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Phosphorylation of synthetic peptides by a tyrosine protein kinase from the particulate fraction of a lymphoma cell line

(phosphotyrosine/pp60^{src})

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ABSTRACT **The particulate fraction from a lymphoma cell line, LSTRA, was found to contain an apparent high level of tyrosine protein kinase activity. When this fraction was incubated with [γ-^{32}P]ATP in the presence of 10 mM $MnCl_2$, hydrolyzed, and assayed, 70–80% of the radioactivity recovered in phosphoamino acids was in phosphotyrosine. Gel electrophoresis of the proteins showed that a large portion of the ^{32}P was in a single protein with a molecular weight of approximately 58,000. The phosphorylated residue in this protein was identified as phosphotyrosine. Detergent extracts of the particulate fraction from LSTRA cells contained both the M_r 58,000 protein and the enzyme responsible for its phosphorylation. These extracts were found to catalyze the phosphorylation of the tyrosine residue in the synthetic peptide, Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly, corresponding to the sequence around the tyrosine that is phosphorylated in pp60^{src}; the K_m for the peptide in this reaction was 5 mM. High-performance liquid chromatography was used to assay for this phosphorylation. A second peptide was synthesized that contained two additional arginine residues whose presence permitted the phosphorylation of the peptide to be measured by a simple assay using phosphocellulose paper. The K_m for this peptide was 3–4 mM, indicating that the presence of the additional arginine residues did not alter the apparent affinity of the kinase for the peptide.**

Recently, a class of protein kinases has been identified that phosphorylates tyrosine residues in proteins (1–4). This type of reaction appears to be relatively uncommon because in comparison with the amounts of phosphoserine and phosphothreonine, the amount of phosphotyrosine recovered from most cells is quite low (1, 5). Protein kinases that phosphorylate tyrosine residues were initially found associated with transforming proteins of RNA tumor viruses. Further work demonstrated that the protein kinase associated with the epidermal growth factor receptor phosphorylated tyrosine residues (6).

When the amino acid sequence around the site of phosphorylation of the tyrosine residue in the Rous sarcoma virus transforming protein, pp60^{src}, became known (7–9), it was of interest to synthesize a peptide corresponding to this region to determine if it would serve as a substrate. Synthetic peptides have been of great value in elucidating the specificity determinants of substrates for the cyclic AMP-dependent protein kinase (10, 11), and it was hoped that they might also be beneficial for similar studies involving tyrosine protein kinases. Moreover, a synthetic peptide would be more readily available than pp60^{src} itself and might serve as a useful substrate for other enzymological studies.

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In this paper, the synthesis of peptides related to the tyrosine phosphorylation site in pp60^{src} and their phosphorylation by a detergent extract of a particulate fraction from a lymphoma cell line are reported.

MATERIALS AND METHODS

Materials. All serum and cell culture supplements were purchased from GIBCO except sodium pyruvate (Microbiological Associates, Bethesda, MD) and potassium penicillin G (Eli Lilly). Phosphothreonine, phosphoserine, Triton X-100, and Hepes were purchased from Sigma. Phosphotyrosine was synthesized according to Rothberg *et al.* (12). The *t*-butyloxycarbonyl derivatives of amino acids were obtained from Peninsula Laboratories (San Carlos, CA) or Vega Biochemicals (Tucson, AZ).

Cell Culture. Rous sarcoma virus-transformed mouse fibroblasts were kindly provided by L. Rohrschneider (Fred Hutchinson Cancer Research Center, Seattle, WA). They were maintained in minimal essential medium supplemented with 10% heat-inactivated fetal calf serum, potassium penicillin G (100 units/ml), and streptomycin (100 mg/ml). The Moloney lymphoma cell line LSTRA was originally induced in BALB/c mice by the Moloney murine leukemia virus (13) and maintained in culture as described (14). Cells were washed once with phosphate-buffered saline (pH 7.2) before homogenization.

