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HETEROGENEITY OF THE CALCIUM-DEPENDENT PHOSPHATIDYLINOSITOL-PHOSPHODIESTERASE OF RAT LIVER AND KIDNEY, AS REVEALED BY COLUMN CHROMATOFOCUSING

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Summary: The Ca^{2+}-dependent phosphatidylinositol-phosphodiesterase of rat liver and kidney has been examined by column chromatofocusing. Five different fractions were eluted with isoelectric points of approximately pH 7.0, pH 6.0, pH 5.0, pH 4.5 and <4, all of which showed activity at pH 5.5. Only the fraction with an isoelectric point about pH 4.5, showed the additional peak of activity at pH 8.0 which was evident upon assay of the original homogenate. The heterogeneity of phosphatidylinositol phosphodiesterase is therefore in both tissues similar to that found in brain. With the modifications introduced here, chromatofocusing can be successfully employed for examining crude tissue supernatants.

There is now little doubt that a very early metabolic event following stimulation of many cell surface receptors is a breakdown of phosphatidylinositol present in the cell membranes (1,2). The Ca^{2+}-dependent phosphatidylinositol phosphodiesterase (phospholipase C-type) probably responsible for this breakdown produces diglyceride, inositol cyclic 1:2 monophosphate and inositol 1-monophosphate (3,4) and is present in the cytosolic supernatant of mammalian tissues, for example, sheep pancreas (5,6), brain (7,8), rat liver (9) and the intestinal mucosa of guinea-pig (10).

We have previously described a considerable heterogeneity of this enzyme in rat brain with respect to its pH profile, calcium dependency and isoelectric points (8,11). In particular we have shown a considerable number of different enzyme fractions can be separated by the use of chromatofocusing, a new column technique which discretely separates proteins on the

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basis of their isoelectric points (11). We report here that in cytosolic supernatants from rat liver and kidney similar isoenzyme profiles are produced to those found in brain.

MATERIALS AND METHODS

Preparation of [^{32}P] and non-labelled phosphatidylinositol

[^{32}P] phosphatidylinositol was prepared as described by Hazlewood & Dawson (12) and Irvine et al (13); non-labelled phosphatidylinositol was also purified from bakers' yeast by the same method.

Assay system

The assay mixture contained 50 mM Tris-Maleate buffer pH 5.5 or 8.0, 129 nmol of mixture of [^{32}P] and non-radioactive phosphatidylinositol, 1 mM $CaCl_2$ and the enzyme fraction in 0.5 ml final volume. The reaction mixture was incubated for 30 minutes at 37°C. The reaction was terminated by the addition of 0.5 ml of trichloroacetic acid and 0.05 ml of 5% bovine serum albumin solutions. After the supernatant was washed twice with diethyl ether, the trichloroacetic acid supernatant radioactivity was measured (13).

Enzyme preparation

An albino female rat (approx. 200g) was sacrificed. 3g of liver or kidney were washed in 50 mM imidazole HCl buffer pH 7.4, containing 1 mM dithiothreitol 0.25 M sucrose (homogenate buffer) after the connective tissue was carefully taken out.

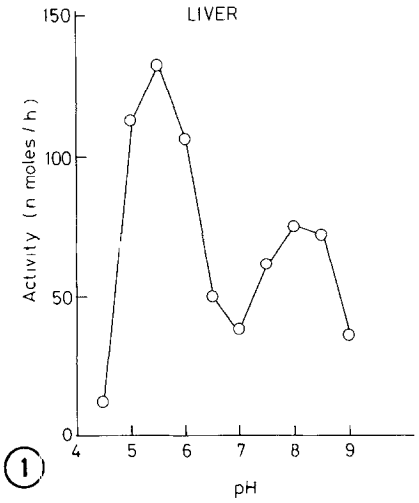
The tissue was homogenized and centrifuged at 100600g for 60 minutes at 4°C. The cytosolic supernatant was used as the enzyme source for chromatofocusing.

