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Characterization of sites for tyrosine phosphorylation in the transforming protein of Rous sarcoma virus (pp60$^{v\text{-}src}$) and its normal cellular homologue (pp60$^{c\text{-}src}$)

(oncogene/tumor virus/protein kinase/phosphotyrosine/*src* gene)

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ABSTRACT **The transforming protein of Rous sarcoma virus (pp60$^{v\text{-}src}$) and its normal cellular homologue (pp60$^{c\text{-}src}$) appear to be protein kinases that phosphorylate tyrosine in a variety of protein substrates. In addition, pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ are themselves phosphorylated on serine and tyrosine. It is likely that these phosphorylations serve to regulate the function(s) of pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. We have therefore characterized the sites of tyrosine phosphorylation in the two proteins. Tyrosine phosphorylation of pp60$^{v\text{-}src}$ in infected cells occurs mainly (if not entirely) at residue 419 in the deduced amino acid sequence of the protein. Surrounding this residue is the sequence Leu-Ile-Glu-Asp-Asn-Glu-Tyr(*P*)-Thr-Ala-Arg. This peptide is distinguished by the fact that three out of the four amino acids that precede the phosphorylated tyrosine are acidic in nature. These results define what may prove to be a widely used site for tyrosine phosphorylation in the regulation of cellular function. The same site was phosphorylated when partially purified pp60$^{v\text{-}src}$ was used in a phosphotransfer reaction *in vitro*. The results with pp60$^{c\text{-}src}$ were more complex. The site of tyrosine phosphorylation *in vitro* appeared to be the same as that found in pp60$^{v\text{-}src}$. By contrast, phosphorylation of pp60$^{c\text{-}src}$ *in vivo* apparently occurred at a different, and currently unidentified, tyrosine residue. It is therefore possible that pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ respond differently to regulatory influences in the intact cell.**

The transforming gene (v-*src*) of Rous sarcoma virus (RSV) encodes a 60,000-dalton protein (pp60$^{v\text{-}src}$) that is capable of phosphorylating tyrosine in a variety of protein substrates (1–7). Uninfected vertebrate cells contain a similar protein [pp60$^{c\text{-}src}$ (8–12)]. Both pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ are phosphorylated on serine and tyrosine; these phosphorylations may regulate the enzymatic activity of the proteins. Phosphorylation of cellular proteins by pp60$^{v\text{-}src}$ presumably plays a major role in the establishment and maintenance of the neoplastic phenotype induced by infection with RSV. It is therefore important to explore all of the means by which the action of this protein might be controlled.

We have characterized and compared the sites of tyrosine phosphorylation in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. Phosphorylation of the proteins was carried out in two ways: by metabolic labeling of cells in culture (4) and by phosphotransfer *in vitro*, using partially purified preparations of pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ (13, 14). Our strategy exploited the availability of a predicted amino acid sequence for the viral protein (15). Tryptic peptides containing phosphotyrosine were isolated by high-performance liquid chromatography (HPLC) and then subjected to sequential Edman degradation to locate the phosphorylated residue in the peptide. The results indicate that phosphorylation of tyrosine in pp60$^{v\text{-}src}$ occurs on the same amino acid residue either *in vitro* or *in vivo* and an apparently identical site is also phosphorylated in pp60$^{c\text{-}src}$ *in vitro*. By contrast, phosphorylation of pp60$^{c\text{-}src}$ *in vivo* apparently occurs at a different site in the protein (16, 17). The significance of this difference remains to be elucidated.

MATERIALS AND METHODS

General Procedures. We have described our procedures for the propagation and isotopic labeling of cultured cells, the preparation of antisera from rabbits bearing tumors induced by the Schmidt–Ruppin strain of RSV, the immunoprecipitation of virus-specific proteins, partial proteolysis by staphylococcus V8 protease, the assay of the protein kinase associated with pp60^{src}, the purification of pp60$^{v\text{-}src}$ by immunoaffinity chromatography, and the fractionation of proteins by electrophoresis in polyacrylamide gels (4, 9, 13, 14). For metabolic labeling of pp60$^{v\text{-}src}$, chicken embryo fibroblasts were infected with a virus stock that was generated by transfection with cloned SRA-2 DNA (18).