Preparation of Cell Fractions and Extracts. Approximately 4×10^7 cells were suspended in 4 ml of cold 5 mM Hepes, pH 7.4/1 mM $MgCl_2$/5 mM 2-mercaptoethanol. After a 10-min incubation on ice, the cells were broken open with 20 strokes of the A pestle in a Wheaton Dounce homogenizer. Sucrose was then added to a final concentration of 0.25 M. The broken cell suspensions were centrifuged for 30 sec at 1300 × *g* at 2°C in order to remove nuclei. The supernatants were removed and centrifuged at 300,000 × *g* for 45 min at 2°C. The pellets were resuspended in 0.3 ml of 25 mM Hepes, pH 7.4/5 mM 2-mercaptoethanol. To prepare detergent extracts of this fraction, Triton X-100 was added to a final concentration of 1% and the material was centrifuged at 4°C for 5 min in a Microfuge. The supernatant was collected and the pellet was discarded.

Peptide Synthesis. Solid-phase peptide synthesis was carried out with the Beckman 990B automated instrument as described (15). Glutamine and asparagine residues were protected with the xanthyl group, and tyrosine was protected as the dichlorobenzyl ether. Peptides were cleaved from the resin and deprotected by incubating the resin in 75% HF/25% anisole for 30 min at 0°C (16). The peptide Ile-Glu-Asp-Asn-Glu-Tyr-Thr-

Abbreviations: SRC-peptide, Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gln-Gly; R-R-SRC-peptide, Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly; HPLC, high-performance liquid chromatography; p58, M_r 58,000 protein.

Ala-Arg-Gln-Gly (SRC-peptide) was purified on DEAE-Sephadex with a linear gradient of 0.1–1.0 M ammonium acetate (pH 7.0). The peptide Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly (R-R-SRC-peptide) was purified on SP-Sephadex with a gradient of 0.1 M ammonium acetate at pH 3.2 to 0.75 M ammonium acetate at pH 9.2. The peptides were desalted by chromatography in 30% acetic acid on Sephadex G-10. The compositions of the peptides were confirmed by amino acid analysis and sequence determination.

Phosphorylation Reactions. Protein phosphorylations were carried out in a volume of 50 μl containing 20–40 μCi of [γ-^{32}P]ATP (2000–4000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), 10 mM $MnCl_2$ or 10 mM $MgCl_2$, 12.5 mM Hepes (pH 7.4), 2.5 mM 2-mercaptoethanol, and 40–80 μg of sample protein. Reactions were incubated for 15 sec at 30°C. For phosphoamino acid analysis, the reactions were terminated by the addition of 10 μl of a solution of 100% trichloroacetic acid. For gel electrophoresis, the reactions were stopped by the addition of 30 μl of 2.5% $NaDodSO_4$/10% 2-mercaptoethanol/0.75 M sucrose/0.025 M Tris, pH 8.0, containing pyronin Y at 2.5 mg/ml. The samples were then placed in a boiling water bath for 5 min.

Peptide phosphorylation reactions were performed in 25 μl containing approximately 25 μg of protein, 50 μM [γ^{32}P]ATP (2–5 Ci/mmol), 10 mM $MnCl_2$ or $MgCl_2$, and the indicated concentration of peptide. Reactions with the SRC-peptide were stopped by the addition of 10 μl of 100% trichloroacetic acid and the protein was pelleted by centrifugation. The protein pellet was washed with 100 μl of 10% trichloroacetic acid and the supernatants were combined. The phosphorylation of the SRC-peptide was assayed by high-performance liquid chromatography (HPLC) on a reverse-phase C_{18} column with 0.48% NaH_2PO_4 (pH 3.0) as the aqueous buffer and acetonitrile as the organic solvent. The trichloroacetic acid supernatant from the reaction was added to 1.5 ml of 4.8% NaH_2PO_4 (pH 3.0) and injected onto the column. The column was washed with 20 ml of aqueous buffer to elute the radioactive ATP and P_i. The peptide was then eluted with a linear gradient of 0–50% acetonitrile over 20 min at 1 ml/min. Kinase reactions with the R-R-SRC-peptide were stopped by the addition of 155 μl of 3.2% trichloroacetic acid. A 20-μl portion of bovine gamma globulin (10 mg/ml) was added and the samples were incubated for 30 min in an ice-water bath. The protein was pelleted and the R-R-SRC peptide phosphorylation was assayed by applying 50 μl of the acid supernatant onto phosphocellulose paper and washing the paper with solutions of acetic acid (17).