Chromatofocusing column technique

The basic principles and procedures have been published as a booklet (Chromatofocusing with Polybuffer and PBE) by Pharmacia Fine Chemicals (Sweden), and some modifications of the procedure have been reported previously (11). Briefly, a crude cytosolic supernatant (above) was loaded onto a column of Polybuffer Exchanger (Pharmacia) equilibrated at pH 7.4, and then eluted by Polybuffer 74 (Pharmacia) adjusted to pH 4.0 with HCl. The pH of the eluent changed by about 0.025 pH units per ml, and 6 ml fractions were collected in 1 ml of 50 mM imidazole-HCl (pH 7.4) to prevent denaturation of proteins caused by prolonged storage at their isoelectric points.

RESULTS AND DISCUSSION

The pH activity profiles of phosphatidylinositol-phosphodiesterase in the cytosolic supernatants from liver and kidney are very similar to each other (Figs. 1 and 2) showing two clear pH optima at 5.5 and 8.0 using 50 mM Tris-Maleate buffers in the presence of 1 mM Ca^{2+}. This double optimum has not been previously described in liver, except by Kemp et al. (9) who reported a minor peak at 6.9 in addition to the major optimum at pH 5.5. In our previous report (11) we found that ammonium sulphate fractionation of brain could result in loss of activity, particularly in the alkaline range;



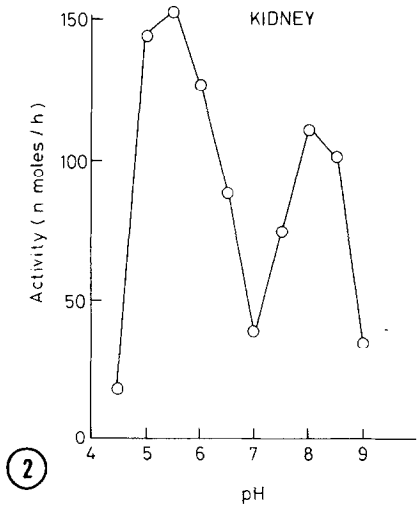


Figure 1 Effect of pH on the hydrolysis of [^{32}P] phosphatidylinositol by a cytosolic supernatant of rat liver

The incubation conditions were as described in Materials and Methods; 50 mM Tris-Maleate buffer was used for all pH ranges.

Figure 2 Effect of pH on the hydrolysis of [^{32}P] phosphatidylinositol by a cytosolic supernatant of rat kidney

See Figure 1 for the details.

Takenawa & Nagai (14), who purified this phospholipase C from rat liver supernatant and found a single optimum of pH 7, presumably only isolated one of these two activities. We have also found (unpublished observations) that rabbit iris-smooth muscle showed two peaks of pH optima under the same conditions, and we suggest that the single optimum at only 5.5 reported by Abdel-Latif et al. (15) was caused by their use of ammonium sulphate fractionation.

Chromatofocusing has been employed for the separation of a crude cytosolic supernatant of rat brain (11) and we show here that it can also be used successfully for fractionation of membrane-free supernatants from liver and kidney (Figs. 3 and 4). We found that 1% (w/v) polyethyleneglycol helps cleave hydrogen bonds between subunits and prevents protein aggregation if it was added to the sample in starting buffer before being applied to the