Hydrolysis of Proteins with Trypsin. ^{32}P-Labeled polypeptides were visualized by autoradiography of dried gels, excised, oxidized with performic acid, and digested with *N*-tosylphenylalanine chloromethyl ketone-trypsin while still in the gel slice, as described by Smart and Ito (19).

Reverse-Phase Chromatography of Tryptic Peptides. Peptides were dissolved for ≈10 min in 100 μl of 90% formic acid, and the mixtures were then diluted with 400 μl of H_2O. The sample was applied to a 4.6 mm × 25 cm Spherisorb ODS (C18) reverse-phase column (Spectra-Physics). The column was run at 40°C and a flow rate of 1.4 ml/min. After injection of the sample, the column was washed for 5 min with 4.5% formic acid (PSC reagent, Baker) and then developed with a linear gradient from 0–62.5% ethanol (Photrex reagent, Baker) in 4.5% formic acid over a period of 95 min on a Spectra-Physics SP8000 HPLC system. Fractions of 0.7 ml (0.5 min) were collected for a total of 200 fractions. Appropriate aliquots were taken from each fraction and mixed with 6 vol of Aquasol (New England Nuclear) for assaying in a liquid scintillation counter. Radioactive peaks were pooled, frozen, and lyophilized. Two milligrams of apomyoglobin was added (before freezing and lyophilization) to peptides that were to be sequenced.

Peptide Sequence Analysis. ^{32}P-Labeled peptides in 2 mg of apomyoglobin were dissolved for ≈10 min in 100 μl of 90% formic acid, and the mixtures were then diluted with 400 μl of H_2O. A 50-μl aliquot was taken for assaying, and the remainder

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Abbreviations: RSV, Rous sarcoma virus; HPLC, high-performance liquid chromatography; v-*src*, transforming gene of RSV; pp60$^{v\text{-}src}$, transforming protein of RSV; pp60$^{c\text{-}src}$, cellular homologue of pp60$^{v\text{-}src}$; >PhNCS, phenylthiohydantoin.

was applied to the spinning cup of a Beckman 890C sequencer. Polybrene (3 mg; Sigma) was applied with the sample to reduce sample washout. Sequence analysis was performed using a 0.1 M Quadrol buffer program (Beckman 030176 and 121078 for the 890C modified with a cold trap). Conversion of the anilinothiazolinone residues was performed immediately on a Sequemat P-6 auto-converter. The resulting phenylthiohydantoin (>PhNCS) derivatives were evaporated to dryness in a Speed Vac Concentrator (Savant). The >PhNCS derivatives were then dissolved in 200 μl of H_2O and 2 ml of Aquasol for assaying in a liquid scintillation counter. To ascertain that the sequencer had functioned properly, 10-μl aliquots of the >PhNCS derivatives at cleavage cycles 1 (valine), 2 (leucine), 9 (leucine), 10 (valine), 11 (leucine), 13 (valine), 17 (valine), and 21 (valine) (values in parentheses are the expected >PhNCS derivatives of apomyoglobin) were chromatographed on a 5-μm Ultrasphere-ODS (Beckman) 4.6 mm × 2.5 cm reverse-phase column as described (20). A zero cycle was included in each run by omitting the heptafluorobutyric acid cleavage step during the first programmed cycle. At the end of the desired number of cycles, the sample remaining in the cup was dissolved in 1 ml of acetic acid and removed from the cup. The cup was washed with an additional 1 ml of acetic acid, which was combined with the first wash. Six milliliters of Aquasol was added, and the sample was assayed in a liquid scintillation counter to determine the percentage of radioactivity remaining in the cup.

RESULTS

Preparation of Phosphorylated pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. Both pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ can be recovered from cellular extracts by immunoprecipitation with antisera raised in newborn rabbits (1). The immunoprecipitated proteins can then be resolved by electrophoresis in polyacrylamide gels and eluted from gel slices for further analysis. Alternatively, the two proteins can be partially purified by adsorption to affinity columns constructed with rabbit antisera directed against pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ (ref. 13; unpublished). When the partially purified proteins are allowed to react with [γ-^{32}P]ATP *in vitro*, tyrosine residues are labeled by phosphorylation (6). Representative results of these procedures have been reported before and are not repeated here.

