JOURNAL OF VIROLOGY, July 1983, p. 253–258
0022-538X/83/070253-06$02.00/0

Vol. 47, No. 1

Specific Proteolytic Fragmentation of $p60^{v\text{-}src}$ in Transformed Cell Lysates

SUSAN K. WELLS AND MARC S. COLLETT*

Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Received 10 January 1983/Accepted 28 March 1983

Work involving the transforming protein, $p60^{v\text{-}src}$, of Rous sarcoma virus has resulted in the extensive characterization of its protein structure and associated phosphotransferase activity. However, in many investigations proteolytic fragments (principally $p52^{v\text{-}src}$) of the *src* protein are actually studied. Here, we emphasize potential problems in the interpretation of experimental results in which the proteolytic fragmentation of $p60^{v\text{-}src}$ may be involved and offer several means for the complete prevention of this $p60^{v\text{-}src}$ degradation.

The protein product of the Rous sarcoma virus (RSV) transforming gene (*src*) is a 60,000-dalton protein called $p60^{v\text{-}src}$. This single viral gene product appears to be directly responsible for transformation of cells in culture and sarcomagenesis in infected animals (13, 26). Since $p60^{v\text{-}src}$ is a protein kinase specific for tyrosine residues (6, 7, 14, 21), it seems likely that phosphorylation of tyrosine sites on cellular proteins plays a crucial role in cellular oncogenesis by RSV (5, 11, 24).

During the past several years since its identification (2, 23), $p60^{v\text{-}src}$ has been the subject of a myriad of studies, all with the ultimate goal of understanding the detailed molecular mechanism of its action in causing malignant transformation. However, workers have often observed the appearance of polypeptides specifically immunoprecipitated by anti-$p60^{v\text{-}src}$ antibodies that have molecular weights less than the $p60^{v\text{-}src}$ protein. The appearance of these polypeptides, the major species usually being 52,000 in molecular weight, seems to be the result of a proteolytic removal of the amino-terminal portion of $p60^{v\text{-}src}$, as demonstrated by various peptide mapping results (10, 17, 18, 20). This proteolytic breakdown of $p60^{v\text{-}src}$, which occurs during various experimental manipulations, has been generally regarded as artifactual and merely a nuisance. No serious attempts have been made to assess the possible consequences of this limited, often variable, but very specific breakdown of the $p60^{v\text{-}src}$ polypeptide with respect to the interpretation of experimental results. Indeed, many studies have failed to report the integrity of the RSV transforming protein under study.

This report is meant to emphasize potential problems in the interpretation of experimental results in which the proteolytic fragmentation of $p60^{v\text{-}src}$ may be involved and to offer several means for the prevention of this $p60^{v\text{-}src}$ degradation.

Studies addressing the subcellular localization of the *src* protein may have been affected by the proteolysis of $p60^{v\text{-}src}$. Many experimental approaches, including a variety of methods such as cell fractionation, immunoelectron microscopy, and immunofluorescence, have been employed to locate the *src* protein in a variety of nonnuclear areas within cells (reviewed in reference 15). The variability of the results may be a reflection of the different techniques used or in fact may indicate that the *src* protein resides in several locations within the transformed cells studied. However, in many cases the location of $p60^{v\text{-}src}$ was claimed to be studied with no regard for the possible involvement of $p52^{v\text{-}src}$. In fact, certain cell fractionation studies have indicated that $p52^{v\text{-}src}$ appears to be a soluble polypeptide under conditions in which $p60^{v\text{-}src}$ is largely plasma membrane bound (17, 18). However, in other subcellular fractionation work, the recoveries of $p60^{v\text{-}src}$ and $p52^{v\text{-}src}$ from various fractions were congruent (8).

The presence and amount of *src*-specific phosphotransferase activity, as measured by the original immune complex protein kinase assay (6, 21), have often been taken as a quantitative estimate of the amount of *src* polypeptide present in the preparation or cellular fraction. However, it appears that preparations containing $p52^{v\text{-}src}$ exhibit a higher phosphotransferase activity in this assay than preparations containing intact $p60^{v\text{-}src}$ (18). We have further investigated this observation by comparing the protein kinase activities of partially purified preparations containing either $p52^{v\text{-}src}$ or intact $p60^{v\text{-}src}$. Our procedures for the purification of the *src* protein by immunoaffinity chromatography have been previously described (9, 10). [^{35}S]methionine-