To prepare phosphorylated peptide for sequence determination and phosphoamino acid analysis, the samples recovered from the C_{18} column were lyophilized and desalted on the C_{18} column with 1.3% formic acid (pH 2.1) as the aqueous buffer. The peptide was eluted by using a gradient of 0–100% acetonitrile at 1 ml/min over 10 min. Sequence analysis of the phosphorylated peptide was carried out as described (18).

Analysis of Phosphoamino Acids. The radioactive material was added to 1 ml of 5.7 M HCl containing 50 μg each of authentic phosphoserine, phosphothreonine, and phosphotyrosine. The samples were sealed under vacuum, heated for 2 hr at 110°C, and lyophilized. The resultant material was redissolved in 10–20 μl of H_2O and analyzed by one-dimensional paper electrophoresis at pH 3.6 (3000 V, 45 min). The standards were localized by ninhydrin staining. To quantify the results, stained spots were cut from the paper and placed in a scintillation vial, the ninhydrin stain was destroyed by heating at 60°C with 0.5 ml of 6% H_2O_2, 10 ml of scintillation fluid was added, and the samples were assayed for radioactivity.

Gel Electrophoresis. Samples were analyzed on 6–12% gradient gels as described by Maizel (19). Molecular weights were determined by using phosphorylase *b* (92,000), bovine serum albumin (68,000), ovalbumin (44,000), and chymotrypsinogen (25,000) as standards. For quantitative experiments, the gel band was cut out and dissolved by incubation at 60°C with 30% H_2O_2 for 12 hr. The resultant material was then dissolved in 0.5 ml of H_2O, and then 10 ml of scintillation fluid was added. Proteins were extracted from dried gels for phosphoamino acid analysis as described (20).

RESULTS

Tyrosine Protein Kinase Activity from LSTRA Cells. Preliminary experiments with the particulate fraction from LSTRA cells indicated that this fraction contained a relatively high amount of tyrosine protein kinase activity. The fraction was incubated with [γ-^{32}P]ATP in the presence of 10 mM $MnCl_2$ and then hydrolyzed and analyzed for the formation of [^{32}P]phosphotyrosine. Under these conditions, 70–80% of the radioactivity recovered in phosphoamino acids was in phosphotyrosine. This experiment was then repeated with particulate fractions from both the LSTRA cells and Rous sarcoma virus-transformed cells. The fraction from the LSTRA cells incorporated 8–10 times more radioactivity into phosphotyrosine than did the particulate fraction from the transformed cells (data not shown).

The proteins in the fraction from LSTRA cells that were labeled with ^{32}P during the phosphorylation reaction were examined by gel electrophoresis. A large percentage of the total radioactivity incorporated was present in a single protein with a molecular weight of 58,000 (p58) (average of several determinations with a range of values from 55,000 to 59,000) (Fig. 1). This protein was the most heavily labeled protein when either Mg^{2+} or Mn^{2+} was used in the reaction mixture although, with the assay conditions used to obtain the results illustrated in Fig. 1, approximately 5-fold more radioactivity was incorporated with Mn^{2+}. This effect of Mn^{2+} was not specific to the phosphorylation of p58 because other proteins were also labeled more heavily in the presence of Mn^{2+} than Mg^{2+}.

p58 was eluted from the gel and partially acid hydrolyzed to identify the phosphorylated amino acid residues. All the radio-

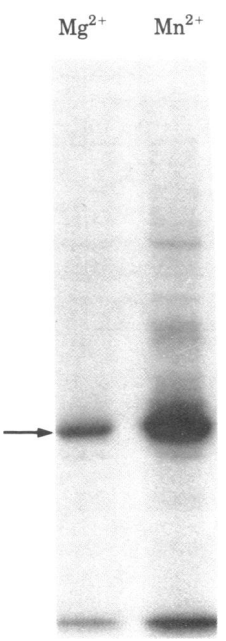

FIG. 1. Autoradiogram showing the proteins phosphorylated in the particulate fraction from LSTRA cells. The phosphorylation reaction was carried out for 15 sec with 53 μg of protein and 0.2 μM [γ-^{32}]ATP (3600 Ci/mmol) in the presence of 10 mM $MgCl_2$ or 10 mM $MnCl_2$. The samples were dissolved in $NaDodSO_4$ solution and subjected to gel electrophoresis. The gel was stained, dried, and autoradiographed by exposing the film to the gel for 30 min. Arrow, location of p58.