Localization of the Phosphotyrosine-Containing Peptide of pp60^{src} Labeled *in Vivo*. Reverse-phase HPLC of the tryptic peptides of pp60^{src} labeled *in vivo* with $^{32}P_i$ gives four ^{32}P-containing peaks, the initial column flow through and three peaks that are eluted by the gradient (Fig. 1). The major eluted peak appeared in fractions 53–60. Sequential Edman degradation of this peptide liberated $^{32}P_i$ at cycle 7 (Fig. 2). The relatively low yield, as well as the continued washout of the cleaved phosphotyrosine, was most probably due to the hydrophilic contribution of the phosphate group on the >PhNCS-(P)Tyr residue (which causes the residue to remain associated with the Polybrene that had been added to the spinning cup to prevent washout of the original peptide). In each sequential Edman degradation run, 200 mg of apomyoglobin was added to the sample as a carrier and to check on the performance of the sequencer at each cycle. In all cases, the expected amino acids of apomyoglobin were observed at the appropriate cycles, with the expected yields, and with very little overlap, indicating that the low yield and repeated washout of the [^{32}P]phosphotyrosine is due to properties of the >PhNCS-(P)Tyr residue itself.

Staphylococcus V8 protease can be used to divide pp60$^{v\text{-}src}$ or pp60$^{c\text{-}src}$ into an NH_2-terminal fragment (M_r 34,000) and a COOH-terminal fragment (M_r 26,000 and 28,000, respectively) (21). The phosphorylated tyrosine resides exclusively in the

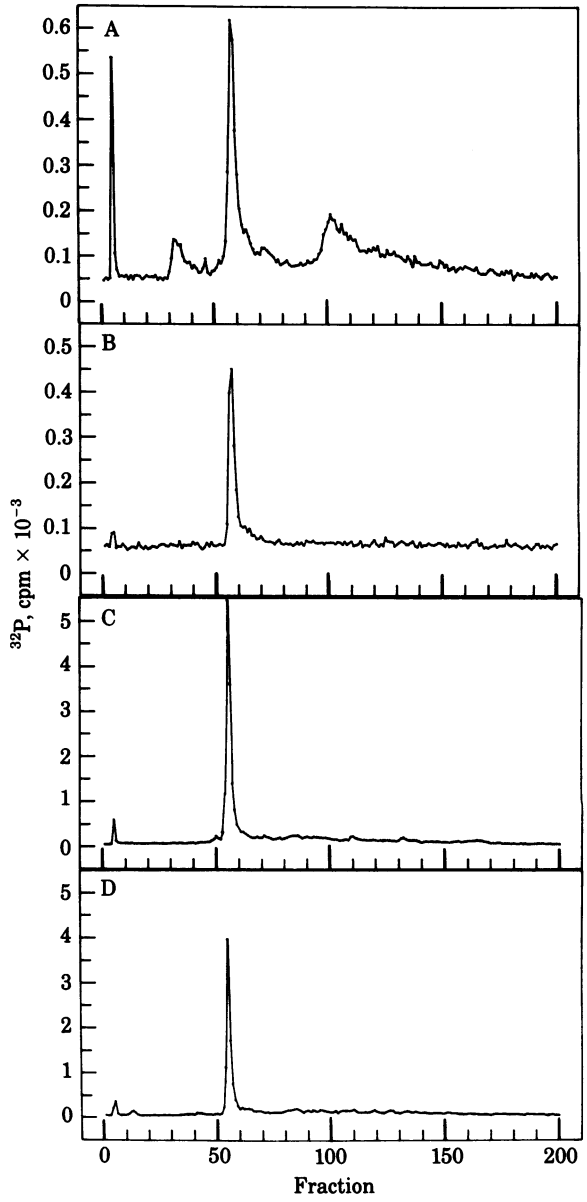

FIG. 1. Reverse-phase chromatography of phosphopeptides. Phosphorylated proteins were prepared from metabolically labeled cells or by *in vitro* reactions, isolated by electrophoresis in polyacrylamide gels, hydrolyzed with trypsin, and subjected to HPLC. (*A*) pp60$^{v\text{-}src}$ labeled *in vivo*. (*B*) COOH-terminal fragment of pp60$^{v\text{-}src}$ labeled *in vivo* and then cleaved with V8 protease. (*C*) pp60$^{v\text{-}src}$ phosphrylated *in vitro*. (*D*) pp60$^{c\text{-}src}$ phosphorylated *in vitro*.

