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Evidence from two transformed cell lines that the phosphorylations of peptide tyrosine and phosphatidylinositol are catalyzed by different proteins

(pp60^{src}/pp56/tyrosine kinase/immunoprecipitation)

M. LYNN MACDONALD*, ELIZABETH A. KUENZEL†, JOHN A. GLOMSET*, AND EDWIN G. KREBS†

Departments of *Biochemistry and †Pharmacology, Howard Hughes Medical Institute Laboratories, University of Washington, Seattle, WA 98195

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ABSTRACT **Two transformed rodent cell lines (RS-1 and LSTRA) were studied *in vitro* to determine if their major protein tyrosine kinases catalyzed the phosphorylation of phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate (PtdIns4*P*), or diacylglycerol. RS-1 cells, transformed by Rous sarcoma virus, contain high levels of pp60^{src}; LSTRA cells, transformed by Moloney murine leukemia virus, contain a tyrosine kinase (pp56) that is the product of an unknown cellular gene. Rates of phosphorylation of peptide tyrosine were elevated more than 20-fold in RS-1 and LSTRA particulate fractions compared to fractions from suitable control cells (N2 and YAC-1), but there was not a proportional increase in rates of phosphorylation of PtdIns, PtdIns4*P*, or diacylglycerol. Heat (34°C) completely inactivated the LSTRA tyrosine kinase, while it enhanced the phosphorylation of PtdIns and PtdIns4*P* and had no effect on the phosphorylation of diacylglycerol. PtdIns4*P* inhibited the phosphorylation of PtdIns but had no effect on tyrosine kinase activity. An antibody, raised against a peptide with a sequence homologous to the autophosphorylation site of pp60^{src}, immunoprecipitated tyrosine kinase activity from RS-1 and LSTRA extracts but had no effect on PtdIns kinase or PtdIns4*P* kinase activity. These results provide evidence that the phosphorylations of tyrosine and PtdIns are catalyzed by different proteins. An additional observation was that a monoclonal antibody that binds to pp60^{src} and pp56 removed PtdIns kinase as well as tyrosine kinase activity from RS-1 and LSTRA particulate extracts. This antibody also removed PtdIns kinase from N2 and YAC-1 extracts, in which tyrosine kinase activity was low or undetectable. Thus, the anti-pp60^{src} monoclonal antibody may recognize the PtdIns kinase in addition to pp60^{src} and pp56.**

Protein tyrosine phosphorylation and enhanced metabolism of phosphatidylinositol (PtdIns) are two processes that are thought to have important roles in cell growth and cell transformation (for reviews, see refs. 1–3). The mechanism by which turnover of phosphoinositides is enhanced is not well understood. Recently, Sugimoto *et al.* (4) reported that pp60^{src}, the protein tyrosine kinase encoded by Rous sarcoma virus, phosphorylated PtdIns to form phosphatidylinositol 4-phosphate (PtdIns4*P*) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] and also phosphorylated diacylglycerol (acyl$_2$Gro) to form phosphatidic acid. Similarly, Macara *et al.* (5) found that immunoprecipitates of the protein tyrosine kinase p68^{ros}, the transforming protein of avian sarcoma virus UR2, were associated with PtdIns kinase activity. Both groups suggested that these activities might be significant in enhancing acyl$_2$Gro formation and activating protein kinase C. If the phosphorylation of PtdIns and PtdIns4*P* is a common property of tyrosine kinases, one might expect that the receptors for insulin, epidermal growth factor, and platelet-derived growth factor would also possess this activity, and in this regard Machicao and Wieland (6) reported that a purified insulin receptor from human placenta phosphorylated PtdIns.