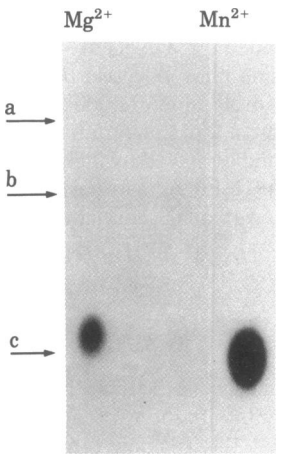

FIG. 2. Analysis of the phosphorylated amino acids present in p58 (the phosphorylation was performed as in Fig. 1) eluted from gels and hydrolyzed in 5.7 M HCl for 2 hr at 110°C. The samples were then analyzed for the ^{32}P-labeled amino acids by paper electrophoresis. Arrows indicate the location of standards: a, phosphoserine; b, phosphothreonine; c, phosphotyrosine.

activity in the protein that was recovered in phosphoamino acid was in phosphotyrosine (Fig. 2). Thus, p58 was the major endogenous substrate protein for the tyrosine protein kinase activity in this particulate fraction.

The time course of the phosphorylation of p58 was examined at a higher initial concentration of ATP (28 μM) than was used in the experiment of Fig. 1 (0.2 μM) in order to investigate the observed stimulation of this reaction by $MnCl_2$. With higher concentrations of ATP, the initial velocity of the phosphorylation reaction in the presence of $MgCl_2$ was actually higher than that seen when $MnCl_2$ was used in the assay but, as the ATP in the assay was depleted, the labeled p58 underwent dephosphorylation in the presence of $MgCl_2$ (Fig. 3). However, when $MnCl_2$ was present the radioactivity in the p58 was not lost as the concentration of ATP declined. These results indicate that the stimulatory effect seen in Fig. 1 with $MnCl_2$ at low ATP concentrations was probably not due to the tyrosine protein kinase having a higher V_{max} with MnATP than with MgATP. The data suggest that this enzyme either has a much lower K_m for ATP in the presence of $MnCl_2$ or, more likely, that the $MnCl_2$ was inhibiting an endogenous phosphoprotein phosphatase that dephosphorylates p58.

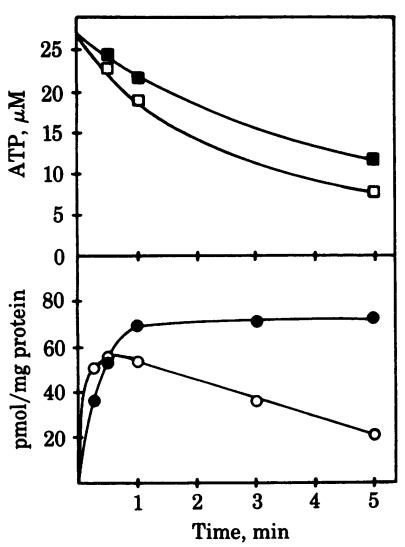

FIG. 3. Time course of phosphate incorporation (○, ●) into p58 and ATP degradation (□, ■) in the particulate fraction from LSTRA in the presence of 10 mM $MgCl_2$ (○, □) or 10 mM $MnCl_2$ (●, ■). Each assay tube contained 34 μg of protein and 28 μM ATP (8 Ci/mmol) in 50 μl. The phosphate in the p58 was quantified by cutting the labeled protein from the gel and analyzing for radioactivity. For the measurement of ATP degradation, the assay reactions were terminated by the addition of 10 μl of a solution of 100% trichloroacetic acid. The precipitated protein was removed by centrifugation and the supernatants were analyzed for the loss of radioactivity capable of binding to charcoal (21).