COOH-terminal fragment (5,11). We therefore used HPLC and Edman degradation to analyze the phosphorylated tryptic peptides derived from the separated 34,000 and 26,000 fragments of pp60$^{v\text{-}src}$. The phosphopeptide eluting in fractions 53–60 was found in the 26,000 COOH-terminal fragment (Fig. 1). Sequential Edman degradation of this peptide confirmed that the phosphotyrosine occurs at residue 7 (data not shown).

Fig. 3 illustrates all of the tyrosine-containing tryptic peptides predicted for the COOH-terminal domain defined by cleavage of pp60$^{v\text{-}src}$ with V8 protease (21). Only one of these peptides contains tyrosine at position 7 [i.e., residue 419 in the NH_2 sequence predicted for pp60$^{v\text{-}src}$ by Czernilofsky *et al.* (15)]. Two other properties of this peptide substantiate its identity (Table 1). First, it should bear a strong negative charge, a property that is confirmed by the behavior of the phosphoty-

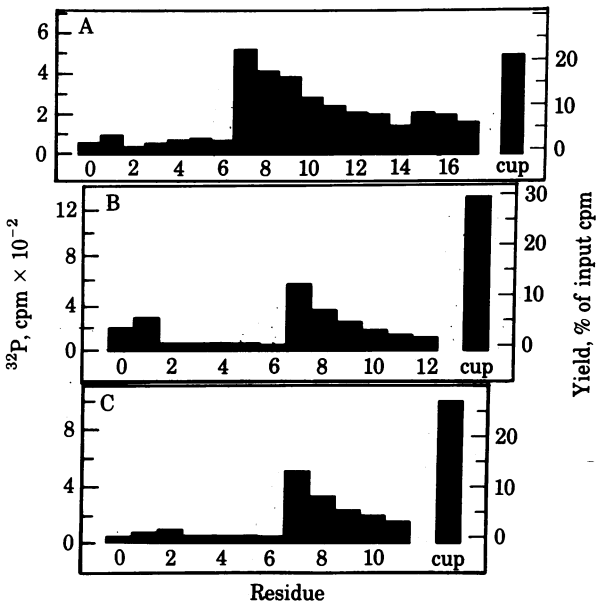

FIG. 2. Automatic sequential Edman degradation of isolated phosphopeptides. Radioactively labeled phosphopeptides were isolated by HPLC as described in the legend to Fig. 1. In each instance, the peptide eluting in fractions 53–60 was taken for analysis by sequential Edman degradation. (*A*) pp60$^{v\text{-}src}$ labeled *in vivo*. (*B*) pp60$^{v\text{-}src}$ phosphorylated *in vitro*. (*C*) pp60$^{c\text{-}src}$ phosphorylated *in vitro*.

rosine peptide in electrophoresis (refs. 11 and 17; see Fig. 4). Second, the peptide contains no methionine, a prediction that was fulfilled when we analyzed the tryptic peptides of pp60$^{v\text{-}src}$ labeled with [^{35}S]methionine (data not shown). We therefore conclude that the principal phosphotyrosine residue of pp60$^{v\text{-}src}$ is located at residue 419 in the predicted amino acid sequence and that the sequence of the tryptic peptide bearing this residue is as follows: -Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg.

