The Subcellular Distribution of Triphosphoinositide Phosphomonoesterase in Guinea-Pig Brain

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(Received 10 January 1972)

1. Some properties of the triphosphoinositide phosphomonoesterase from the homogenates of guinea-pig brain were studied. The enzyme has an optimum pH range 6.7–7.3, is stimulated with KCl at a concentration of 0.1M, and under these conditions has $K_m 1.43 \times 10^{-4}$ M. 2. A factor from the 'pH5 supernatant' of guinea-pig brain stimulates the enzyme activity over and above the stimulation produced by KCl. Subcellular fractions of guinea-pig brain varied in their response to the 'pH5 supernatant'. Maximum stimulation was observed with the P_1 fraction, containing myelin and nuclei. 3. An assay system for the enzyme was developed that contained optimum concentrations of both KCl and the 'pH5 supernatant'. Acid phosphatases were inhibited by NaF, but, in contrast with previous work, no EDTA was added to the assay system to inhibit the alkaline phosphatases. This reagent inhibited the triphosphoinositide phosphomonoesterase. It was estimated that the remaining fraction of non-specific phosphatases can account for only 14% of the observed triphosphoinositide phosphomonoesterase activity. 4. Subcellular fractions of guinea-pig brain were characterized by electron microscopy and subcellular markers. The triphosphoinositide phosphomonoesterase exhibited a distribution between the fractions similar to that of 5'-nucleotidase, but different from that of alkaline phosphatase.

Although polyphosphoinositides are minor constituents of brain lipids, they derive their importance from the following observations. (1) They are mainly localized in myelin (Eichberg & Dawson, 1965), but they can also be found in non-myelinic structures (Sheltawy & Dawson, 1966, 1969a). (2) In the presence of Ca^{2+} , triphosphoinositide is capable of binding with the basic proteins of myelin to give complexes that possess many of the properties of proteolipids (Palmer & Dawson, 1969). (3) In experiments with ^{32}P , the rate of turnover of triphosphoinositide is high when compared with other lipids [in brain (Eichberg & Dawson, 1965; Kai & Hawthorne, 1966), in sciatic nerve (Sheltawy & Dawson, 1969b) and in kidney (A. Sheltawy, unpublished work)].

Work on the subcellular distribution of enzymes involved in the metabolism of polyphosphoinositides led to conflicting observations. Phosphatidylinositol kinase (Kai *et al.*, 1966) and triphosphoinositide phosphodiesterase (Keough & Thompson, 1970) were found to be distributed in brain organelles in a fashion similar to that of 5'-nucleotidase and $Na^+ + K^+$ -stimulated adenosine triphosphatase, both known to be cell (plasma) membrane markers. Diphosphoinositide kinase (Kai *et al.*, 1968) and triphosphoinositide phosphomonoesterase (Salway *et al.*, 1967; Harwood & Hawthorne, 1969) of the brain were found to be localized in the particle-free cyto-

plasmic fraction. However, triphosphoinositide phosphomonoesterase of the kidney was found enriched in the endoplasmic reticulum (Lee & Huggins, 1968).

None of the studies on the triphosphoinositide phosphomonoesterase (Scheme 1) seem to have taken into consideration that the 'supernatant fraction' contains a factor that is capable of stimulating the hydrolysis of triphosphoinositide by the enzyme (Dawson & Thompson, 1964). Triphosphoinositide phosphodiesterase (Scheme 1) is not subject to the same stimulation. We have therefore undertaken to develop an assay system for the phosphomonoesterase that takes this fact into account, and to re-examine the subcellular distribution of this enzyme in guinea-pig brain.