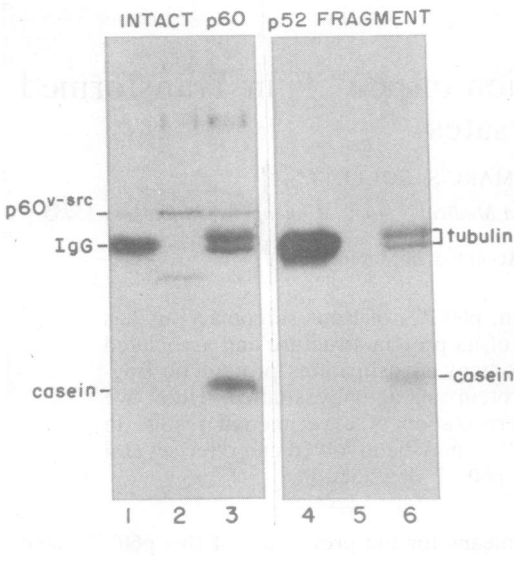

FIG. 1. Phosphorylation by partially purified preparations of either intact $p60^{v\text{-}src}$ or $p52^{v\text{-}src}$. RSV-transformed vole cells (*Microtus agrestis*) were radiolabeled with [^{35}S]methionine for 2 h as previously described (2, 4). Cells were harvested, divided into two equal portions, and pelleted. One cell pellet was suspended in RIPA buffer (12), and the other was suspended in hypotonic buffer (20 mM Tris-hydrochloride [pH 7.2], 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.05% Triton X-100). After 5 min at 4°C, the samples were Dounce homogenized and centrifuged at 3,000 × *g* for 10 min. The cleared lysates were incubated at 4°C for 14 h before application to a column containing immobilized anti-*src* IgG. Immunoaffinity chromatography was performed exactly as previously described (9, 10). Aliquots of the resultant enzyme preparations were subjected to SDS-polyacrylamide gel electrophoresis (19), and the [^{35}S]methionine-labeled *src* protein bands were localized by fluorography (3). The enzyme preparation derived from the RIPA lysate contained only intact $p60^{v\text{-}src}$, whereas the hypotonic buffer lysate contained principally $p52^{v\text{-}src}$, with only a minor amount (about 10%) of a further proteolytic fragment, p49 (10). No intact $p60^{v\text{-}src}$ was detectable in this latter preparation. The radioactivity present in the respective *src* protein bands was quantitated by liquid scintillation counting. Equivalent amounts of *src* polypeptide from each of the two enzyme preparations were then assayed in several protein kinase reactions as previously described (7). IgG derived from tumor-bearing rabbit serum (2) was present at 1 mg/ml (tracks 1 and 4), and tubulin and casein (7) were present at 700 μg/ml and 1 mg/ml, respectively (tracks 3 and 6). Tracks 2 and 5 contained no exogenously added protein. After 10 min at 22°C, reactions were terminated by the addition of SDS sample buffer. The phosphorylated proteins were resolved by electrophoresis on an SDS–10% polyacrylamide gel and detected by autoradiography. The [^{35}S]methionine radioactivity present in the enzyme preparations was sufficiently low so as not to be detectable in the autoradiograms of the phosphorylated proteins. No protein kinase activity was detected in reactions performed with either IgG, tubulin, or casein incubated in the absence of the *src* enzyme preparations.

labeled transformed cells were processed as described in the legend to Fig. 1 to obtain cellular lysates containing only intact $p60^{v\text{-}src}$ or only $p52^{v\text{-}src}$. These extracts were then subjected to parallel immunoaffinity chromatography. The kinase activities of the two resultant enzyme preparations were then evaluated by incubating equal quantities of the respective *src* polypeptides in our standard analytic protein kinase reactions, alone or in the presence of several phosphate acceptor protein substrates. Figure 1 shows an autoradiogram of the phosphorylated proteins resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. When the $p60^{v\text{-}src}$ preparation was incubated without exogenously added protein substrates, $p60^{v\text{-}src}$ itself became phosphorylated (Fig. 1, track 2), as previously described (7, 9, 10, 22). No polypeptides appeared to be phosphorylated in a similar reaction with the $p52^{v\text{-}src}$ enzyme preparation (Fig. 1, track 5). However, there was a considerable amount of *src*-specific protein kinase activity in these preparations since anti-*src* immunoglobulin G (IgG), but not preimmune IgG (not shown), was extensively phosphorylated (Fig. 1, track 4). Also, two proteins previously shown to be substrates for the *src*-specific protein kinase activity, tubulin and casein (7), were phosphorylated by the $p52^{v\text{-}src}$ enzyme preparations (Fig. 1, track 6), but the extent of phosphorylation of the various protein substrates by the two enzyme preparations appeared to differ. The enzyme preparation containing the proteolytic breakdown product of $p60^{v\text{-}src}$ more vigorously phosphorylated the anti-*src* IgG, whereas both casein and tubulin appeared to be phosphorylated to a lesser degree by $p52^{v\text{-}src}$ compared with their phosphorylation by intact $p60^{v\text{-}src}$ (Table 1). We are currently pursuing these initial observations by investigating, in detail, enzymatic differences between these two preparations of the *src* protein kinase.

