Purification and Characterization of Phosphoinositide 3-Kinase from Rat Liver*

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Phosphoinositide 3-kinase was purified 27,000-fold from rat liver. The enzyme was purified by acid precipitation of the cytosol followed by chromatography on DEAE-Sepharose, S-Sepharose, hydroxyapatite, Mono-Q, and Mono-S columns. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified phosphoinositide 3-kinase preparation contained an 80-kDa protein and a protein doublet of ~110 kDa. The 85- and 110-kDa proteins focus together on native isoelectric focusing gels and are cross-linked by dithiobis(succinylamide propionate), showing that the 110- and 85-kDa proteins are a complex. The apparent size of the native enzyme, as determined by gel filtration, is 190 kDa. The 85-kDa subunit is the same protein previously shown to associate with polyoma virus middle T antigen and the platelet-derived growth factor receptor (Kaplan, D. R., Whitman, M., Schaffhausen, D., Dallas, D. C., White, M., Cantley, L., and Roberts, T. M. (1987) Cell 50, 1021-1029). The two proteins co-migrate on two-dimensional gels; and, using a Western blotting procedure, 32P-labeled middle T antigen specifically blots the 85-kDa protein. The purified enzyme phosphorylates phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate. The apparent $K_m$ values for ATP were found to be 60 μM with phosphatidylinositol as the substrate and 30 μM with phosphatidylinositol 4-phosphate or phosphatidylinositol 4,5-bisphosphate as the substrate. The apparent $K_m$ for phosphatidylinositol is 60 μM, for phosphatidylinositol 4-phosphate is 9 μM, and for phosphatidylinositol 4,5-bisphosphate is 4 μM. The maximum specific activity using phosphatidylinositol as the substrate is 0.8 μmol/mg/min. The enzyme requires Mg$^{2+}$ with an optimum of 5 mM. Substitution of Mn$^{2+}$ for Mg$^{2+}$ results in only ~10% of the Mg$^{2+}$-dependent activity. Physiological calcium concentrations have no effect on the enzyme activity. Phosphoinositide 3-kinase has a broad pH optimum around 7.

Polyphosphoinositides and their metabolites are crucial intracellular signals in the responses to a number of hormones and growth factors (1, 2). The recent discovery of a phosphatidylinositol kinase that phosphorylates PI$^1$ at the D-3 position of the inositol ring uncovered a new pathway of PI metabolism and potential intracellular signals (3). This pathway is distinct from the pathway which leads from PI to PI-4,5-P$_2$ and then to diacylglycerol and inositol 1,4,5-trisphosphate through the action of phospholipase C.

Phosphoinositide 3-kinase was first discovered in pp60$^{src}$ immunoprecipitates from transformed cells (4). It was subsequently found in middle T immunoprecipitates from polyoma middle T antigen-transformed cells (5) and more recently has been found to coimmunoprecipitate with PDGF (6-8), colony-stimulating factor 1 (9), and insulin receptors (10) in ligand-stimulated cells. Analysis of mutants has closely linked the PI 3-kinase pathway to transformation by several oncogene products and to the mitogenic response to PDGF. Mutants of middle T antigen with which PI 3-kinase does not associate are nontransforming, and PDGF receptor mutants that do not associate with PI 3-kinase do not have a mitogenic response to PDGF (5-7, 11-16).

Although these studies indicate that PI 3-kinase produces a crucial second messenger, the signal(s) from this pathway have not yet been identified. In addition to producing PI-3-P, immunoprecipitates of middle T antigen or the PDGF receptor also have enzymatic activities that phosphorylate PI-3-P and PI-4,5-P$_2$ to produce PI-3,4-P$_2$ and PIP$_3$ (probably phosphatidylinositol 3,4,5-bisphosphate) (12, 17). Quiescent smooth muscle cells and fibroblasts have detectable levels of PI-3-P, but the levels of this lipid do not change appreciably with stimulation by PDGF or transformation by middle T antigen. PI-3,4-P$_2$ and PIP$_3$ are not detectable prior to stimulation or transformation, but reach significant levels after stimulation with PDGF or expression of middle T antigen (12, 17). Similarly, Chinese hamster ovary cells, transfected with the human insulin receptor, show elevated levels of PI-3,4-P$_2$ and PIP$_3$ in response to insulin (10). No phospholipase