Methods

Preparation of subcellular fractions

Guinea pigs were killed by spinal dislocation and decapitated. The cerebral hemispheres were removed and weighed (2.5–3.0g/animal). Sufficient 0.32M-sucrose was added to produce a 20% (w/v) homogenate, and the tissue was initially homogenized lightly in a loose-fitting glass homogenizer. Final homogenization was then carried out at 4°C by using

Scheme 1. Enzymic hydrolysis of triphosphoinositide

a power-driven Perspex pestle in Viridia glass tubing (all-round clearance 0.25 mm). The pestle was rotated at 900 rev./min and six complete up-and-down strokes were carried out in a total time of 1 min. Sufficient 0.32M-sucrose was then added to make a 10% (w/v) homogenate.

Subcellular fractions were prepared at 4°C as described by Eichberg *et al.* (1964). The P_1 fraction (1000g for 11 min) was subfractionated by layering (in 0.32M-sucrose) on a discontinuous density gradient (0.8M- and 1.2M-sucrose) and centrifuging at 53000g for 2 h. On examination by electron microscopy, the top band (P_1A) consisted of myelin with some smaller vesicular elements, and the intermediate band (P_1B) of nuclei together with some mitochondria. The pellet consisted mainly of tissue debris and blood cells and was always rejected. The mitochondrial fraction (P_2), obtained at 17500g for 1 h, contained also small myelin fragments, endoplasmic reticulum and synaptosomes (pinched-off nerve endings). It was not further fractionated, since it was not particularly rich in the triphosphoinositide phosphomonoesterase (see the Results section). The microsomal fraction (P_3) was obtained at 105000g for 1 h, leaving a particle-free supernatant fraction (S).

Preparation of 'pH 5 supernatant'

A portion of the 105000g supernatant obtained by the above procedure was mixed with an equal volume of 0.264M-tris-HCl buffer, pH 5.0, and the mixture adjusted to pH 5.0 at 4°C with 1M-HCl. It was kept at 4°C for 10 min, and the precipitate formed was centrifuged at 3000 rev./min in an 8 × 50 rotor of the MSE 18 centrifuge. The supernatant was removed and adjusted to pH 7.0 with solid tris. Sometimes the 'pH 5 supernatant' was concentrated by freeze-drying.

Preparation of the substrate

The ammonium salt of triphosphoinositide was prepared by the method of Dittmer & Dawson (1961). This involved extracting the tissue with neutral organic solvents and then with acidified organic sol-

vents. When the extracts were examined by t.l.c. (Gonzalez-Sastre & Folch-Pi, 1968) two bands were apparent. The major one had R_F 0.11 and was identified as triphosphoinositide by comparison with a standard. A faint band with R_F 0.22 was identified as diphosphoinositide.

The yield of substrate from one ox brain (300g) varied from 1.5 to 3.7 mg of P. Highest yields were obtained by using distilled methanol in all the extractions. This minimizes the loss of polyphosphoinositides to the initial 'neutral-solvent extracts' (detected by t.l.c. examination; see above).

Final method of assay for the triphosphoinositide phosphomonoesterase

A modification of the method described by Dawson & Thompson (1964) was used. The incubation system contained appropriate amounts of 'pH 5 supernatant', which stimulates the phosphomonoesterase with no effect on the diesterase. Further, GSH, which stimulates the first enzyme but inhibits the latter (Dawson & Thompson, 1964), was added to the incubation system. To avoid stimulating the alkaline phosphatase no Mg^{2+} was added to the system; the amount provided by the homogenate or subcellular fractions was considered sufficient for the phosphomonoesterase activity. Acid phosphatases were inhibited by NaF.

To constitute the system, a portion of a chloroform solution of the substrate was evaporated to dryness *in vacuo* and the solid film was dispersed in water. KCl and other constituents were then added to the final concentrations indicated below. If the solid film was initially dispersed in KCl or buffer solutions instead of water, incomplete emulsification of the substrate was obtained. The final incubation system contained: triphosphoinositide (0.54M, i.e. 50 µg of P), GSH neutralized to pH 7.2 with solid tris (12.5mM), KCl (0.1M) and NaF (1mM). Optimum concentrations of 'pH 5 supernatant' and the enzyme preparations were then added. The system was buffered with tris-HCl (42mM) to pH 7.2 and had a final volume of 1 ml. Incubation was carried out at 37°C for 30 min.