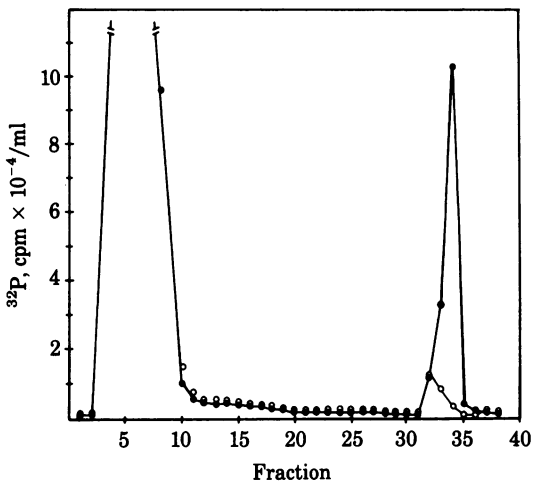

FIG. 4. HPLC assay for the phosphorylation of the SRC-peptide by Triton X-100 extracts of the particulate fraction from LSTRA cells. Reaction mixtures (25 μl) were incubated for 5 min in the presence of 50 μM [γ-^{32}]ATP (2.3 Ci/mmol), 10 mM $MnCl_2$, and 26 μg of protein in the absence (○) or presence (●) of 2.5 mM SRC-peptide. The protein was precipitated with trichloroacetic acid and the soluble material was applied to a C_{18} column. The column was washed with the aqueous phosphate buffer to remove $^{32}P_i$ and [γ-^{32}]ATP, which did not stick to the column. Starting with the fraction 20, the column was then eluted with a gradient of acetonitrile to release any phosphorylated peptide. Fraction volume, 1 ml.

When the particulate fraction from the LSTRA cells was treated with 1% Triton and the nonsolubilized material was removed by centrifugation, phosphorylation of p58 was observed in the detergent extract (data not shown). Thus, these extracts contained both p58 and the enzyme that phosphorylates it.

Phosphorylation of SRC-Peptide by Triton X-100 Extracts of the Particulate Fraction from LSTRA Cells. Triton X-100 extracts of the particulate fraction from LSTRA cells not only contained p58 and the enzyme(s) that would phosphorylate it but also catalyzed the phosphorylation of the SRC-peptide. In a typical experiment, the SRC-peptide was incubated with the extract in the presence of 10 mM $MnCl_2$ and 50 μM [γ-^{32}]ATP. After precipitation of the protein with trichloroacetic acid, the soluble material was analyzed by HPLC for the formation of ^{32}P-labeled peptide. Incubation of the extract with the SRC-peptide resulted in the formation of a component that appeared as a new

Table 1. Distribution of radioactivity after partial acid hydrolysis of ^{32}P-labeled SRC-peptide

Product	cpm	% of total
P_i	5260	53
Phosphothreonine	370	3.7
Phosphotyrosine	3350	34
Incompletely digested peptides	970	9.7

The phosphorylated peptide was isolated by HPLC. A total of 10,000 cpm from the acid hydrolysate was applied to the paper.

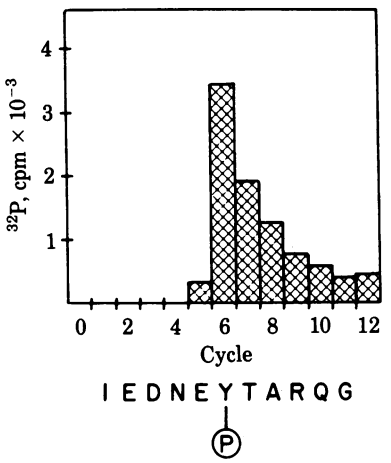

FIG. 5. Sequence analysis of ^{32}P-labeled SRC-peptide. The peptide was phosphorylated and purified by HPLC as in Fig. 4. A total of 77,000 cpm was applied to the cup in the presence of 173 nmol of apomyoglobin. Of the total cpm in the cup, 4.5% were released at the sixth cycle of the Edman degradation. The sequence of the peptide is given by the one-letter code for amino acid residues.

peak of radioactivity in the HPLC chromatogram (Fig. 4), suggesting that the peptide was being phosphorylated by a kinase present in the Triton extract.

The peak of phosphorylated material obtained from the C_{18} column was analyzed for the presence of [^{32}P]phosphotyrosine. Although a small amount of radioactivity was found in phosphothreonine, the majority of the ^{32}P recovered in phosphoamino acids was in phosphotyrosine (Table 1). The small peak of radioactivity that appears on the chromatogram in Fig. 4 in the absence of peptide yielded only $^{32}P_i$ upon acid hydrolysis. It appears to be a minor contaminant of the [γ-^{32}P]ATP. The result of this experiment strongly indicates that the detergent-extracted material from the LSTRA cells contained an enzyme that would phosphorylate the tyrosine residue of the SRC-peptide. The ^{32}P-labeled material was then subjected to sequential Edman degradation. A peak of radioactivity was released after the sixth cycle of the Edman degradation (Fig. 5), consistent with the ^{32}P-labeled material being SRC-peptide phosphorylated on the tyrosine residue.