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1 The abbreviations used are: PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; PIP$_2$, phosphatidylinositol bisphosphate; PI-3-P, phosphatidylinositol 3-phosphate; PI-3,4-P$_2$, phosphatidylinositol 3,4-bisphosphate; PI-3,4,5-P$_3$, phosphatidylinositol 3,4,5-trisphosphate; HEPES, 4-(hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; HPLC, high performance liquid chromatography; gPI-3-P, glycerophosphoinositol 3-phosphate; gPI-3,4-P$_2$, glycerophosphoinositol 3,4-bisphosphate; gPI-3,4,5-P$_3$, glycerophosphoinositol 3,4,5-trisphosphate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PDGF, platelet-derived growth factor; DSP, dithiobis(succinylamide propionate); MES, 2-(N-morpholino)ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; PS, phosphatidylserine; PC, phosphatidylcholine; PA, phosphatidic acid; DAG, diacylglycerol.

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C has been found which will cleave any of these phosphoinositides (18, 19), suggesting that PI-3,4,5-P₃ and/or PIP₃ themselves are the crucial signals emanating from this pathway, rather than inositol polyphosphates.

Although there is a strong correlation between PI 3-kinase activity and cell growth, D-3 phosphorylated phosphoinositides have also been found in nongrowing cells. PIP₃ (probably phosphatidylinositol 3,4,5-trisphosphate) is found in neutrophils stimulated by iMet-Let-Phe (20, 21). Platelets also contain PI 3-kinase activity² and form PI-3,4,5-P₃ when stimulated by thrombin (22). PI-3,4-P₂ has been found to appear in a Leydig tumor cell line after stimulation with epidermal growth factor, which acts as a differentiating, rather than mitogenic, factor for these cells (23). These findings suggest that D-3 phosphorylated polyphosphoinositides are also involved in nonmitotic activation of terminally differentiated cells.

Previous work (6, 7, 13, 16) has implicated an 85-kDa phosphoprotein as the PI 3-kinase because of the correlation of its presence with PI kinase activity in middle T antigen and PDGF receptor mutants. This 85-kDa protein is phosphorylated on serine, threonine, and tyrosine in response to PDGF stimulation or middle T antigen transformation (6).

In this paper, we show that the PI 3-kinase purified from rat liver cytosol is a heterodimer of the previously described 85-kDa protein and a 110-kDa protein.

**MATERIALS AND METHODS AND RESULTS**³

85- and 110-kDa Proteins in Purified PI 3-Kinase—SDS-PAGE analysis of fractions from the Mono-S column revealed an 85-kDa protein and a protein doublet at 110 kDa that both peaked with PI 3-kinase activity. A silver-stained SDS-polyacrylamide gel of the middle two fractions from the activity peak of the Mono-S column eluate is shown in Fig. 2 (upper). Early fractions of PI kinase activity peak from the Mono-S column contained primarily the lower 110-kDa protein, whereas the later fractions contained primarily the upper band. PI kinase activity and the amount of 85-kDa protein present with PI 3-kinase activity is correlated with the sum of the amount of protein in the lower and upper 110-kDa bands. In some preparations, trace amounts of protein were seen at 65, 55, and 45 kDa that seem to be proteolytic fragments of the 85-kDa protein (data not shown). Only the 110- and 85-kDa bands reproducibly correlate with PI 3-kinase activity.

To determine whether the 110- and 85-kDa proteins copurified because they are in a complex or simply because they behave similarly through the purification steps, we did two-dimensional gels with native isoelectric focusing in the first dimension and SDS-PAGE in the second dimension. The 110- and 85-kDa proteins focused together under native, but not denaturing, isoelectric focusing conditions, they appear to exist as a complex is provided by cross-linking studies. When the 85-kDa phosphoprotein has been implicated as a component of the PI 3-kinase (Fig. 4), which is consistent with a heterodimer of the 110- and 85-kDa proteins. On silver-stained SDS-PAGE of the gel filtration column fractions, the 110- and 85-kDa proteins co-migrated with PI 3-kinase activity (data not shown).