We have investigated the possible dual specificities of protein tyrosine kinases in two transformed cell lines: RS-1 and LSTRA. The RS-1 cell line is a rat embryo fibroblast line transformed by the Schmidt–Ruppin D strain of Rous sarcoma virus; these cells express high levels of pp60^{src}, the protein tyrosine kinase encoded by the *src* gene (7). The LSTRA cell line is a mouse lymphoma in which the original transforming agent was the Moloney leukemia virus (8, 9). LSTRA cells contain elevated levels of a protein tyrosine kinase, pp56 (10–13), which is thought to be the product of a cellular gene because the Moloney virus does not encode a protein kinase (8, 9). The amino acid sequence of the autophosphorylation site in pp56 is highly homologous or identical to that in pp60^{src} (10). Recently, Voronova *et al.* (14) reported that another Moloney virus-transformed cell line also has an elevated level of pp56.

In the studies reported here, relative rates of phosphorylation of protein tyrosine kinase substrate and PtdIns or PtdIns-derived substrates were determined in membrane preparations of RS-1 and LSTRA cells and compared to suitable control cells that exhibit no elevation in tyrosine kinase activity. The heat labilities of the LSTRA protein tyrosine kinase and lipid kinases were compared. The effect of a PtdIns kinase inhibitor (PtdIns4*P*) on tyrosine phosphorylation was tested. The effect on PtdIns kinase activity of antibodies that immunoprecipitate pp60^{src} and pp56 was assessed. The results provide evidence that the phosphorylations of PtdIns, PtdIns4*P*, and acyl$_2$Gro are catalyzed by kinases that are distinct from the major protein tyrosine kinases of these cells.

MATERIALS AND METHODS

Materials. The peptide Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly (RR-SRC), which has a sequence based on the autophosphorylation site in pp60^{src}, was synthesized as described (13) and was used as a substrate for protein tyrosine kinase assays. Pig liver PtdIns was from Serdary Research Laboratories (London, ON, Canada). PtdIns4*P*, PtdIns(4,5)P_2, and 1,2-dioleoylglycerol (Ole$_2$Gro) were from Sigma. Mouse monoclonal antibody against pp60^{src} (hybridoma no. 261) (15) was a gift from Joan Brugge (State

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Abbreviations: pp56, phosphoprotein of molecular weight 56,000 from LSTRA cells; pp60^{src}, phosphoprotein product of the Rous sarcoma virus *src* gene; PtdIns, phosphatidylinositol; PtdIns4*P*, phosphatidylinositol 4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; acyl$_2$Gro, diacylglycerol; Ole$_2$Gro, 1,2-dioleoylglycerol; Staph A, *Staphylococcus aureus* absorbent; Mops, 3-(*N*-morpholino)propanesulfonic acid.

University of New York at Stony Brook). Affinity-purified anti-peptide antibody was prepared as described (12) against the synthetic peptide Lys-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly, which contains the sequence around the site of tyrosine phosphorylation in $pp60^{v\text{-}src}$ and pp56. Fixed *Staphylococcus aureus* (Staph A) Cowan I cells, which contain protein A, were from Miles.

Cell Culture. The lymphoma cell lines LSTRA (9) and YAC-1 (16) were maintained in culture as described (11). Normal (N2) and Rous sarcoma virus-transformed (RS-1) fibroblasts were a gift from Byron Gallis of this laboratory. The production, culture, and properties of these cells have been described (7, 17).

Preparation of Cell Particulate Fractions and Detergent Extracts. For particulate fractions, sonicated cell suspensions were sedimented and the pellet was homogenized in cold buffer [40 mM 3-(*N*-morpholino)propanesulfonic acid (Mops), pH 7.0/10% (vol/vol) glycerol/1 mM dithiothreitol] with 20 strokes of the pestle in a Dounce homogenizer. Final concentrations of protein in the particulate fractions were 0.8–2.0 mg/ml. For the comparison of kinase activities in transformed cells and corresponding control cells, protein concentrations of the particulate fractions were made equal. For "detergent extracts," the particulate fraction was homogenized in cold 40 mM Mops, pH 7.0, containing 1 mM dithiothreitol and either 1% Triton X-100 or 1% Triton X-100/1% sodium deoxycholate/0.1% $NaDodSO_4$/150 mM NaCl. The extracts were centrifuged at 100,000 × *g* for 30–60 min to remove insoluble material.