The above studies corroborate earlier observations of *src* kinase activity differences in cell lysates (18) and further illustrate the potential for misinterpretation of certain experimental data. For example, depending on the physical state of the *src* polypeptide, studies involving the quantitation of *src* protein or protein kinase activity by the immune complex assay may be misleading.

Early work on the purification of $p60^{v\text{-}src}$ (9, 10) revealed the problem of proteolytic fragmentation of the *src* protein upon prolonged manipulation. Enriched protein preparations contained various ratios of $p60^{v\text{-}src}$ and $p52^{v\text{-}src}$ and, often, additional lower-molecular-weight species of the

src protein. Purification of *src* proteins from tumor tissues (as opposed to *src* proteins from cultured cells) also appears to result in a preparation containing only $p52^{v\text{-}src}$ (1), completely devoid of intact $p60^{v\text{-}src}$. Thus, in examining the various functional capabilities of the purified *src* protein, one must be clearly aware of the physical state of the *src* polypeptide under study. This is of particular significance in many of our studies, since (i) as described above, we have found that enzyme preparations containing only intact $p60^{v\text{-}src}$ and preparations containing only $p52^{v\text{-}src}$ exhibit different degrees of phosphotransferase activities under certain assay conditions (Fig. 1 and Table 1), and (ii) we are interested in correlating various structural features of the *src* polypeptide with its functional capabilities.

The proteolytic breakdown of $p60^{v\text{-}src}$ appears to be dependent, at least in part, on the exact conditions of cellular disruption. Much of the work involving the immunoprecipitation of $p60^{v\text{-}src}$ from radiolabeled cells in culture has employed the detergent-containing RIPA buffer (2, 12) for cell disruption. An example of such an immunoprecipitation of a [^{35}S]methionine-labeled RIPA cell lysate is shown in Fig. 2, tracks 1 and 2. Intact $p60^{v\text{-}src}$ can clearly be seen, along with Pr76, the precursor polypeptide to the viral structural proteins. However, in many studies the presence of detergents is not desirable. In such cases cellular disruption may be accomplished by some mechanical means (e.g., Dounce homogenization), often in a hypotonic buffer solution. An immunoprecipitation of [^{35}S]methionine-labeled cells disrupted in this manner is shown in Fig. 2, tracks 3 and 4. The amount of $p60^{v\text{-}src}$ was reduced, and a new polypeptide, $p52^{v\text{-}src}$, appeared. Furthermore, Pr76 was no longer apparent in the immunoprecipitate. When this same cellular lysate was allowed to stand at 4°C for 3 h before immunoprecipitation, the yield of $p60^{v\text{-}src}$ was much reduced, whereas the amount of $p52^{v\text{-}src}$ increased (Fig. 2, tracks 7 and 8).

Thus, the conditions of cell lysis and the time of manipulation before analysis (immunoprecipitation) are important factors concerning the integrity of the *src* polypeptide. Tables 2 and 3 expand on these two factors. Table 2 shows the effect of different concentrations of nonionic detergent (Triton X-100) present in the cellular disruption buffer on the ratio of $p60^{v\text{-}src}$ and $p52^{v\text{-}src}$ after a prolonged incubation period (14 h) before immunoprecipitation. Table 3 shows the effect of time of incubation on the breakdown of $p60^{v\text{-}src}$ to $p52^{v\text{-}src}$ in a hypotonic buffer containing 0.05% Triton X-100. It can clearly be seen that under certain conditions of cell lysis, frag-