**Fig. 2. One- and two-dimensional gels of purified PI 3-kinase.** Upper, silver-stained 7.5% SDS-PAGE of two fractions of Mono-S eluate activity peak. Center, silver-stained two-dimensional gel of purified PI 3-kinase. The first dimension was native isoelectric focusing, and the second dimension was SDS-PAGE: b indicates the basic end and a the acidic end of the isoelectric focusing gel. Lower, silver-stained two-dimensional gel of purified PI 3-kinase. The first dimension was isoelectric focusing done under denaturing conditions, and the second dimension was SDS-PAGE. b indicates the basic end and a the acidic end of the isoelectric focusing gel.

in the lower band eluting first from the Mono-S cation-exchange column (Fig. 2, upper). Since the 85- and 110-kDa proteins focused together under native, but not denaturing, isoelectric focusing conditions, they appear to exist as a complex in the native state. Additional evidence that the proteins are a complex is provided by cross-linking studies. When the purified material was incubated with dithiothreitol (succinylamide propionate), a reversible cross-linking agent, a protein complex of 200 kDa was seen when analyzed by one-dimensional SDS-PAGE. When cross-linked material, separated in a first dimension tube gel, was separated in a second dimension slab gel with β-mercaptoethanol to reverse the cross-linking, it was evident that the 85- and 110-kDa proteins were cross-linked in the same complex (Fig. 3). When cross-linking was done on less pure preparations of PI 3-kinase, other proteins present did not cross-link into the complex with the 85- and 110-kDa proteins.

The apparent molecular mass of PI 3-kinase by gel filtration is ~190 kDa (Fig. 4), which is consistent with a heterodimer of the 110- and 85-kDa proteins. On silver-stained SDS-PAGE of the gel filtration column fractions, the 110- and 85-kDa proteins co-migrated with PI 3-kinase activity (data not shown).

**Purified 85-kDa Protein Is the Same 85-kDa Protein That Associates with Middle T Antigen and PDGF Receptor**—An 85-kDa phosphoprotein has been implicated as a component of PI 3-kinase because its presence correlates with PI 3-kinase activity in middle T antigen and PDGF receptor immunopre-
Polyoma Middle T Antigen Specifically Associates with 85-kDa Subunit of Purified PI 3-Kinase.—More dramatic evidence that the 85-kDa subunit of purified PI 3-kinase is the protein previously shown to associate with middle T antigen is provided by a middle T antigen blotting experiment. Cohen et al. (29) previously showed that if middle T antigen, expressed in a baculovirus system along with c-src, is phosphorylated by pp60<sup>-src</sup> and then purified, middle T antigen will form a tight complex with the 85-kDa protein from cell lysates. In addition, if proteins from total cell lysates are separated by SDS-PAGE and transferred to nitrocellulose, <sup>32</sup>P-labeled middle T antigen can be used to blot the proteins, and only an 85-kDa protein is detected. To determine whether the blotted protein is the 85-kDa subunit of PI 3-kinase, this procedure was used with a rat liver homogenate and purified PI 3-kinase. As shown in Fig. 5 (lower), middle T antigen specifically blotted an 85-kDa band in the rat liver homogenate (lane A) that was dramatically enhanced in the purified PI 3-kinase preparation (lane B). This is a remarkable result and indicates that phosphorylated middle T antigen specifically associates with the SDS-denatured 85-kDa protein from cell lysates even though the protein is low in abundance. On some blots of purified PI 3-kinase, weak bands at 65, 55, and 45 kDa were visible. It is likely that these are proteolytic products of the 85-kDa protein. The 110-kDa proteins did not blot, indicating that they are not precursors of the 85-kDa protein.

Characteristics of Enzyme—PI 3-kinase activity was found to be maximal at an Mg<sup>2+</sup> concentration of 5 mM (Fig. 6). Activity using Mn<sup>2+</sup> instead of Mg<sup>2+</sup> was 10% or less of the Mg<sup>2+</sup>-dependent activity. Calcium at physiological concentrations (1–100 μM) had no effect on activity. The pH optimum is broad and centered around 7 (Fig. 7).