Tyrosine Phosphorylation of pp60$^{c\text{-}src}$ *in Vivo*. Previous reports have suggested that the tryptic peptide-bearing phosphotyrosine in pp60$^{c\text{-}src}$ may be different from that in pp60$^{v\text{-}src}$ (11, 17). We therefore prepared the NH_2- and COOH-terminal domains of ^{32}P-labeled pp60$^{c\text{-}src}$ by cleavage with V8 protease, hydrolyzed the isolated fragments with trypsin, and analyzed the resulting peptides by thin-layer electrophoresis and chromatography (Fig. 4). (Our efforts to obtain reproducible results with HPLC were thwarted by the small amounts of pp60$^{c\text{-}src}$ recoverable from uninfected cells.) Single and apparently identical phosphopeptides were obtained from the NH_2-terminal domains of pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ (Fig. 4, lanes 1, 2, 5, and 6). The results with the COOH-terminal domains were more complex. Electrophoretic analysis showed a single phosphopeptide from pp60$^{c\text{-}src}$ that appeared to be similar to the major phosphopeptide obtained from pp60$^{v\text{-}src}$ (lanes 3 and 4). By contrast, chromatography showed the major phosphotyrosine peptides of pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ to have quite different properties; instead, a less abundant phosphopeptide from pp60$^{v\text{-}src}$ comigrated with the sole phosphopeptide from pp60$^{c\text{-}src}$ (lanes 7 and 8). Two explanations of these findings are possible: (*i*) either pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ are each phosphorylated on a single tyrosine residue, and the less abundant peptide from pp60$^{v\text{-}src}$ is an artifact due to incomplete hydrolysis or (*ii*) a relatively minor site of tyrosine phosphorylation in pp60$^{v\text{-}src}$ is analogous to the sole site of tyrosine phosphorylation in pp60$^{c\text{-}src}$. In any event, the discrepancies between the results obtained with the viral and cellular forms of pp60^{src} are in accord with previous reports and sustain the impression that tyrosine phosphorylation *in vivo* occurs at different sites in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. We were therefore unprepared for the results obtained when we turned to the analysis of phosphorylation *in vitro*.

Tyrosine Phosphorylation of pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ *in Vitro*. Immunoaffinity chromatography was used to isolate pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ (13, 14). Both proteins were then phosphorylated *in vitro*; the reaction labels tyrosine exclusively (6). Analysis of tryptic peptides by HPLC revealed that both pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ had been labeled on a peptide that eluted in fractions 53–60 (Fig. 1), as found previously for the phosphotyrosine peptide from pp60$^{v\text{-}src}$ labeled *in vivo*. Moreover, sequential Edman degradation released $^{32}P_i$ at residue 7 of the peptide from either pp60$^{v\text{-}src}$ or pp60$^{c\text{-}src}$ phosphorylated *in vitro* (Fig. 2). As before, the >PhNCS derivative of phosphotyrosine was obtained in relatively low yield and washed out in a repetitive manner with the release of subsequent residues (Fig. 2).

DISCUSSION

Phosphorylation of Tyrosine in the Protein Products of v-*src* and c-*src*. The protein kinase activity attributed to pp60$^{v\text{-}src}$ offers an attractive explanation for the pleiotropic effects of neoplastic transformation induced by RSV. One protein, by a single enzymatic function, could affect the structure or activity of numerous cellular proteins. Similarly, it is assumed that the kinase activity of pp60$^{c\text{-}src}$ serves pleiotropic regulatory functions in the normal cell. Many protein kinases are subject to regulation,

329, 338, 343: lys|leu val gln leu[tyr]ala val val ser glu glu pro ile[tyr]ile val ile glu[tyr]met ser lys|gly ser
360: gly glu met gly lys|tyr|leu arg|leu pro gln
379: cys ile arg|met ala|tyr|val glu arg|met asn
385: val glu arg|met asn|tyr|val his arg|asp leu
*419: |leu ile glu asp asn glu|tyr|thr ala arg|gln gly
437: |trp thr ala pro glu ala ala leu|tyr|gly arg|phe thr ile
466: lys|gly arg|val pro|tyr|pro gly met gly asn gly glu val leu asp arg|
482: arg|val glu arg|gly|tyr|arg|met pro cys pro
515: |asp pro gly gly ala ala his phe ser ser[tyr]leu gln ala gln leu leu pro ala cys val leu glu val ala glu.

FIG. 3. Tryptic peptides containing tyrosine in the COOH-terminal domain of pp60$^{v\text{-}src}$. Partial cleavage of pp60$^{v\text{-}src}$ divides the protein into a 34,000-dalton NH_2-terminal fragment and a 26,000-dalton COOH-terminal fragment (21). The amino acid sequences surrounding all of the tyrosine residues contained in the 26,000-dalton fragment, as predicted from the nucleotide sequence reported by Czernilofsky *et al.* (15), are illustrated. Potential sites of cleavage by trypsin are marked by vertical bars. Negatively charged residues in close vicinity of tyrosine are underlined. Numbers indicate positions of tyrosine residues in the full amino acid sequence. *, Tyrosine residue that apparently is phosphorylated.