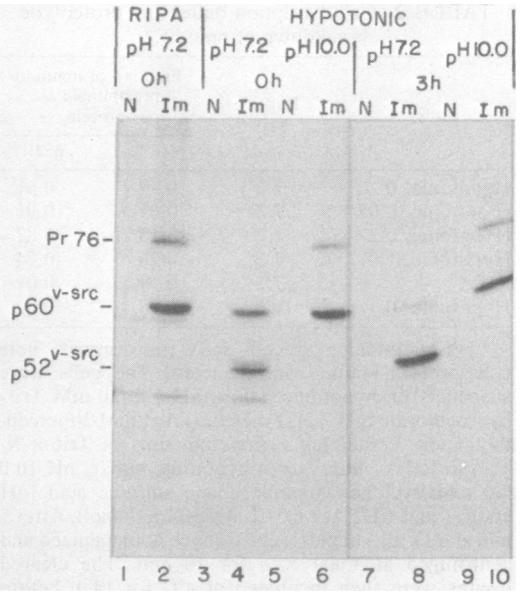

FIG. 2. Proteolytic breakdown of $p60^{v\text{-}src}$ under various conditions of cellular disruption. RSV-transformed vole cells, radiolabeled with [^{35}S]methionine, were divided into equal portions and pelleted. The cells were suspended in either RIPA buffer (150 mM NaCl, 20 mM Tris-hydrochloride [pH 7.2], 1% Triton X-100, 1% deoxycholate, 0.1% SDS), tracks 1 and 2; hypotonic buffer, pH 7.2 (20 mM Tris-hydrochloride [pH 7.2], 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.05% Triton X-100), tracks 3, 4, 7, and 8; or hypotonic buffer, pH 10.0 (20 mM CAPS [pH 10.0], 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.05% Triton X-100), tracks 5, 6, 9 and 10. Cells suspended in hypotonic buffers were allowed to swell at 4°C for 5 min before disruption by Dounce homogenization. All samples were cleared at 3,000 × *g* for 10 min. The cleared lysates were either immediately immunoprecipitated with normal rabbit serum (N) or tumor-bearing rabbit serum (Im) (2) or incubated at 4°C for 3 h before immunoprecipitation. All samples were adjusted to RIPA buffer before the addition of serum. Immunoprecipitation was carried out as previously described (2), and samples of the precipitated materials were subjected to electrophoresis on an SDS–10% polyacrylamide gel (19). The gel was stained with Coomassie brilliant blue, destained, prepared for fluorography (3), and exposed to Cronex 4 X-ray film at −70°C.

TABLE 1. Relative protein kinase activity of $p52^{v\text{-}src}$ and $p60^{v\text{-}src}$ [a]

Protein substrate	Relative activity ($p52^{v\text{-}src}/p60^{v\text{-}src}$)
Anti-*src* IgG	5.8
Casein	0.3
Tubulin	0.6

[a] The phosphorylated protein bands shown in Fig. 1 were excised and quantitated by liquid scintillation spectrometry. The ratio of the corresponding values from the two enzyme phosphorylation reactions is presented.

TABLE 2. Cell disruption buffer and proteolytic breakdown of $p60^{v\text{-}src}$ [a]

Buffer	Buffer pH	Fraction of immunoprecipitable *src* protein	
		$p60^{v\text{-}src}$	$p52^{v\text{-}src}$
Hypotonic, 0[b]	7.2	0.39	0.61
Hypotonic, 0.05	7.2	0.09	0.91
Hypotonic, 0.2	7.2	0.18	0.82
Hypotonic, 1.0	7.2	0.26	0.74
RIPA	7.2	0.99	0.01
Hypotonic, 0	10.0	0.98	0.02

[a] [^{35}S]methionine-labeled RSV-transformed vole cells were aliquoted and pelleted. The cells were suspended in hypotonic buffer, pH 7.2 (20 mM Tris-hydrochloride [pH 7.2], 1 mM EDTA, 1 mM 2-mercaptoethanol), containing various amounts of Triton X-100, in RIPA buffer, or in hypotonic buffer, pH 10.0 (20 mM cyclohexylaminopropane sulfonic acid [pH 10.0], 1 mM EDTA, 1 mM 2-mercaptoethanol). After 5 min at 4°C, all samples were Dounce homogenized and centrifuged at 3,000 × *g* for 10 min. The cleared lysates were then incubated at 4°C for 14 h before immunoprecipitation as outlined in the legend to Fig. 2. After polyacrylamide gel electrophoresis and fluorographic localization, the *src* protein bands were excised, and the amount of radioactivity in each band was determined by scintillation spectrometry.