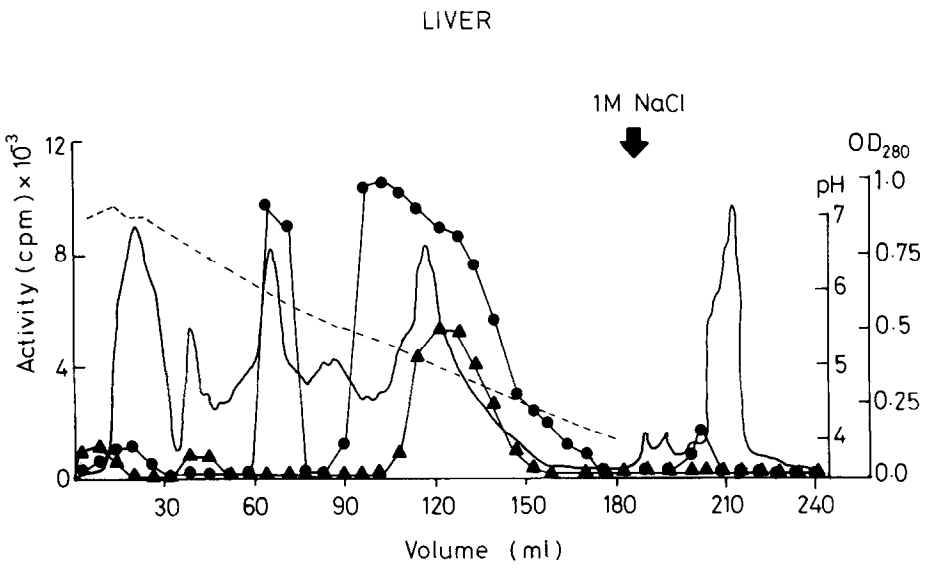


Figure 3 Chromatofocusing of a rat liver supernatant

O.D. at 280 nm (——). The activity is presented when assayed at pH 5.5 (●), pH 8.0 (▲) in the presence of 1 mM Ca^{2+}. The broken line shows the pH measurement of the eluent. The arrow (⟶) marks the point at which 1 M NaCl was applied to the column. The products of hydrolysis of all activity peaks were analysed, and all produced only inositol monophosphate and diacylglycerol, indicating phosphodiesterase activity.

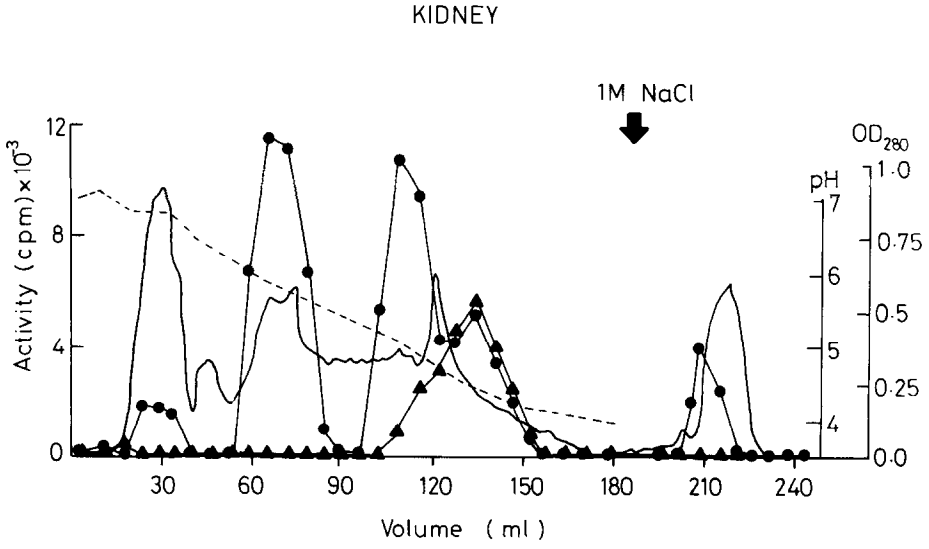


Figure 4 Chromatofocusing of a rat kidney supernatant

The presentation is the same as Figure 3.

column. We have also introduced a modification of the chromatofocusing buffer concentration (final buffering power 0.015 mmol/pH unit/ml); this is twice the concentration recommended by the supplier and it made a steeper, and steadier, pH curve (intervals approx. 0.15 pH units when the fractions were collected as 6 ml). As the ampholyte itself is a zwitterionic compound it probably also contributed to the stability of the proteins by increasing the ionic strength of the solutions. In early experiments applying a crude tissue supernatant according to recommended methods, we lost significant and variable amounts of enzyme activity. However, after our modifications, we obtained highly reproducible patterns of isoenzymes from each tissue.

The chromatofocusing profiles of the cytosolic supernatants from both liver and kidney were broadly similar to each other and to that of brain. The minor quantitative differences between peaks in liver and kidney versus each other and versus brain (11) may reflect a different isoenzyme composition of each tissue.

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