. & Hawthorne, J. N. (1975) *Biochem. J.* **150**, 537–551

Ellis, R. B. & Hawthorne, J. N. (1961) *Biochim. Biophys. Acta* **51**, 385–387

Garbus, J., De Luca, H. G., Loomans, M. E. & Strong, F. M. (1963) *J. Biol. Chem.* **238**, 59–63

Garrett, N. E., Garrett, R. J. B., Talwalkar, R. T. & Lester, R. L. (1975) *J. Cell. Physiol.* **87**, 63–69

Glynn, I. M. & Chappell, J. B. (1964) *Biochem. J.* **90**, 147–149

Gonzalez-Sastre, F. & Folch-Pi, J. (1968) *J. Lipid Res.* **9**, 532–533

Hajra, A. K., Seguin, E. B. & Agranoff, B. W. (1968) *J. Biol. Chem.* **243**, 1609–1616

Hanahan, D. J. (1960) *Lipide Chemistry*, pp. 30–32, John Wiley and Sons, New York

Harwood, J. L. & Hawthorne, J. N. (1969*a*) *Biochim. Biophys. Acta* **171**, 75–88

Harwood, J. L. & Hawthorne, J. N. (1969*b*) *J. Neurochem.* **16**, 1377–1387

Hawthorne, J. N. & White, D. A. (1975) *Vitam. Horm. (N.Y.)* **33**, 529–573

Hokin, L. E. & Hokin, M. R. (1964) *Biochim. Biophys. Acta* **84**, 563–575

Kai, M. & Hawthorne, J. N. (1966) *Biochem. J.* **98**, 62–67

Kai, M., White, G. L. & Hawthorne, J. N. (1966) *Biochem. J.* **101**, 328–337

Kai, M., Salway, J. G. & Hawthorne, J. N. (1968) *Biochem. J.* **106**, 791–801

Lefebvre, Y. A., White, D. A. & Hawthorne, J. N. (1976) *Can. J. Biochem.* in the press

Long, C. & Owens, K. (1962) *Biochem. J.* **85**, 34P

Santiago-Calvo, E., Mulé, S. J., Redman, C. M., Hokin, M. R. & Hokin, L. E. (1964) *Biochim. Biophys. Acta* **84**, 550–562

Tou, J.-S. & Huggins, C. J. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 539

Tou, J.-S., Hurst, M. W. & Huggins, C. J. (1969) *Arch. Biochem. Biophys.* **131**, 596–602

Tou, J.-S., Hurst, M. W., Huggins, C. J. & Foor, W. E. (1970) *Arch. Biochem. Biophys.* **140**, 492–502

Vignais, P. M., Vignais, P. V. & Lehninger, A. L. (1964) *J. Biol. Chem.* **239**, 2011–2021