The reaction was normally stopped by adding 0.2 ml of 5% (w/v) bovine serum albumin and 1 ml of cold 10% (v/v) perchloric acid. A portion was taken from the supernatant for determination of P_i liberated by the method of Fiske & SubbaRow (1925). Under the conditions used, no organically bound P (e.g., inositol di- or tri-phosphate) was measured. In one experiment (Fig. 1c) the reaction was stopped by adding one drop of conc. HCl and the lipids were extracted by the method of Bligh & Dyer (1959). P_i and total water-soluble phosphate were determined in a portion of the upper phase, and polyphosphoinositides were determined by quantitative t.l.c. (Gonzalez-Sastre & Folch-Pi, 1968) of the lower phase.

The presence of sucrose (up to 250 mM) did not interfere with results from this assay system.

Measurement of other enzyme activities

To assay succinate dehydrogenase (EC 1.3.99.1) the final incubation system consisted of the following, in a final volume of 3.9 ml: phosphate buffer, pH 7.4 (65 mM), the oxidized form of 2,6-dichlorophenol-indophenol (15 μ g), KCN (39 μ M), EDTA (disodium salt; 1.5 mM), disodium succinate (64 mM). Enzyme activities were measured in terms of the rate of decrease in E_{600} . Disodium succinate was added only after the endogenous substrate was totally consumed or its rate of oxidation accurately determined.

Lactate dehydrogenase (L-lactate-NAD oxidoreductase, EC 1.1.1.27) was assayed by the method of Bergmeyer *et al.* (1963) with 10 μ l of each subcellular fraction. 5'-Nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) was assayed by the method of Michell & Hawthorne (1965) with the method of Fiske & SubbaRow (1925) being used for the determination of P_i released. Alkaline phosphatase (EC 3.1.3.1) was assayed by measurement of the rate of hydrolysis of *p*-nitrophenyl phosphate in glycine buffer, pH 10 (50 mM), in the presence of $MgCl_2$ (5 mM).

Determination of RNA and protein

RNA. This was determined by extracting 1 ml of the subcellular fraction with 2 ml of aq. 90% (w/v) phenol. The phases were separated by repeated freezing and thawing followed by centrifugation. The aqueous phase was mixed with 3 vol. of ethanol and the RNA was precipitated overnight at -16°C . The precipitate was washed once with aq. 80% (v/v) ethanol and dissolved in 2 ml of 1 mM-NaOH. After it had been placed in a boiling-water bath for 10 min, it was mixed with 3 ml of an orcinol reagent (ferric alum, 1 mg/ml, and orcinol, 10 mg/ml, in conc. HCl). The mixture was heated in a boiling-water bath for 5 min together with standards of sodium RNA (25–125 μ g) treated in a

similar manner. The blue colour developed was measured at 660 nm.

Protein. The following modifications were introduced into the method of Lowry *et al.* (1951). Sodium dodecyl sulphate [0.1 ml of a 5% (w/v) solution per tube] was added to disperse the different subcellular fractions. Omission of this reagent may lead to underestimation of the protein content in the particulate fractions. The colour was developed to completion by heating at 60°C for 10 min, and measured at 730 nm. The absorption maximum is different from that produced without heating (660 nm). The presence of sucrose in concentrations up to 250 mM did not interfere with protein determinations in which this modification was used.