Table 1. Properties of the tryptic peptides containing tyrosine in the COOH-terminal domain of pp60$^{v\text{-}src}$

Tyrosine position			
In pp60^{src}	In tryptic peptide	Net charge of tryptic peptide	Methionine present
329 (326)	5	−2	Yes
338 (335)	14	−2	Yes
343 (340)	19	−2	Yes
360 (357)	1	+1	No
379 (376)	3 (17)	0 (−1)	Yes
385 (382)	3	+1	Yes
419 (416)	7*	−2*	No*
439 (436)	9	0	No
466 (463)	3	−1 (+1)	Yes
482 (479)	2	+1	No
515 (511)	11 (1)	−3 (−2)	No

The information summarized here was deduced from the amino acid sequence proposed for pp60^{src} by Czernilofsky *et al.* (15). Values in parentheses were deduced from the amino acid sequence of pp60^{src} of PrC RSV proposed by D. Schwartz (personal communication). Only the COOH-terminal domain of the M_r 26,000 polypeptide was analyzed (21).

* Properties of the tryptic peptide that apparently bears the phosphotyrosine.

often by phosphorylation (22, 23). It appears that pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ conform to this rule. Both are phosphorylated on serine and tyrosine (5, 21), and these phosphorylations are likely to affect their enzymatic activity (22, 23).

Phosphorylation of tyrosine is newly described (5, 24, 25), and nothing is known of the sites at which this form of phosphorylation occurs in proteins. We have therefore characterized the site of tyrosine phosphorylation in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. Our results indicate that phosphorylation of pp60$^{v\text{-}src}$ both *in vivo* and *in vitro* occurs at residue 419 in the proposed amino acid sequence of the molecule (15). (Unpublished data suggest that correction of errors in the published sequence will slightly change the position of the phosphorylated residue relative to the NH_2 terminus, but the amino acid sequence that surrounds the phosphotyrosine would remain unchanged.)

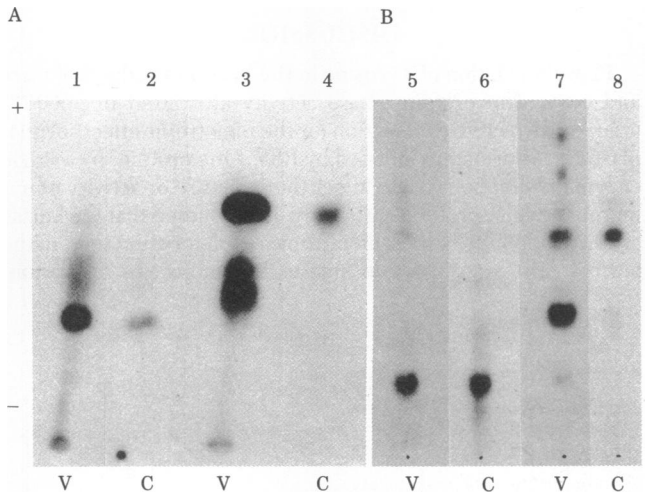

FIG. 4. Analysis of phosphopeptides in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ on cellulose thin layers: autoradiograms of thin-layer plates. Immunoprecipitation and electrophoresis in polyacrylamide gels were used to recover pp60$^{v\text{-}src}$ (V) and pp60$^{c\text{-}src}$ (C) from ^{32}P-labeled cells (9). Hydrolysis with V8 protease was used to divide the isolated proteins into a 34,000-dalton NH_2-terminal domain containing phosphoserine and a 26,000-dalton domain containing phosphotyrosine. The separated fragments were then hydrolyzed with trypsin, and the resulting peptides were fractionated by thin-layer electrophoresis at pH 8.9 (*A*) or thin-layer chromatography (*B*), using the solvents described by Hunter and Sefton (5). Lanes: 1 and 5, NH_2-terminal domain of pp60$^{v\text{-}src}$; 2 and 6, NH_2-terminal domain of pp60$^{c\text{-}src}$; 3 and 7, COOH-terminal domain of pp60$^{v\text{-}src}$; 4 and 8, COOH-terminal domain of pp60$^{c\text{-}src}$.