Phosphorylation of Peptide, PtdIns, PtdIns4*P*, and $Acyl_2Gro$. Phosphorylation assays were carried out for 2 min at 32°C in 35–45 μl. A 5-μl aliquot of the cell preparation containing 4–10 μg of protein was the source of enzyme. Other assay constituents were (in final concentrations) 50 mM Mops at pH 7.0, 1 mM dithiothreitol, 150 μM [γ-^{32}P]ATP (600–2800 cpm/pmol), 14 mM $MgCl_2$, 0.14% Triton X-100 (for the phosphorylation of RR-SRC, PtdIns, or PtdIns4*P*) or 1 mM deoxycholate (for the phosphorylation of $acyl_2Gro$), and the following concentrations of substrates: RR-SRC, 2.2 mM; PtdIns, 4 mM; PtdIns4*P*, 1 mM; Ole_2Gro, 1 mM. These conditions gave rates of phosphorylation that were essentially linear with time and with protein concentration. Concentrated solutions of PtdIns and PtdIns4*P* in $CHCl_3$ were dried under nitrogen, resuspended in the appropriate volume of 1.2% Triton X-100, and sonicated for 15 sec in a bath sonicator. For assays of $acyl_2Gro$ kinase, Ole_2Gro was suspended in buffer that contained 1 mM deoxycholate and sonicated with a probe for 5 min on ice. In some experiments, $acyl_2Gro$ kinase activity was estimated from the phosphorylation of endogenous $acyl_2Gro$ in the absence of detergent.

Rates of phosphorylation of peptide were measured by a phosphocellulose paper assay essentially as described (13). Reactions with PtdIns, PtdIns4*P*, or $acyl_2Gro$ were stopped with $CHCl_3/CH_3OH$/12 M HCl (66:33:1, vol/vol). Phases were separated by centrifugation and the lower phase was washed two or three times with $CH_3OH/H_2O/CHCl_3$ (48:47:3, vol/vol). After the addition of carrier lipids (PtdIns, PtdIns4*P*, PtdIns(4,5)P_2, and phosphatidic acid) the washed lower phase was dried and resuspended in $CHCl_3/CH_3OH/H_2O$ (75:25:2, vol/vol). Lipids were separated by cellulose TLC (18). Plates were stained with Nile blue A, bands were excised, and radioactivity was determined by liquid scintillation spectrometry. This procedure gave complete separation of the lipid standards.

Immunoprecipitations. Detergent extracts of particulate fractions were used for immunoprecipitations. Immunoprecipitations with monoclonal antibody (15) and with anti-peptide antibody (12) were conducted essentially as described. Immune complexes were collected by adding Staph A. After the Staph A was sedimented, the supernate was removed and an aliquot was assayed for kinase activities as described above.

RESULTS

Relative Activities of Protein Tyrosine Kinase and Lipid Kinases in Different Cell Lines. If protein tyrosine kinases account for a substantial part of the lipid phosphotransferase activity in cells, one would expect that an increase in the amount of protein tyrosine kinase would be paralleled by a proportionate increase in rates of phosphorylation of lipids. To examine this possibility, rates of phosphorylation of RR-SRC and of lipids were measured in the particulate fractions of two cell lines, RS-1 and LSTRA, that exhibit high levels of $pp60^{src}$ and pp56, respectively. Kinase activities in the RS-1 particulate fraction were compared to those for the untransformed cell line, N2. For LSTRA, the YAC-1 cell line was used as a control.