[b] Percentage of Triton X-100.

mentation of $p60^{v\text{-}src}$ is very rapid and extensive and progresses with time.

Under certain other conditions of cell disruption, the conversion of $p60^{v\text{-}src}$ to $p52^{v\text{-}src}$ did not occur. As discussed above, cell lysis in RIPA buffer resulted in the appearance of intact $p60^{v\text{-}src}$ (Fig. 2), even after prolonged periods of lysate incubation before immunoprecipitation (Table 2). Another cell lysis condition we have found useful in preventing the proteolytic conversion of $p60^{v\text{-}src}$ to $p52^{v\text{-}src}$ is shown in Fig. 2, tracks 5 and 6. Cellular lysis at a high pH, with or without detergents present, resulted in the maintenance of the integrity of $p60^{v\text{-}src}$ (as well as Pr76), even after long incubation times before analysis (Fig. 2, tracks 9 and 10; Table 2).

The proteolytic breakdown of $p60^{v\text{-}src}$ appears to be highly specific, suggesting the involvement of a specific protease activity. We have found that this protease activity is present in normal cells and is able to act on the normal cell homolog to the RSV *src* protein, $p60^{c\text{-}src}$ (data not shown). Furthermore, this protease does not require monovalent or divalent cations and is independent of ATP. We have observed the proteolytic activity over a wide range of pH values (pH 5 to 8); however, above pH 8.5, it appears to be largely inactive.

We have screened a variety of agents that might act as inhibitors of the proteolysis of $p60^{v\text{-}src}$ (Fig. 3). Under lysis conditions in which extensive breakdown of $p60^{v\text{-}src}$ was observed (Fig. 3, track 2), a variety of potential inhibitors was added to the hypotonic lysis buffer before cell disruption. Several commonly used protease inhibitors failed to prevent the breakdown of $p60^{v\text{-}src}$ (e.g., L-1-tosylamide-2-phenylethyl chloromethyl ketone, phenylmethylsulfonyl fluoride, and aprotinin), whereas others were very effective (e.g., Nα-*p*-tosyl-L-lysine chloromethyl ketone, antipain, leupeptin, and soybean trypsin inhibitor). Interestingly, $NaPP_i$ appeared to prevent proteolysis of $p60^{v\text{-}src}$ (Fig. 3, track 14). $HgCl_2$ (and other mercurials) also inhibited $p60^{v\text{-}src}$ breakdown, suggesting that a critical sulfhydryl group was required for this proteolytic activity. The inhibition of $p60^{v\text{-}src}$ proteolysis by $HgCl_2$ was reversible. Incubation of cell lysates in hypotonic buffer in the absence of $HgCl_2$ resulted in the extensive breakdown of $p60^{v\text{-}src}$ to $p52^{v\text{-}src}$ (Fig. 4, tracks 1 to 4). This breakdown was completely inhibited by the addition of 1 mM $HgCl_2$ to the lysis buffer (Fig. 4, track 6). However, if, after 2 h of incubation of the lysate in the presence of $HgCl_2$, 2-mercaptoethanol was added and incubation continued for an additional 2 h, extensive proteolysis of $p60^{v\text{-}src}$ resulted (Fig. 4, track 8).

We have attempted in this report to summarize certain of our observations of the specific proteolytic breakdown of $p60^{v\text{-}src}$ upon cellular disruption. Although this proteolysis appears to be very specific, it is also highly variable. Our observations over the last several years suggest that some RSV-transformed cells exhibit higher levels of proteolytic activity than others. Furthermore, the same cell line at different times may vary in the extent to which $p60^{v\text{-}src}$ is fragmented. The reasons for this variability are unknown.

TABLE 3. Time course of $p60^{v\text{-}src}$ proteolytic breakdown[a]

Time (h) at 4°C	Fraction of immunoprecipitable *src* protein	
	$p60^{v\text{-}src}$	$p52^{v\text{-}src}$
0	0.66	0.34
2	0.58	0.42
5	0.36	0.64
14	0.09	0.91

[a] Pelleted [^{35}S]methionine-labeled RSV-transformed vole cells were suspended in a hypotonic buffer (20 mM Tris-hydrochloride [pH 7.2], 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.05% Triton X-100), homogenized, and centrifuged as described in the legend to Fig. 2. The cleared lysate was incubated at 4°C for various periods of time, after which equal portions were removed, adjusted to RIPA buffer, and immunoprecipitated. The fraction of immunoprecipitable *src* proteins was determined as described in Table 2, footnote *a*.