Results

Properties of the triphosphoinositide phosphomonoesterase from homogenates of guinea-pig brain

In agreement with previous work on the enzyme from ox brain (Dawson & Thompson, 1964), the optimum pH of the guinea-pig brain enzyme was found to lie in the range 6.7–7.3 (Fig. 1a). In this respect it differs from the triphosphoinositide phosphomonoesterase of rat kidney cortex, which has an optimum of pH 8.0 (Lee & Huggins, 1968). The enzyme activity in the guinea-pig brain homogenate was markedly stimulated with KCl and maximum activation occurred at a concentration of 0.1 M (similar to the results of Dawson & Thompson, 1964). The activity was inhibited at concentrations of salt higher than 0.2 M (Fig. 1b), and this could be due to a fall in the solubility of the substrate when dispersed in solutions of high KCl concentrations. Evidence in support of this hypothesis was obtained by evaporating portions of a chloroform solution of triphosphoinositide and shaking them with solutions of various KCl concentrations. The extent of the dispersion of triphosphoinositide was assayed by total phosphorus determination on the clear supernatant obtained by centrifugation at 1000g (Fig. 1b). The amount of triphosphoinositide dispersed decreased with increasing salt concentration. During the fall-off phase, the amount of triphosphoinositide hydrolysed was greater than that dispersed, owing to the enzyme shifting the equilibrium between the dispersed and non-dispersed substrate.

The rate of change of reactants and products was studied in a system that contained brain homogenate (1 mg of protein) and 0.1 M-KCl (Fig. 1c). Some phosphodiesterase activity was observed (total water-soluble phosphate minus P_i) but this did not apparently change with time. A reaction time of 30 min was therefore selected to minimize the influence of this on the measured phosphomonoesterase activity.

Fig. 1. Some properties of the triphosphoinositide phosphomonoesterase of guinea-pig brain homogenates

Enzyme assays were carried out as described in the text. (a) Optimum pH of the enzyme. (b) Stimulation of the enzyme with KCl: ●, P_i released/30min; ○, triphosphoinositide dispersed at a given KCl concentration. (c) Change of reactants and products with time: ○, triphosphoinositide; △, diphosphoinositide; ●, P_i ; ▲, total water-soluble phosphate. (d) Saturation of enzyme with the substrate.

Maximum hydrolysis in the system stimulated with KCl alone occurred when the substrate concentration was 0.54mM (50 μg of triphosphoinositide P; Fig. 1d). A Lineweaver-Burk plot indicated that the guinea-pig brain triphosphoinositide phosphomonoesterase had K_m 1.43×10^{-4} M.

The triphosphoinositide phosphomonoesterase activity (P_i released) was inhibited by EDTA up to a concentration of 4mM, where only 30% of the original activity remained (Fig. 2). The release of total water-soluble phosphate was similarly inhibited in response to the addition of EDTA.

Stimulation of the triphosphoinositide phosphomonoesterase with 'pH5 supernatant'

To test the magnitude of stimulation of the enzyme in homogenates by the 'pH5 supernatant' (Dawson & Thompson, 1964), different volumes were added to the basic incubation medium, which in addition contained different concentrations of KCl (Fig. 3a). The presence of KCl was essential to the activation with 'pH5 supernatant'. No stimulation was observed at a salt concentration of 0.025M, whereas at 0.05M-KCl maximal stimulation was attained with 0.2ml of the 'pH5 supernatant'. At 0.1M-KCl, how-

ever, maximal stimulation was obtained with only 0.1 ml of 'pH5 supernatant'. The stimulation effect at 0.2M-KCl was less in magnitude than that when 0.1M-KCl was used. The stimulation was over and above that due to KCl alone, and the effect was more apparent with aged (2 days at 4°C) homogenates that were initially less active.

Different subcellular fractions varied in their response to the 'pH5 supernatant'. No stimulation was observed when the microsomal fraction (P_3) was used as a source of the enzyme. For the P_2 and S fractions, only a small stimulation occurred at low concentrations of the 'pH5 supernatant', followed by an inhibition at higher concentrations (Fig. 3b). Maximum stimulation (80%) was observed with the P_1 fraction, which indicated that most of the activity of the 'pH5 supernatant' observed in the total homogenate could be accounted for by its effect on this fraction.