Table 1 shows that tyrosine kinase activity, measured by the phosphorylation of RR-SRC, was greatly elevated in the particulate fractions of RS-1 and LSTRA cells compared to their respective control cells. For RS-1 cells the particulate fraction had 20 times more tyrosine kinase activity than for N2 cells. The LSTRA particulate fraction also exhibited high levels of tyrosine kinase activity, while this activity was not detectable for YAC-1 cells.

Rates of phosphorylation of PtdIns and PtdIns4*P* were higher for RS-1 and LSTRA cells than for their respective control cells, but the increase was only moderate (50–100%) compared to the 20-fold or greater increase in tyrosine phosphorylation in these cell lines. Therefore, the elevation in protein tyrosine kinase activities in RS-1 and LSTRA cells was not followed by a proportionate increase in PtdIns and PtdIns4*P* kinase activities. $Acyl_2Gro$ kinase activity was higher for RS-1 cells than for N2 cells but was lower for LSTRA cells than for YAC-1 cells. Thus, this enzyme activity also did not follow a pattern consistent with the changes in tyrosine kinase activity.

Heat Lability of the Tyrosine Kinase and Lipid Kinases from LSTRA Cells. To assess whether the tyrosine kinase and lipid kinases had similar properties, the heat lability of the enzyme activities from LSTRA cells was studied. The LSTRA particulate fraction was incubated at 34°C for various times up to 20 min. Assay components were then added and rates of phosphorylation of peptide, PtdIns, PtdIns4*P*, and $acyl_2Gro$ were determined. Fig. 1 shows that incubation of

Table 1. Rates of phosphorylation of peptide, PtdIns, PtdIns4*P*, and $acyl_2Gro$ by particulate fractions of RS-1 and LSTRA cells and control cells (N2 and YAC-1)

Cell line	Phosphate incorporated,* pmol/min per mg of protein			
	Peptide	PtdIns	PtdIns4*P*	Ole_2Gro
RS-1	8062 ± 328†	250 ± 32	1203 ± 48†	27 ± 2†
N2	352 ± 44	188 ± 30	938 ± 59	9 ± 1
LSTRA	5677 ± 272	172 ± 10†	99 ± 2†	33 ± 2†
YAC-1	Undetectable‡	88 ± 1	43 ± 3	120 ± 13

The particulate fraction was incubated for 2 min with 2.2 mM RR-SRC peptide, 4 mM PtdIns, or 1 mM PtdIns4*P* (all in 0.14% Triton X-100) or with 1 mM Ole_2Gro in 1 mM deoxycholate. Assay mixtures contained 14 mM $MgCl_2$, 4–10 μg of protein, and 150 μM [γ-^{32}P]ATP. Protein concentrations of the particulate fractions were 0.8 mg/ml for RS-1 and N2 and 1.9 mg/ml for LSTRA and YAC-1.

*Values are means ± SEM; n = 3 or 4.

†Significantly different from the value obtained for control cells ($P < 0.05$).

‡The amount of ^{32}P bound to phosphocellulose paper when peptide was present was not greater than the amount bound when peptide was absent.