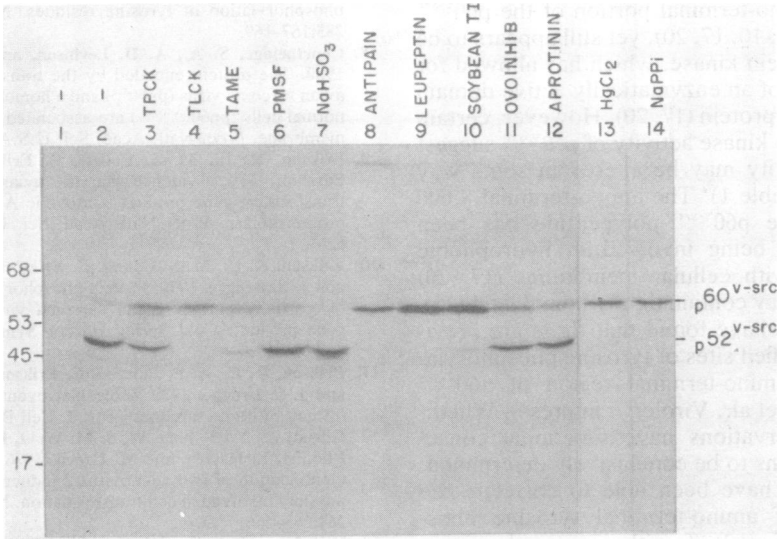

FIG. 3. Inhibition of $p60^{v\text{-}src}$ degradation. [^{35}S]methionine-labeled RSV-transformed vole cells were aliquoted into tubes and pelleted. The cells were then suspended in hypotonic buffer, pH 7.2, containing various agents. The cells were allowed to swell for 5 min before Dounce homogenization and centrifugation at 3,000 × *g* for 10 min. The cleared lysates were incubated at 4°C for 5 h before the solutions were adjusted to RIPA buffer and immunoprecipitated with either normal rabbit serum (track 1) or tumor-bearing rabbit serum (tracks 2 to 14). Subsequent electrophoresis on a polyacrylamide gel and fluorographic processing were as described in the legend to Fig. 2. The various agents added to the hypotonic lysis buffer were as follows: tracks 1 and 2, no additions; track 3, 10 mM L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK); track 4, 10 mM Nα-*p*-tosyl-L-lysine chloromethyl ketone (TLCK); track 5, 10 mM *p*-tosyl-L-arginine methyl ester (TAME); track 6, 2 mM phenylmethylsulfonyl fluoride (PMSF); track 7, 10 mM sodium bisulfite ($NaHSO_3$); track 8, 100 μg of antipain per ml; track 9, 100 μg of leupeptin per ml; track 10, 100 μg of soybean trypsin inhibitor per ml; track 11, 100 μg of ovoinhibitor per ml; track 12, 100 kallikrein inactivator units of aprotinin per ml; track 13, 1 mM mercuric chloride; track 14, 20 mM $NaPP_i$. All peptide (analog) inhibitors were obtained from Sigma Chemical Co. The numbers at the left represent the positions of molecular weight standards in thousands.