Non-specific phosphatases

Non-specific phosphatases present in the homogenate or in the subcellular fractions may cause apparently high rates of triphosphoinositide phosphomonoesterase activity and therefore interfere with the accurate localization of the enzyme. NaF (1mM) was added to the incubation medium to inhibit the acid phosphatases. This anion up to a concentration of 3mM does not inhibit the triphosphoinositide

phosphomonoesterase (Dawson & Thompson, 1964). Observed activities in the different subcellular fractions were decreased by the addition of 1mM-NaF. Maximum inhibition occurred in the P_2 and P_3 fractions (68 and 83% of the original activities were lost respectively). Almost half the observed activity of the non-particulate fraction (S) was lost, and no

Fig. 2. Effect of EDTA on the release of P_i and total water-soluble phosphate by guinea-pig brain homogenates

Experimental details are given in the text. ○, Release of P_i (triphosphoinositide phosphomonoesterase activity); ●, release of total water-soluble phosphate.

Fig. 3. Effect of 'pH5 supernatant' on the triphosphoinositide phosphomonoesterase activity of whole guinea-pig brain homogenates and its subcellular fractions

(a) Whole-brain homogenate. The KCl concentrations were: ■, 0.025M; ●, 0.05M; ○, 0.1M; □, 0.2M. (b) Subcellular fractions. The subcellular fractions were: ●, P_1 ; ○, P_2 ; ■, P_3 ; □, S. The 'pH5 supernatant' used was concentrated tenfold by freeze-drying.

decrease in activity was observed with the P_1 fraction.

EDTA was not used to inhibit the alkaline phosphatases in the present system since this reagent also inhibits the triphosphoinositide phosphomonoesterase (Fig. 2). Mg^{2+} , which is essential to the activity of alkaline phosphatases, was not added; ions provided by the homogenate or subcellular fractions were considered sufficient for the triphosphoinositide phosphomonoesterase activity. The contribution of alkaline phosphatases to the measured activity of this enzyme was estimated as follows. Inositol triphosphate ($59\mu g$ of P_i ; prepared by acid hydrolysis of triphosphoinositide) was incubated with brain homogenate for 30 min in the presence of F^- under the same conditions described for the triphosphoinositide phosphomonoesterase. Only 6% of the monoesterified phosphate was liberated into the medium as P_i , probably as a result of the action of alkaline phosphatases. Under identical conditions, nearly 45% of the triphosphoinositide monoesterified phosphate was hydrolysed. Our values are therefore likely to be a maximum of 14% too high as a consequence of

non-specific phosphatases surviving the treatment with F^- .

Finally, the subcellular distribution of alkaline phosphatase was determined at pH 10 in the presence of $MgCl_2$ and was found to exhibit a different profile from that of the triphosphoinositide phosphomonoesterase (Fig. 4). The subcellular distribution of this enzyme described below cannot therefore be due to the action of alkaline phosphatase.

Subcellular distribution of triphosphoinositide phosphomonoesterase and other subcellular markers in guinea-pig cerebral hemispheres

Fig. 4 illustrates the subcellular distribution of triphosphoinositide phosphomonoesterase, 5'-nucleotidase, alkaline phosphatase, succinate dehydrogenase, RNA and lactate dehydrogenase in guinea-pig cerebral hemispheres. Recovery of the triphosphoinositide phosphomonoesterase assayed under the present conditions varied from 83 to 86% in four different experiments. The enzyme (Fig. 4a) showed a distribution very similar to that of 5'-

Fig. 4. Distribution of triphosphoinositide phosphomonoesterase and markers in the subcellular fractions of guinea-pig brain

Scales on the ordinates are expressed relative to the homogenate specific activities. These were: for (a) triphosphoinositide phosphomonoesterase assayed by our method, 12.6 nmol/min per mg of protein; for (b) 5'-nucleotidase, 56 nmol/min per mg of protein; for (c) alkaline phosphatase, 1.211 μ mol/min per mg of protein; for (d) triphosphoinositide phosphomonoesterase assayed by the method of Salway *et al.* (1967), 6.1 nmol/min per mg of protein; for (e) succinate dehydrogenase, 9.1 nmol/min per mg of protein; for (f) RNA, 70 μg /mg of protein; for (g) lactate dehydrogenase, 2.17 μ mol/min per mg of protein. Fractions are denoted as described in the Methods section. The abscissae express the concentration of protein in a given fraction collected from 1 g fresh wt. of brain, the full scale representing 100 mg of protein. Average protein recoveries from the combined subcellular fractions were 85% of that present in the whole homogenate (six determinations).