The <i>K<sub>m</sub></i><sub>(app)</sub> for ATP is 60 μM when PI is used as the substrate and 30 μM when PI-4-P or PI-4,5-P<sub>2</sub> is used as the substrate (Fig. 8). We also determined the dependence of the reaction on phosphoinositide concentration (keeping the molar ratio of phosphoinositide to PS at 1) (Fig. 9). The <i>K<sub>m</sub></i><sub>(app)</sub> for PI is 80 μM, for PIP is 10 μM, and for PIP<sub>2</sub> is 4 μM. The maximum specific activity (based on the data shown in Fig. 8) for PI 3-kinase activity is 0.8 μmol/mg/min, for PI-4-P kinase activity is 0.13 μmol/mg/min, and for PI-4,5-P<sub>2</sub> kinase activity is 0.22 μmol/mg/min. Table I presents the specific activities for each step of the purification when all three substrates are present simultaneously and also under conditions of maximal specific activity when only one substrate is present.

The effect of phospholipid composition of the liposomes on substrate utilization was further investigated. When all three substrates are present in equimolar amounts with 40% PS as carrier, the relative rate of formation of product is: PIP<sub>2</sub>, 100%; PIP<sub>3</sub>, ~20%; and PIP<sub>4</sub>, ~40%. PI-4-P and PI-4,5-P<sub>2</sub> are very poor substrates when used without a carrier lipid such as PS. Unexpectedly, when crude brain phosphoinositides (PS:PI:PIP:PIP<sub>2</sub> of ~30:30:15:15) were used as substrates, PIP<sub>2</sub> was the preferred substrate (Table I). Crude brain phosphoinositides (Sigma) seem to contain a compound that specifically enhances PIP<sub>2</sub> kinase activity. This phenomenon also seems to be batch-dependent.

Nonionic detergents inhibit PI 3-kinase when present above the critical micellar concentration. When used in concentrations below the critical micellar concentration, there is an activation of the enzyme (Fig. 10). We also examined the effects of other lipids on enzyme activity and found that
addition of phosphatidylcholine, phosphatidic acid, lysophosphatidic acid, or cardiolipin inhibited activity and that diacylglycerol had little effect (Table II). We found minimal inhibition of the enzyme by adenosine at concentrations up to 100 μM, as previously shown (8).

DISCUSSION

PI 3-kinase is a heterodimer of 110- and 85-kDa proteins. We have not been able to identify which subunit has PI kinase activity. We have been unable to separate the two proteins under native conditions and have also been unable to renature activity once they have been separated by SDS-PAGE. The 85-kDa component of the heterodimer is the same 85-kDa protein that is found in association with middle T antigen and the PDGF receptor, based on the blot done with labeled middle T antigen and the identical migration of the proteins on two-dimensional gels (Fig. 5). Previous work (6) has shown that the 85-kDa proteins associated with middle T antigen and the PDGF receptor are the same. Whether the 110-kDa subunit of PI 3-kinase associates with middle T antigen and/or the PDGF receptor along with the 85-kDa subunit is not yet known. Proteins of ~110 kDa have been found to associate with the PDGF receptor, however (13).

The two 110-kDa proteins seem to be very closely related, but not identical proteins. They have similar isoelectric points, and both appear to associate with the 85-kDa protein. They also have similar but not identical protease V8 fragments (data not shown). Protein sequence analysis of protease V8 fragments reveals what seems to be similar, but not identical amino acid sequences, suggesting that the upper and lower 110-kDa bands are products of different genes.

PI 3-kinase is found in the cytosol and apparently is recruited to the membrane when oncogene products are present or receptors are activated (6, 8, 29). Recruitment to the membrane may allow modulation of the enzyme's activity or substrate specificity through protein-protein interactions or phosphorylation. At the membrane, PI 3-kinase would be in close proximity to its substrates.

All other PI kinases which have been purified are membrane-associated, and all phosphorylate PI at the D-4 position of the inositol ring (8, 31–34). The PI 3-kinase which has been purified from human erythrocytes phosphorylates PI-4-P at the D-5 position of the inositol ring (35). These enzymes are in the classic phosphatidylinositol pathway which leads to synthesis of PI-4,5-P2.