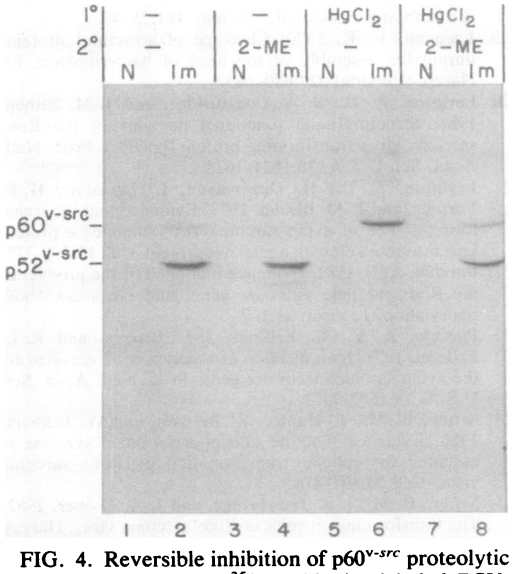

FIG. 4. Reversible inhibition of $p60^{v\text{-}src}$ proteolytic breakdown by $HgCl_2$. [^{35}S]methionine-labeled RSV-transformed vole cells were aliquoted into tubes and pelleted. Cells were suspended in either hypotonic buffer containing Triton X-100 (20 mM Tris-hydrochloride [pH 7.2], 1 mM EDTA, 0.2% Triton X-100) (tracks 1 to 4) or hypotonic buffer containing Triton X-100 plus 1 mM $HgCl_2$ (tracks 5 to 8). After Dounce homogenization and clearing, the lysates were incubated at 4°C for 2 h (primary [1°] incubation). One set of tubes was then immunoprecipitated with normal rabbit serum (N, track 1) or tumor-bearing rabbit serum (Im, track 2). The other tubes were further incubated at 4°C for 2 h (secondary [2°] incubation) after the addition of 2-mercaptoethanol to a final concentration of 60 mM (tracks 3, 4, 7, and 8) or after no additions (tracks 5 and 6). After this second incubation period, the samples were immunoprecipitated and analyzed as described in the legend to Fig. 2. To samples not receiving 2-mercaptoethanol, an equivalent amount of 2-mercaptoethanol was added before immunoprecipitation.

We have demonstrated several means for the control of the breakdown of $p60^{v\text{-}src}$. Various detergent combinations, pH, and specific inhibitors, when present in cell disruption buffers, can completely prevent $p60^{v\text{-}src}$ breakdown. These should be useful to many workers observing $p60^{v\text{-}src}$ proteolysis in various studies.

The biological significance of the proteolysis of $p60^{v\text{-}src}$, if any, is totally unclear. $p52^{v\text{-}src}$

lacks the amino-terminal portion of the $p60^{v\text{-}src}$ polypeptide (8, 10, 17, 20), yet still appears to be an active protein kinase, which has allowed for the definition of an enzymatically active domain on the $p60^{v\text{-}src}$ protein (17, 20). However, certain features of the kinase activity of $p52^{v\text{-}src}$ suggest that this activity may be altered in some way (Fig. 1 and Table 1). The amino-terminal 8,000 daltons of the $p60^{v\text{-}src}$ polypeptide has been implicated as being involved in hydrophobic interactions with cellular membranes (17, 20) and, in fact, may contain tightly bound lipid (25). Moreover, we have found that there are previously unidentified sites of tyrosine phosphorylation in the amino-terminal region of $p60^{v\text{-}src}$ (M. S. Collett et al., Virology, in press). Whether these observations have functional consequences remains to be conclusively determined. However, we have been able to correlate the appearance of amino-terminal tyrosine phosphorylation of $p60^{v\text{-}src}$ with increased protein kinase activity (A. F. Purchio et al., submitted for publication). Furthermore, Krueger et al. (16) have recently shown that certain recovered avian sarcoma viruses, whose *src* gene products exhibit an altered amino-terminal region, have reduced pathogenic capabilities in vivo. Therefore, the potential importance of the amino-terminal portion of $p60^{v\text{-}src}$ is clearly suggested, and any modification of this region of the polypeptide should be seriously considered.

This investigation was supported by Public Health Service grant 5R01-CA29041-02 awarded by the National Cancer Institute and by a grant from the Leukemia Research Fund, University of Minnesota Foundation.

We thank Cindy Kosman for assistance in manuscript preparation.