nucleotidase (Fig. 4b), and may therefore be present in the outer cell membrane. Although some alkaline phosphatase activity (Fig. 4c) was detected in the P_1A fraction (myelin), the bulk of the activity was present in the nuclear fraction (P_1B) and the rather heterogeneous P_2 fraction. This pattern differs from that of either triphosphoinositide phosphomonoesterase or 5'-nucleotidase.

The difference between our results and those of previous authors (Salway *et al.*, 1967) may possibly be attributed to differences in the conditions of brain homogenization or methods of subcellular fractionation. To eliminate this possibility, the subcellular distribution of triphosphoinositide phosphomonoesterase was studied in the present work by the enzyme-assay method devised by those authors. Although the measured activities (Fig. 4d) were lower than those reported by Salway *et al.* (1967), the distribution obtained was similar to theirs. The highest specific activity was found in the non-particulate fraction. This agreement indicated that the conditions for homogenization and subcellular fractionation were similar in both studies, and that the differences observed (compare Figs. 4a and 4d) were due to the different conditions of enzyme assay (see the Discussion section).

The other subcellular markers showed the distribution expected from previous work on brain tissue (Salway *et al.*, 1967; Keough & Thompson, 1970). The RNA found in the myelin fraction (P_1A) may represent the cytoplasmic inclusions usually observed in myelin on electron-microscopic examination (Hirano & Dembitzer, 1967).

Discussion

Composition of the present system for assay of the triphosphoinositide phosphomonoesterase

In the present assay system, we monitored the triphosphoinositide phosphomonoesterase activity by measuring the rate of liberation of P_i alone. This excludes measurement of the phosphodiesterase activity. However, in the presence of the latter enzyme the apparent rate of the phosphomonoesterase activity is likely to be decreased. Agents are at present known to stimulate selectively but not to inhibit either of these enzymes (Dawson & Thompson, 1964; Thompson & Dawson, 1964). Consequently, the incubation medium in our study contained 'pH 5 supernatant' and GSH to stimulate the triphosphoinositide phosphomonoesterase activity. Further, no cetyltrimethylammonium bromide or ether, which stimulate the phosphodiesterase, was included. It is not clear, however, whether the constant apparent triphosphoinositide phosphodiesterase activity observed in the present work was in fact representative of that enzyme, since it did not appear to change with time.

Non-specific phosphatases may increase the apparent triphosphoinositide phosphomonoesterase activity. F^- , which inhibits the acid phosphatase, was added to the present system, but EDTA, which inhibits the alkaline phosphatase, was not included since it also inhibits the triphosphoinositide phosphomonoesterase. Instead, Mg^{2+} , which is essential to the action of alkaline phosphatase, was omitted. It was estimated that these remaining non-specific phosphatase activities may account for only 14% of the observed monoesterase activity of the whole homogenate. This may still be an overestimate since it was necessarily determined with a water-soluble and not a lipid substrate.

Under the present assay conditions, the triphosphoinositide phosphomonoesterase was saturated with 0.54 mM-triphosphoinositide, giving a K_m ($1.43 \times 10^{-4} M$) very similar to that of the enzyme from rat kidney cortex (Lee & Huggins, 1968). This is different from the values quoted by Salway *et al.* (1967) for the rat brain enzyme (saturated at 3.23 mM-triphosphoinositide). The activity in their system may have been inhibited, since EDTA was included in the incubation medium. This is supported by several other observations. Mg^{2+} was found to be essential to the activity, but at a much higher concentration (7 mM) than that found by Dawson & Thompson (1964). Moreover, the characteristic stimulation of the enzyme with KCl or NaCl (Fig. 1b; Dawson & Thompson, 1964) was not observed, and in fact the enzyme was inhibited by Na^+ (Salway *et al.*, 1967).