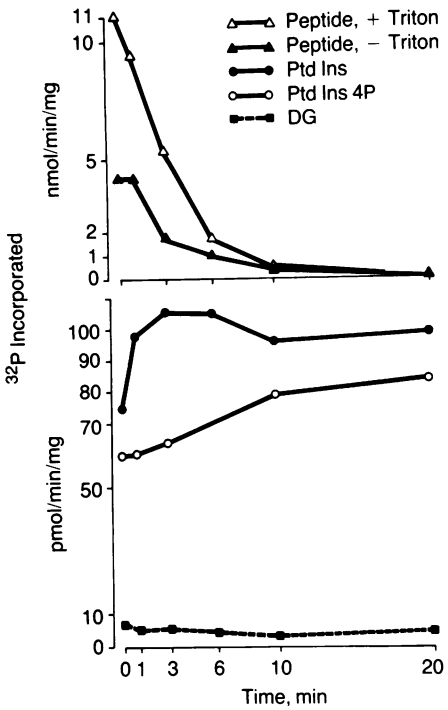

FIG. 1. Effect of mild heat treatment on the phosphorylation of peptide RR-SRC, PtdIns, PtdIns4*P*, and acyl$_2$Gro (DG) by the particulate fraction of LSTRA cells. Tubes containing aliquots of the particulate fraction on ice were transferred to a 34°C water bath and incubated for the times indicated. Assay constituents were then added and phosphorylation was allowed to proceed for 2 min. Assay mixtures contained 2.2 mM peptide, 4 mM PtdIns, or 1 mM PtdIns4*P* (all in 0.14% Triton X-100). Peptide phosphorylation was also measured without Triton X-100 (− Triton) for comparison with the phosphorylation of acyl$_2$Gro. Acyl$_2$Gro phosphorylation was measured without Triton X-100 by the phosphorylation of endogenous acyl$_2$Gro present in the particulate fraction. Units of phosphorylation are nmol or pmol of phosphate incorporated into substrate per min per mg of protein.

the LSTRA particulate fraction at 34°C progressively inactivated the tyrosine kinase. Half-maximal inactivation occurred within 3 min. In contrast, the incorporations of ^{32}P into PtdIns and PtdIns4*P* were enhanced 50–100% by the mild heat treatment, and the phosphorylation of acyl$_2$Gro was unchanged. Thus, complete inactivation of the tyrosine kinase did not adversely affect the rates of phosphorylation of the lipids.

Effect of PtdIns4*P* on the Phosphorylation of PtdIns and of Peptide. PtdIns4*P* was used as an inhibitor (19) to test whether inhibition of the PtdIns kinase would affect tyrosine kinase activity. Table 2 shows that 1 mM PtdIns4*P* completely inhibited the phosphorylation of the endogenous PtdIns in the particulate fraction. With 0.2 mM PtdIns as the added substrate, 1 mM PtdIns4*P* reduced the phosphorylation of PtdIns from 247 pmol/min per mg to 69 pmol/min per mg (72% inhibition). With 2 mM PtdIns4*P* the PtdIns kinase was inhibited by 83%. The inhibition of the PtdIns kinase by PtdIns4*P* could be overcome by increasing the concentration of added PtdIns to 2 mM, indicating that the inhibition was of a competitive type. PtdIns(4,5)P_2 did not inhibit the phosphorylation of PtdIns (although it did inhibit the phosphorylation of PtdIns4*P*; data not shown). It was of interest to determine if the inhibition of PtdIns phosphorylation would have any effect on peptide phosphorylation, as might be expected if these reactions were catalyzed by the same enzyme. Table 1 shows that peptide phosphorylation was unaffected by PtdIns4*P* at a concentration (2 mM) that inhibited the phosphorylation of PtdIns by more than 80%.

Table 2. Effects of PtdIns4*P* and PtdIns(4,5)P_2 on the rates of phosphorylation of PtdIns and of peptide tyrosine by the particulate fraction of LSTRA cells

Substrate	Conc. of added substrate, mM	Other additions	^{32}P incorporated into substrate, pmol/min per mg of protein
PtdIns	0	None	97
	0	1.0 mM PtdIns4*P*	0
	0	1.0 mM PtdIns(4,5)P_2	105
	0.2	None	247
	2.0	None	278
	2.0	2.2 mM peptide	282
	0.2	0.2 mM PtdIns4*P*	103
	0.2	1.0 mM PtdIns4*P*	69
	0.2	2.0 mM PtdIns4*P*	41
	2.0	1.0 mM PtdIns4*P*	385
Peptide	1.1	None	2258
	1.1	4.0 mM PtdIns	2009
	1.1	2.0 mM PtdIns4*P*	2596
	1.1	1.0 mM PtdIns(4,5)P_2	2777

Data are compiled from several experiments. Duplicate assays were performed; values are means. All assay mixtures contained 0.14% Triton X-100. When PtdIns was not added, the ^{32}P incorporated into PtdIns represents the phosphorylation of endogenous PtdIns present in the particulate fraction.