The time course of the phosphorylation of SRC-peptide is shown in Fig. 6. Even in the presence of 10 mM $MnCl_2$ and 50 μM ATP the reaction was linear for less than 3 min. After 5 min

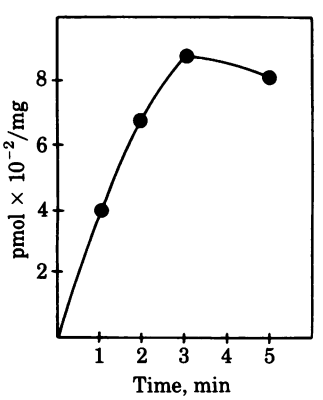

FIG. 6. Time course of the phosphorylation of the SRC-peptide. Phosphorylation of the peptide was followed using the HPLC assay as described in the legend to Fig. 4. The incubation was performed with 25 μg of protein and 2.5 mM peptide in the presence of 10 mM $MnCl_2$ and 50 μM [γ-^{32}P]ATP.

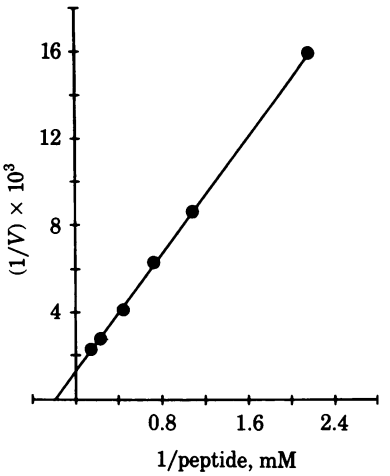

FIG. 7. Apparent K_m for the phosphorylation of the SRC-peptide by the tyrosine protein kinase activity present in the Triton X-100 extracts from LSTRA cells. The incubations were carried out for 2 min with 24 μg of protein and 10 mM $MnCl_2$. V is in pmol/min per mg.

the amount of phosphate incorporated into the peptide had begun to decline. Based on this type of experiment, it was arbitrarily decided to use a 2-min time point in measuring enzyme activity with this peptide. This was chosen as a compromise between linearity and the necessity to incorporate a sufficient amount of radioactivity above background. The result of an experiment in which an apparent K_m for the peptide substrate was determined is shown in Fig. 7; the linear double-reciprocal plot gave a K_m of 5 mM.

Phosphorylation of R-R-SRC-Peptide. In order to develop a simple assay in which phosphocellulose ion exchange paper could be used to separate the ^{32}P-labeled peptide from [^{32}P]ATP and $^{32}P_i$ (17) a second, more basic, peptide was synthesized. This was done by extending the peptide on the amino-terminal side by two amino acids to include a leucine and another arginine residue, which occur in the natural sequence (9), and then adding still one additional arginine residue at the amino terminus. In addition, the threonine residue was replaced by an alanine so that the tyrosine would be the only residue that could act as a phosphate acceptor. Finally, in order to simplify the synthesis and improve yields, the asparagine residue was also replaced by an alanine and the glutamine near the carboxyl terminus was omitted. Thus, this new peptide had the sequence Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly.

The second peptide, like SRC-peptide, was phosphorylated by the Triton X-100 extracts of the particulate fraction from LSTRA cells (Table 2). The phosphorylated peptide was retained

Table 2. Phosphorylation of R-R-SRC-peptide

Conditions	Metal	cpm
No peptide	Mg^{2+}	6,780
2.8 mM R-R-SRC	Mg^{2+}	93,257
No peptide	Mn^{2+}	5,364
2.8 mM R-R-SRC	Mn^{2+}	40,145

Incubations were carried out for 30 sec with Mg^{2+} and for 1 min with Mn^{2+}. The assay mixture contained 22.5 μg of detergent-solubilized protein and 50 μM [γ-^{32}P]ATP (4.5 Ci/mmol). After precipitation of the protein with trichloroacetic acid, one-fourth of the supernatant was applied to a piece of phosphocellulose paper (2 × 2 cm) which was then washed with acetic acid. The results are the average of duplicate determinations and were confirmed by analyzing aliquots from the trichloroacetic acid supernatants for phosphorylated peptide by HPLC as described for the SRC-peptide.