The sequence of the tryptic peptide that contains the phosphotyrosine is Leu-Ile-Glu-Asp-Asn-Glu-Tyr(P)-Thr-Ala-Arg. In the case of cyclic AMP-dependent protein kinase, it has been shown that the amino acid sequences at the phosphorylation sites in physiologically significant protein substrates fall into two categories: (*i*) -Lys-Arg-X-X-Ser-X- and (*ii*) -Arg-Arg-X-Ser-X- [in which X stands for any amino acid (except that the amino acid residues immediately adjacent on either side of the serine usually have hydrophobic side chains) (22)]. In contrast, the tyrosine kinase target site of pp60$^{v\text{-}src}$ is distinguished by the fact that three of the four amino acids immediately preceding the phosphotyrosine are acidic.

The results with pp60$^{c\text{-}src}$ were more complex and not entirely explicable. When the protein was labeled *in vitro* by an associated protein kinase activity, phosphorylation occurred on a single amino acid residue that appeared to be the same as the tyrosine residue phosphorylated in pp60$^{v\text{-}src}$, either *in vitro* or *in vivo*. By contrast, phosphorylation of pp60$^{c\text{-}src}$ *in vivo* occurred at an ostensibly different and currently unidentified site, in accord with previous reports (11, 17). These findings raise the possibility that the viral protein and its cellular homologue are not entirely identical; they may be subject to different regulatory modifications within the intact cell.

How can we account for the phosphorylation of different tyrosines in pp60$^{c\text{-}src}$ *in vitro* and *in vivo*? It is generally acknowledged that the phosphorylation of proteins *in vitro* may use inauthentic substrate sites. Moreover, the predicted amino acid sequence of the COOH-terminal domain of pp60$^{v\text{-}src}$ presents a series of tyrosine residues whose chemical environments are similar (Fig. 3). For example, five of these tyrosine residues—including the site of phosphorylation in pp60$^{v\text{-}src}$ (residue 419)—are preceeded by glutamine at the fourth position upstream in the sequence (Fig. 3). We cannot explain, however, why one of these sites might be preferred *in vivo*, another *in vitro*, and we cannot be certain that all of these sites occur in pp60$^{c\text{-}src}$ because its amino acid sequence is not yet known.

What Is the Role of Tyrosine Phosphorylation in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$. The enzyme responsible for phosphorylation of tyrosine in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ has not been definitively identified. In some instances, partially purified preparations of pp60$^{v\text{-}src}$ can be phosphorylated on tyrosine *in vitro* by an associated protein kinase activity, as if the phosphorylation might be due to an autocatalytic activity (13, 14); in other instances, phosphorylation of pp60$^{v\text{-}src}$ has not been observed under these circumstances (7). Recent work has identified a new protein kinase (denoted PK_S) that may be responsible for tyrosine phosphorylation in pp60$^{c\text{-}src}$ (26, 27). It is possible that the same kinase phosphorylates tyrosine in pp60$^{v\text{-}src}$.

What is the function of tyrosine phosphorylation in pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$? Previous proposals that the phosphorylated residue might represent an intermediate in phosphotransfer have not been adequately tested. It seems just as likely that tyrosine phosphorylation in the pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ serves a regulatory function. For example, phosphorylation of tyrosine may be required for the enzymatic activation of these proteins (28). Our characterization of the phosphorylated tyrosine in pp60$^{v\text{-}src}$ defines what may prove to be a widely used site for tyrosine phosphorylation in the regulation of cellular functions.

Tyrosine Phosphorylation in Other Retrovirus-Transforming Proteins. Three classes of avian sarcoma viruses have been defined recently on the basis of distinctive oncogenes and their products (29, 30). The oncogene products of all three classes of

viruses are phosphorylated at tyrosine residue(s), display an associated tyrosine kinase activity, and are themselves phosphorylated when incubated with [γ-^{32}P]ATP (5–7, 29–33). Neil *et al.* have found that the oncogene products of the class III viruses are phosphorylated *in vitro* at a tyrosine residue contained in a tryptic peptide that appears to be identical to the phosphotyrosine-containing tryptic peptide of pp60$^{v\text{-}src}$ (34). These data, as well as preliminary data on the transformation-specific proteins of the class II avian sarcoma viruses (PRCII and Fujinami) (unpublished observations), suggest that the occurrence of acidic amino acids adjacent to the phosphoacceptor tyrosine may be a general property of the target site for tyrosine-specific protein kinases. The apparent conservation of these target sites in the otherwise distinct transforming proteins of classes I and III avian sarcoma viruses supports the hypothesis that such sites may play a central role in regulating the function(s) of both the viral transforming proteins and their homologues in normal cells.

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