The physiologically relevant substrate for PI 3-kinase is not known. Undoubtedly, PI-3-P is produced by this enzyme in vivo. The purified protein phosphorylates PI, PIP, and PIP2 so it is possible that the PI-3,4-P2 and PIP2 found in vivo could be produced by phosphorylation of PI-4-P and PIP2-4,5-P2 at the D-3 position by PI 3-kinase. It is also possible that some or all of the PI-3,4-P2, found in vivo is synthesized by the phosphorylation of PI-3-P at position 4. Three types of PI kinases have been described (8, 31). Type I is PI 3-kinase. Types II and III are PI 4-kinases that can be distinguished by detergent and adenosine sensitivity and a specific inhibitory antibody.**" Type II PI kinase from human red blood cells does not phosphorylate PI-3-P.** 1 This is the predominant PI 4-kinase in most cells, and it seems to function solely in the "classic" PI turnover pathway. Whether there are PI 4-kinases that can phosphorylate PI-3-P remains to be determined. The correlation between the association of PI 3-kinase with the PDGF receptor and the elevation of PI-3,4-P2 and PIP2 (but not PI-3-P) levels in smooth muscle cells suggests that the PDGF receptor enhances the ability of PI 3-kinase to utilize PI-4-P and PI-4,5-P2 as substrates in vivo (17). There is also apparently an inhibitor of PIP and PIP2 kinase activities in crude PI 3-kinase preparations. The presence of an inhibitor of these two activities fits well with a model in which PI-3,4-P2 and PIP2 are the signaling molecules since the inhibitor would provide an additional regulatory point.

The ability of the polyoma middle T protein to associate exclusively with the SDS-denatured 85-kDa subunit is a surprising result. It indicates that phosphorylated middle T antigen has a very high specificity for a domain of the 85-kDa protein which either renatures or has little secondary structure. This remarkable specificity of middle T antigen for the 85-kDa protein further strengthens the evidence from mutation studies that the association of middle T antigen with PI 3-kinase is essential for transformation by the oncogene.

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Supplemental material to: Purification and characterization of phosphoinositide 3-kinase from rat liver.

**Materials and Methods**

**Materials:** Male Sprague-Dawley rats weighing about 250 grams were purchased from Taconic Laboratories. [3H]-Mositol and phosphoinositide standards were purchased from NEN, New England Nuclear, as [3H]-mositol and phosphoinositide standards. 50 mM Hepes, pH 7.2, and 1 mM EDTA, mixed with a vortex apparatus and then sonicated for 5 minutes in a batch sonicator. Assays were done during the purification steps contained 10 μl of sample, 5 mM MgCl2, 50 μM ATP (0.4 μCi/μmol), 0.25 mM EDTA, 20 mM HEPES, pH 7.2 and 200 μM PI in a volume of 25 μl. The assays were run for 5 minutes and then stopped by the addition of 10 μl of 10% trichloroacetic acid. The protein concentrations were measured by the method of Bradford.

**Methods:**

**Sample preparation:** Sample preparation was done as previously described. PI-3-P synthesis also detects PI-4 kinase activity. During the development of the purification scheme the deacylated products were analyzed by HPLC, which will separate gPI-3-P from gH-4-P. PI 3-kineae did not separate from PI 3-kineae at any one step, but spread through many fractions in the first 2 columns, while PI 4-kineae activity formed distinct peaks.

**Purification:**

**Purification of the protein:** Livers were removed from rats after ether anesthesia and exsanguination. The livers were cut into small pieces and homogenized in a Waring blender in 2.5 volumes of a buffer containing 20 mM MOPS, pH 7.4, 2 mM MgCl2, 1 mM EDTA, 1 μg/ml leupeptin and 1 μg/ml pepstatin A. The homogenate was centrifuged at 10,000 g for 15 minutes. The supernatant was then diluted to 0.05 M KCl in sample buffer with p-

**Results:**

**Purification of the enzyme:** We first surveyed extracts from a number of tissues for PI 3-kinase activity and found the highest specific activity in liver and spleen. Cell fractionation studies showed that in liver PI 3-kinase was almost exclusively cytosolic, while in PGH the enzymatic fraction showed that 50% of the PI 3-kinase activity was in the absence of detergent. The detergent-associated PI 3-kinase produced almost exclusively PI-3-P. We therefore used rat liver cytosol as the source for purification of PI 3-kinase.