Also, if tyrosine residues and PtdIns were phosphorylated at the same catalytic site of a single protein, one would expect that these substrates would compete for binding and phosphorylation. However, Table 2 shows that there was no apparent competition between PtdIns and RR-SRC for phosphorylation. That is, added PtdIns at a saturating concentration (4 mM) did not affect the phosphorylation of peptide. Similarly, there was no effect of 2.2 mM peptide on the phosphorylation of PtdIns.

Immunoprecipitation of Tyrosine Kinase by Anti-peptide Antibodies. To determine if antibody to the tyrosine kinases also immunoprecipitated PtdIns kinase, experiments were conducted with an antibody that had been prepared against a peptide with a sequence homologous to the autophosphorylation site in pp60^{src} and pp56. The anti-peptide antibody binds to pp56 (12) and to pp60^{src} (L. Rohrschneider, personal communication) and inhibits autophosphorylation and the phosphorylation of exogenous substrates. When Staph A is added, the antigen–antibody complex precipitates (12). Thus, if PtdIns kinase activity resided in the pp60^{src} or pp56 protein chains, the PtdIns kinase would be immunoprecipitated along with the tyrosine kinase. Fig. 2 shows that 85% of the RS-1 tyrosine kinase activity and 100% of the LSTRA tyrosine kinase activity was inhibited by the addition of antibody alone. When Staph A was added and the immune complex was sedimented, no tyrosine kinase activity remained in either the RS-1 or LSTRA particulate extracts. In contrast, PtdIns kinase activity was unaffected by antibody whether Staph A was added or not. Neither was the phosphorylation of PtdIns4*P* affected by antibody (data not shown). Taken together, the results described above strongly suggest that the protein tyrosine kinases of RS-1 and LSTRA cells contribute little if anything to the total phosphorylation of PtdIns, PtdIns4*P*, or acyl$_2$Gro in these cells.

Effect of Monoclonal Antibody on Tyrosine Kinase and PtdIns Kinase Activities. A monoclonal antibody against pp60^{src} was also used to immunoprecipitate the tyrosine kinases from RS-1 and LSTRA particulate extracts. This antibody immunoprecipitates pp60^{src} encoded by the Schmidt-Ruppin D and Prague strains of Rous sarcoma virus and also immunoprecipitates p90^{yes} (15) and pp56 (J. Brugge, personal communication). Fig. 3 shows that 30 μl of mono-

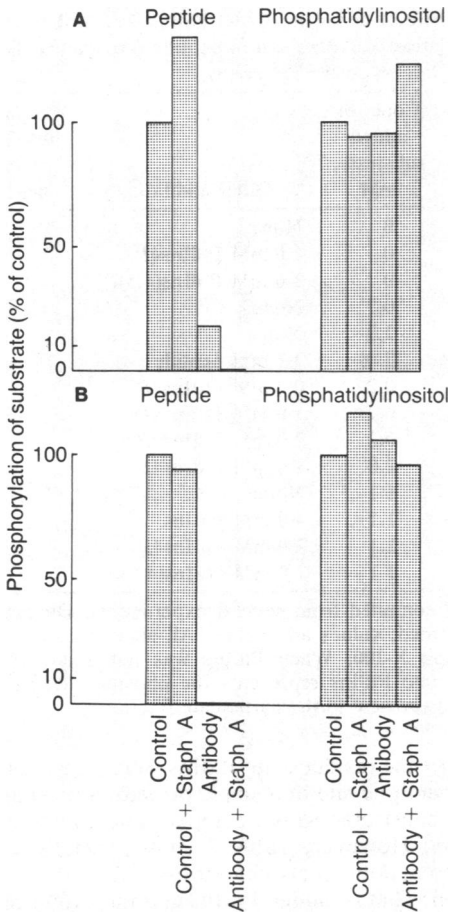

FIG. 2. Effect of anti-peptide antibody on the phosphorylation of peptide and PtdIns. (*A*) RS-1; (*B*) LSTRA. A 30-μl aliquot of detergent extract was incubated with 67 μl of antibody for 2–3 hr. Staph A was added and, after 20 min, immune complexes were sedimented and the supernates were assayed for kinase activities. In the controls, phosphate-buffered saline replaced an equal volume of antibody or Staph A. Assays were performed in duplicate; values are means.