Subcellular distribution of triphosphoinositide phosphomonoesterase

The subcellular distribution of triphosphoinositide phosphomonoesterase in guinea-pig brain found by us differs from that found by previous authors. In the present work the enzyme was found to possess a distribution similar to that of 5'-nucleotidase and may therefore be localized in the cell-surface membrane. Salway *et al.* (1967) and Harwood & Hawthorne (1969) reported its presence in the non-particulate fraction of both rat and guinea-pig brain respectively. This is in spite of the fact that the studies used two different assay systems.

Dawson & Thompson (1964) first demonstrated that the 'pH 5 supernatant' from ox brain stimulated the triphosphoinositide phosphomonoesterase but not the phosphodiesterase activity of that tissue. We have confirmed this observation for the guinea-pig brain, and also demonstrated that the triphosphoinositide phosphomonoesterase activities in its subcellular fractions varied in their response to the 'pH 5 supernatant'. None of the previous studies took this fact into account, an omission that would result in

overestimation of the activity of the particle-free supernatant fraction (Fig. 4d).

The nature of the factor in the 'pH 5 supernatant' is not clear. It was found to possess no triphosphoinositide phosphomonoesterase activity of its own, and when heated at 100°C for 5 min the stimulating activity was still retained. Its calculated content of bivalent cations or tris buffer cannot account for its stimulating activity. Dawson & Thompson (1964) point out that it survives prolonged dialysis, and that the activation produced is higher than that obtained by optimum Mg^{2+} concentrations. They also point out that, although the triphosphoinositide phosphomonoesterase and phosphodiesterase were both activated by KCl and bivalent cations, only the monoesterase was stimulated by the 'pH 5 supernatant'.

The subcellular distribution of triphosphoinositide phosphomonoesterase found in the present work is therefore similar to that of triphosphoinositide phosphodiesterase (Keough & Thompson, 1970) and phosphatidylinositol kinase (Kai *et al.*, 1966), all being located in the cell-surface membrane. Triphosphoinositide itself may possess a similar distribution, although direct evidence for this is lacking owing to its rapid hydrolysis in animal tissues *post mortem*. However, the remaining fraction of this phospholipid surviving hydrolysis was recovered in myelin, a modified cell-surface membrane (Eichberg & Dawson, 1965). By freezing the tissues to avoid hydrolysis *post mortem*, triphosphoinositide was also found in non-myelinated nerve fibres (Sheltawy & Dawson, 1966) and in the brain before myelination (Sheltawy & Dawson, 1969a). It was also demonstrated in non-neuronal tissues, particularly those, such as the kidney (Eichberg & Dawson, 1965), showing numerous proliferations of the cell-surface membrane.

There are, however, some exceptions to the above-mentioned pattern. Diphosphoinositide kinase (Kai *et al.*, 1968) in the brain was found located in the non-particulate fraction, and triphosphoinositide phosphomonoesterase in the kidney (Lee & Huggins, 1968) was found located in the microsomal fraction. The reasons for this observation in the kidney may be manifold, but it is possible that on vigorous homogenization fragments of the cell-surface membrane can be recovered in the microsomal fraction (Thuneberg & Rostgaard, 1968).

Polyphosphoinositides may therefore be located in the cell-surface membrane in a complex with many of the enzymes involved in their metabolism. The

rapid turnover of these phospholipids (see the introduction) indicates that the complex could be performing a function important to the role of this membrane.

This work was supported by a grant from the Multiple Sclerosis Society of Great Britain. We are grateful for Mr. Steve George, who carried out the assays for the alkaline phosphatase and the electron-microscopic examination of the subcellular fractions.

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