Table 3. Comparison of kinetic constants for phosphorylation of SRC-peptide and R-R-SRC peptide

Peptide	Metal	K_m, mM	V_{max},† nmol/min/mg
SRC	Mn^{2+}	5.0	0.62
R-R-SRC	Mn^{2+}	2.8	1.4
R-R-SRC	Mg^{2+}	4.4	7.9

Peptides were assayed with 22-24 μg of Triton-extracted protein in the presence of 50 μM [γ-^{32}P]ATP and 10 mM metal ion. The assay time for R-R-SRC was 1 min with Mn^{2+} and 30 sec with Mg^{2+}. The data for the SRC-peptide are from Fig. 7.

† Only the two V_{max} values obtained with R-R-SRC are comparable because they were obtained with the same preparation of Triton extract.

quantitatively (data not shown) on the phosphocellulose ion exchange paper, providing a convenient assay system. An apparent K_m for the phosphorylation of the R-R-SRC peptide was determined with either Mg^{2+} or Mn^{2+} in the incubation mixture. The reactions with Mg^{2+} present were incubated for shorter times (30 sec as opposed to 1–2 min) because with this ion the reaction is linear for even less time than with Mn^{2+} due to the apparent rapid dephosphorylation of the peptide that occurs after 2–3 min of incubation in the presence of Mg^{2+}. The apparent K_m for the R-R-SRC-peptide was 3–4 mM (Table 3), indicating that the changes in the structure of this peptide from the SRC-peptide had little effect on the apparent affinity of the kinase for the peptide. In addition, in these crude extracts the kinase that is responsible for the phosphorylation of these peptides had about 5-fold greater activity (V_{max}) in the presence of Mg^{2+} than in the presence of Mn^{2+}.

DISCUSSION

The particulate fraction from the cell line utilized in this study has a high level of tyrosine protein kinase activity and an endogenous protein substrate with a molecular weight of 58,000. Based on the results in Fig. 3 it can be calculated that this protein constitutes 0.4% of the total protein in the crude particulate fraction if it is assumed that the protein is phosphorylated at only one site. The LSTRA cell line was originally obtained from a mouse infected with Moloney murine leukemia virus. However, it is not possible to ascribe the high level of the p58 to this agent alone because the cell line has undergone countless passages since its initial isolation. Moreover, it has been reported that cells transformed with this virus do not have increased levels of phosphotyrosine (22).

The tyrosine protein kinase present in the detergent extract from the LSTRA cells readily phosphorylated peptides with sequence based on the site of tyrosine phosphorylation in pp60^{src}. In terms of their apparent K_m values, these peptides are poor substrates compared to peptide substrates used in studying the cyclic AMP-dependent protein kinase. However, this provides no indication as to how effectively the enzyme in this extract would phosphorylate pp60^{src}. Thus, these results do not provide any direct insight into the controversy concerning the identity of the enzyme responsible for the phosphorylation of the tyrosine residue in pp60^{src} (4, 23). In spite of the high K_m, these peptides should prove useful in an investigation of the specificities of various tyrosine protein kinases. It is important to note that one difficulty in studying the phosphorylation of these peptides in crude extracts is the presence of apparently high amounts of phosphatase activity (24). Even in the presence of 10 mM Mn^{2+}, conditions in which phosphatase activity was less than that seen in the presence of 10 mM Mg^{2+}, the phosphorylated peptide was subject to some action by endogenous phosphatases. Thus, the peptide phosphorylation was found to be linear for only a short period of time, making it difficult to perform rigorous K_m and V_{max} determinations. Hence, it should be emphasized that the kinetic constants estimated here must be viewed as preliminary in view of the fact that competing reactions are present in the crude extracts.

The p58 in the particulate fraction from LSTRA cells may be related to pp60^{src}. In addition to the closeness in their molecular weights, both proteins are phosphorylated on tyrosine residues.

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