clonal antibody completely removed tyrosine kinase activity from detergent extracts from RS-1 and LSTRA cells. A surprising result was that, in both cell lines, PtdIns kinase activity was also removed and was, in fact, more sensitive to antibody concentration than was tyrosine kinase activity. The same result was obtained whether extracts were prepared in buffer containing Triton X-100 alone or a mixture of Triton X-100, deoxycholate, and $NaDodSO_4$ (data not shown). In contrast, the rate of phosphorylation of PtdIns4*P* was not affected by the antibody. Since all tubes contained anti-mouse IgG and Staph A, the immunoprecipitation of PtdIns kinase appeared to be a specific effect of the antibody.

Because the results shown in Fig. 2 demonstrated that the immunoprecipitation of $pp60^{src}$ and pp56 by anti-peptide antibody does not result in the removal of PtdIns kinase activity from cell extracts, the effect of monoclonal antibody on PtdIns kinase activity cannot be explained simply by coprecipitation of the PtdIns kinase with the tyrosine kinases. An alternative explanation is that the monoclonal antibody binds directly to the PtdIns kinase. If so, it might be expected that the antibody would also immunoprecipitate the PtdIns kinase from cells that had little or no protein tyrosine kinase activity. Therefore, we tested the effect of the antibody in N2 and YAC-1 particulate extracts. Fig. 4 shows that the antibody removed PtdIns kinase activity from these extracts.

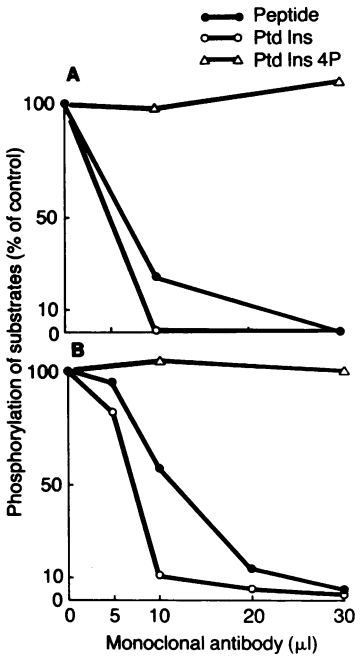

FIG. 3. Effect of monoclonal antibody on the phosphorylation of peptide, PtdIns, and PtdIns4*P*. (*A*) RS-1; (*B*) LSTRA. Detergent extracts of the cell particulate fractions were incubated with monoclonal antibody for 45 min. Volumes of antibody were made to 30 μl by the addition of phosphate-buffered saline. After immunoprecipitation with anti-mouse IgG and Staph A, the supernates were assayed for kinase activities. Immunoprecipitations were performed in duplicate; values are means. Similar results were obtained in two separate experiments.

DISCUSSION

The results of the present studies strongly suggest that protein tyrosine kinases make little or no contribution to the total phosphotransferase activity toward PtdIns, PtdIns4*P*, or $acyl_2Gro$ in RS-1 and LSTRA cells. The increased tyrosine kinase activity in RS-1 and LSTRA cells was not paralleled by a similar increase in the phosphorylation of the lipids. The tyrosine kinase and lipid kinases of LSTRA cells could be distinguished further by their heat labilities. The tyrosine protein kinase was completely inactivated by incubation at 34°C for 20 min, while PtdIns kinase and PtdIns4*P* kinase activities were enhanced by this treatment and $acyl_2Gro$ kinase activity was unchanged. PtdIns4*P* inhibited the phosphorylation of PtdIns but did not affect the phosphorylation