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**Fig. 1.** Representative column profiles. Columns were run and PI kinase activity was assayed as described in Materials and Methods. The A$_{280}$ was determined using an on-line UV detector. The dotted line indicates the salt gradient; the solid line the A$_{280}$ and the PI kinase activity. A, profile of DEAE-Sepharose fast flow column. The column was run at 1.5 ml/min and 14.5 ml fractions were collected. B, profile of S-Sepharose fast flow column. The column was run at 1.5 ml/min and 7 ml fractions were collected. C, profile of hydroxylapatite HR column. The column was run at 0.25 ml/min and 3 ml fractions were collected. D, profile of Mono-Q column. The column was run at 0.5 ml/min and 1 ml fractions were collected. E, profile of Mono-S column. The column was run at 0.5 ml/min and 1 ml fractions were collected.

**Fig. 4.** Gel filtration of purified PI 3-kinase. A sample of the purified PI 3-kinase was run on a Superose 6 column as described in Materials and Methods. The column was calibrated with apotelin (M=$443,000$), B-amylase (M=200,000), yeast alcohol dehydrogenase (M=150,000), bovine serum albumin (M=66,000) and carbonic anhydrase (M=29,000) as standards.

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Fig. 6. Mg dependence of PI 3-kinase activity. PI ( ), PIP ( ) and PIP2 ( ) kinase activities were measured separately under the standard assay conditions, using phosphoinositide-PS (1:1) as substrate. Each point represents the mean of two or three determinations.

Fig. 7. pH dependence of PI 3-kinase activity. PI ( ), PIP ( ) and PIP2 ( ) kinase activities were measured separately under the standard assay conditions, using phosphoinositide-PS (1:1) as substrate. Each point represents the mean of two or three determinations. The buffer used was MES for pH 5-6.5, HEPES for pH 7-7.5 and Tris for pH 8-9.

Fig. 8. ATP dependence of PI 3-kinase reactions. PI, PIP and PIP2 kinase activities were measured separately under the standard assay conditions, using phosphoinositide-PS (1:1) as substrate. Each point represents the mean of two or three determinations. The standard assay conditions were used with phosphoinositide-PS (1:1) except that the reactions were terminated after 1 minute. Double reciprocal plots of ATP dependence of: A, PI kinase activity; B, PIP kinase activity; C, PIP2 kinase activity.
Phosphoinositide 3-Kinase from Rat Liver

Fig 9. Phospholipid dependence of PI 3-kinase reactions. PI, PIP and PIP2 reactions were measured separately under the standard assay conditions, using phosphoinositide PS (1:1) as substrate. Each point represents the mean of two or three determinations. The standard assay conditions were used with phosphoinositide PS (1:1) except that the reactions were terminated after 1 minute and 200μM ATP was used. Double reciprocal plots of A, PI kinase activity; B, PIP kinase activity; C, PIP2 kinase activity.

**TABLE I**

| Step | Protein (mg) | Density (g/ml) | Volume (ml) | Purification (fold) | Yield (%) |
|------|--------------|----------------|-------------|--------------------|-----------|
| Cyno | 12.450 | 86 | ND | ND | 100 |
| And | 9.2 | 80 | 3.9 | 9.1 | 0.867 |
| Dis| 108 | 51 | 2.0 | 64 | 0.509 |
| E.L. | 106 | 51 | 2.0 | 64 | 0.509 |
| Hydrol | 8.950 | 6.2 | 2.4 | 35.5 | 0.8 |
| Meno- | 8.310 | 6.1 | 1.9 | 73 | 6.5 |
| Meno-5 | 8.090 | 6.2 | 2.9 | 35.6 | 124 (100) |

**TABLE II**

Effect of added lipids on enzyme activity

| Added lipid | PI, PIP, PIP2 | PI, PIP, PIP2, PIP3 | PI, PIP, PIP2, PIP3, PIP4, PIP5 |
|-------------|---------------|---------------------|---------------------------------|
| 1-ASR (BSO) | 100 | 100 | 100 |
| 5% PC | 81 | 80 | 75 |
| 1% PC | 91 | 79 | 75 |
| 5% PA | 56 | 42 | 40 |
| 5% 1,2-PA | 275 | 180 | 108 |
| 5% CASG | 94 | 41 | 40 |
Purification and characterization of phosphoinositide 3-kinase from rat liver.
C L Carpenter, B C Duckworth, K R Auger, B Cohen, B S Schaffhausen and L C Cantley

J. Biol. Chem. 1990, 265:19704-19711.

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