LITERATURE CITED

1. **Blithe, D. L., N. D. Richert, and I. H. Pastan.** 1982. Purification of a tyrosine-specific protein kinase from Rous sarcoma virus-induced rat tumor. J. Biol. Chem. **257**:7135–7142.
2. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346–347.
3. **Chamberlain, J. P.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. Anal. Biochem. **98**:132–136.
4. **Collett, M. S., J. S. Brugge, R. L. Erikson, A. F. Lau, R. A. Krzyzek, and A. J. Faras.** 1979. *Src* gene product in both transformed and morphological revertants of ASV-infected mammalian cells. Nature (London) **281**:195–198.
5. **Collett, M. S., E. Erikson, A. F. Purchio, and R. L. Erikson.** 1981. Characterization of the avian sarcoma virus protein responsible for malignant transformation and its homologue in normal cells, p. 105–122. *In* F. E. Arrighi, P. N. Rao, and E. Stubblefield (ed.), Genes, chromosomes, and neoplasia: 33rd Annual Symposium on Fundamental Cancer Research. Raven Press, New York.
6. **Collett, M. S., and R. L. Erikson.** 1978. Protein kinase activity associated with the avian sarcoma virus *src* gene product. Proc. Natl. Acad. Sci. U.S.A. **75**:2021–2024.
7. **Collett, M. S., A. F. Purchio, and R. L. Erikson.** 1980. Soluble protein kinase activity of partially purified avian sarcoma virus-transforming protein, $pp60^{src}$, results in the phosphorylation of tyrosine residues. Nature (London) **285**:167–169.
8. **Courtneidge, S. A., A. D. Levinson, and J. M. Bishop.** 1980. The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. **77**:3783–3787.
9. **Erikson, R. L., M. S. Collett, E. Erikson, and A. F. Purchio.** 1979. Evidence that the avian sarcoma virus transforming gene product is a cyclic AMP-independent protein kinase. Proc. Natl. Acad. Sci. U.S.A. **76**:6260–6264.
10. **Erikson, R. L., M. S. Collett, E. Erikson, A. F. Purchio, and J. S. Brugge.** 1979. Protein phosphorylation mediated by partially purified avian sarcoma virus transforming gene product. Cold Spring Harbor Symp. Quant. Biol. **44**:907–917.
11. **Erikson, R. L., A. F. Purchio, E. Erikson, M. S. Collett, and J. S. Brugge.** 1980. Molecular events in cells transformed by Rous sarcoma virus. J. Cell Biol. **87**:319–325.
12. **Gilead, Z., Y.-H. Jeng, W. S. M. Wold, K. Sugawara, H. Rho, M. L. Harter, and M. Green.** 1976. Immunological identification of two adenovirus 2-induced early proteins possibly involved in cell transformation. Nature (London) **264**:263–266.
13. **Hanafusa, H.** 1977. Cell transformation by RNA tumor viruses, p. 401–483. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 10. Plenum Publishing Corp., New York.
14. **Hunter, T., and B. M. Sefton.** 1980. The transforming protein of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. U.S.A. **77**:1311–1315.
15. **Hynes, R. O.** 1980. Cellular location of viral transforming proteins. Cell **21**:601–602.
16. **Krueger, J. G., E. A. Garber, A. R. Goldberg, and H. Hanafusa.** 1982. Changes in amino-terminal sequences of $pp60^{src}$ lead to decreased membrane association and decreased in vivo tumorigenicity. Cell **28**:889–896.
17. **Krueger, J. G., E. Wang, E. A. Garber, and A. R. Goldberg.** 1980. Differences in intracellular location of $pp60^{src}$ in rat and chicken cells transformed by Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **77**:4142–4146.
18. **Krueger, J. G., E. Wang, and A. R. Goldberg.** 1980. Evidence that the *src* gene product of Rous sarcoma virus is membrane associated. Virology **101**:25–40.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Levinson, A. D., S. A. Courtneidge, and J. M. Bishop.** 1981. Structural and functional domains of the Rous sarcoma virus transforming protein ($pp60^{src}$). Proc. Natl. Acad. Sci. U.S.A. **78**:1624–1628.
21. **Levinson, A. D., H. Oppermann, L. Levintow, H. E. Varmus, and J. M. Bishop.** 1978. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. Cell **15**:561–572.
22. **Purchio, A. F.** 1982. Evidence that $pp60^{src}$, the product of the Rous sarcoma virus *src* gene, undergoes autophosphorylation. J. Virol. **41**:1–7.
23. **Purchio, A. F., E. Erikson, J. S. Brugge, and R. L. Erikson.** 1978. Identification of a polypeptide encoded by the avian sarcoma virus *src* gene. Proc. Natl. Acad. Sci. U.S.A. **75**:1567–1571.
24. **Sefton, B. M., T. Hunter, K. Beemon, and W. Eckhart.** 1980. Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. Cell **20**:807–816.
25. **Sefton, B. M., I. S. Trowbridge, and J. A. Cooper.** 1982. The transforming proteins of Rous sarcoma virus, Harvey sarcoma virus and Abelson virus contain tightly bound lipid. Cell **31**:465–474.
26. **Vogt, P. K.** 1977. The genetics of RNA tumor viruses, p. 341–455. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 9. Plenum Publishing Corp., New York.