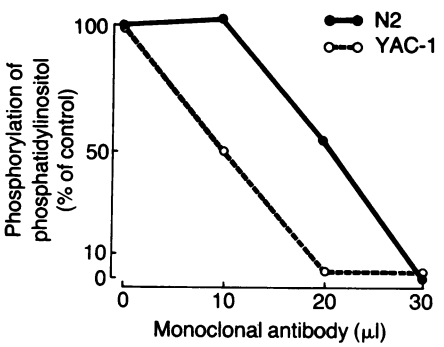

FIG. 4. Effect of monoclonal antibody on the phosphorylation of PtdIns by particulate extracts of control cells (N2 and YAC-1). Immunoprecipitations were performed as described for Fig. 3. Assays were performed in duplicate; values are means. Similar results were obtained in two separate experiments. The antibody had no effect on PtdIns4*P* kinase activity (data not shown).

of peptide. Also, the tyrosine kinase activity in RS-1 cells and LSTRA cells could be abolished by the addition of antibody that binds to the autophosphorylation site of $pp60^{src}$ and pp56, while PtdIns kinase and PtdIns4*P* kinase activities were unaffected. Taken together, these results indicate that the phosphorylations of PtdIns, PtdIns4*P*, and $acyl_2Gro$ in these cells are catalyzed by distinct enzymes that are not associated with the tyrosine kinases. Our studies do not rule out the possibility that purified protein tyrosine kinases might have low phosphotransferase activity toward lipids. However, it should be noted that in the studies by Sugimoto *et al.* (4) and Macara *et al.* (5) the degree of purity of the $pp60^{src}$ and $p68^{ros}$ preparations was not reported. Therefore, an alternative explanation for their findings is that the phosphorylations of lipids were catalyzed by distinct lipid kinases that were present in addition to the protein tyrosine kinases.

We found that a monoclonal antibody to $pp60^{src}$, which also binds to pp56, effectively removed PtdIns kinase activity from detergent lysates from RS-1 and LSTRA cells. The antibody also removed PtdIns kinase activity from extracts from N2 and YAC-1 cells, which have little or no tyrosine kinase activity. Several explanations for this are possible. The apparent antibody effect may be nonspecific, such as a trapping of the PtdIns kinase in the antibody complex, or an effect of a hybridoma protein that is not an antibody. These possibilities can be tested in further studies with appropriate control hybridoma media. If the apparent immunoprecipitation is a specific effect of the antibody, the antibody may bind directly to the PtdIns kinase or, alternatively, the PtdIns kinase may be immunoprecipitated because it is associated with $pp60^{src}$ and with pp56. The latter explanation is untenable because antipeptide antibodies, which also immunoprecipitate $pp60^{src}$ and pp56, did not immunoprecipitate the PtdIns kinase; also, the monoclonal antibody removed PtdIns kinase activity from cell extracts that had little or no tyrosine kinase activity. Therefore, it seems likely that the monoclonal antibody specifically recognizes the PtdIns kinase.

The mechanism by which turnover of PtdIns and PtdIns4*P* is enhanced remains to be established. Although it is generally agreed that phospholipase C is responsible for breaking down the phosphoinositides to form $acyl_2Gro$, the way in which this enzyme is regulated is not known. Also, although the incorporation of ^{32}P into PtdIns4*P* and PtdIns(4,5)P_2 is increased in response to hormones (e.g., see ref. 20), it is not known whether the PtdIns and PtdIns4*P* kinases are directly hormone responsive or whether their role is simply to replete the membranes with PtdIns phosphates as they are broken down. An important step will be to identify the regulated enzymes of PtdIns metabolism so that the possible role of tyrosine kinases in the PtdIns response can be studied.

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