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Biochemistry

Thermolabile protein kinase molecules in a temperature-sensitive murine sarcoma virus pseudotype

(actin-binding kinase/viral transformation/temperature-sensitive phosphorylation)

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ABSTRACT **Murine sarcoma virus-associated protein kinases that bind to actin have been purified by affinity chromatography on actin coupled to Sepharose. Heat inactivation studies showed the presence of thermolabile enzyme activity in pseudotypes containing a temperature-sensitive mutant of murine sarcoma virus (MSV) but not in two independent wild-type MSV pseudotypes. Studies with Sephadex G-75 column fractions showed that a low molecular weight form, ≈15,000, is the major thermolabile kinase in the temperature-sensitive MSV virions. Antibodies raised against the MSV-coded p60 protein, when added to the *in vitro* reaction mixtures, showed specific phosphorylation of the IgG heavy chain and a simultaneous reduction in the extent of phosvitin phosphorylation catalyzed by the various MSV pseudotype kinases. Thus a transforming retrovirus-coded enzyme activity that interacts directly with a major cytoskeletal protein and whose activity parallels the transforming ability of a conditional MSV mutant has now been identified.**

We reported that murine sarcoma virus (MSV) pseudotype virions package a low molecular weight (LMW) protein kinase activity that binds to actin. This activity was found in association with the MSV pseudotypes and was absent in nontransforming helper virus particles (1). We now report the presence of a thermolabile actin-binding protein kinase activity packaged in virion particles containing a temperature-sensitive (ts) MSV. Protein kinases, obtained by affinity chromatography on actin-Sepharose conjugate, from ts or wild-type (wt) MSV particles were compared for their thermal stabilities. The ts MSV pseudotype-associated enzyme preparation was inactivated up to ≈50% at 43°C with a half time of ≈40 min. A parallel enzyme preparation from wt MSV was not inactivated even after incubation for up to 2 hr at 43°C. Gel filtration of actin-binding kinases showed the LMW form to be the major thermolabile species (>80% inactivation in 1 hr at 43°C). Antibodies raised against the m1MSV-specified p60 protein, purified from a m1MSV–feline leukemia virus (FeLV) pseudotype (2), inhibited both the wt and the ts MSV-associated kinase.

MATERIALS AND METHODS

Cells and Viruses. The ts 110 MSV (3) was made into a pseudotype with Moloney murine leukemia virus (MuLV) by an acute infection (multiplicity of infection > 10) of the rat nonproducer line (clone 6M2-31) and virus was collected at 32°C. The MSVcl349–MuLV complex (4) and the m3MSV(IC–MuLV) complex (5) were obtained from producer mouse cell lines. The cultures were grown and maintained in Dulbecco's modification of Eagle's medium or minimal Eagle's medium supplemented with glutamine and 10% fetal calf serum (GIBCO).

Preparation of Virion-Associated Protein Kinases. Virus was concentrated by isopycnic banding (1.16 g/cm^3) and then disrupted in 0.4% Triton X-100/0.3 M KCl/5 mM $MgCl_2$/10 mM dithiothreitol/20 mM Tris·HCl (pH 8.0) at 4°C. The extract was exhaustively dialyzed against 20 mM Tris·HCl (pH 8.0)/0.05 M KCl/5 mM $MgCl_2$. The dialyzed extract was applied to an actin-Sepharose column (1) pre-equilibrated with the above dialysis buffer and was washed with 5–10 column volumes of the above buffer. The actin-binding kinase activity was eluted with 0.35 M KCl.

Protein Kinase Assays. Reaction mixtures, 50 μl, contained 20 mM Tris·HCl (pH 8.0)/0.05 M KCl/5 mM $MgCl_2$/1 μM [γ-^{32}P]ATP at ≈20 Ci/mmol (New England Nuclear; 1 Ci = 3.7×10^{10} becquerels); phosvitin was added to a final concentration of 250 μg/ml. Assays were incubated at the indicated temperatures for 30 min. Radioactive phosphoprotein precipitated by 10% trichloroacetic acid was measured by liquid scintillation spectrometry. When necessary, reaction products were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis according to Laemmli (6).

RESULTS

Actin-binding protein kinases from the wt and ts MSV pseudotypes

We reported (1) that a fraction (≈20%) of total MSV-associated kinase binds to actin molecules *in vitro*. Here we examined the thermolability of these actin-binding kinase molecules obtained from the wt and the ts MSV virions. Crude viral extracts were applied to actin-Sepharose conjugates; the bound enzyme activity was eluted with 0.35 M KCl. In agreement with our previous results, the actin-bound enzyme preparation was enriched for the LMW kinase, when compared to the total viral extract. With phosvitin as the substrate and [γ-^{32}P]ATP as the phosphate donor, 30–40% of the substrate-phosphorylating activity in the actin-bound enzyme could be recovered at ≈15,000 M_r.

The thermolability of the actin-bound enzyme preparations was tested by preincubating the enzyme at the desired temperatures for various lengths of time, in 0.02 M Tris·HCl (pH 8.0)/0.05 M KCl/5 mM $MgCl_2$. Phosvitin (250 μg/ml) and [γ-^{32}P]ATP (1 μM) were added and the mixtures were incubated, for 30 min, at the respective temperatures of preincubation. Trichloroacetic acid-precipitable ^{32}P-labeled phosphoprotein was then measured. In a preliminary series of experiments using the enzyme from m3MSV(IC-MuLV) pseudotype (5), reaction mixtures incubated at various temperatures without any preincubation indicated that the optimal temperature for the phosphorylation reaction is 33–35°C.

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Abbreviations: MSV, murine sarcoma virus; MuLV, murine leukemia virus; FeLV, feline leukemia virus; LMW and HMW, low and high molecular weight protein, respectively; ts, temperature-sensitive; wt, wild-type.

Thermal inactivation patterns of the MSV-associated actin-bound kinases

Actin-bound kinases were prepared from different MSV pseudotypes, preincubated at various temperatures between 22°C and 50°C for 30 min, and then assayed at the respective temperatures. The results are presented in Table 1, with the incorporation at 22°C normalized to 100%. Preincubation at >46°C for 30 min inactivated both preparations; at 43°C, however, there was a difference between the wt and the ts virion enzymes. In fact, when the extent of inhibition at either temperature was calculated with the incorporation catalyzed at that temperature without any preincubation as 100%, no detectable inactivation could be seen with the wt enzyme at 43°C; the enzyme from ts 110 MSV showed ≈25% inhibition. Crude viral extracts were also fractionated by Sephadex G-75 gel filtration to test the thermolability of the high molecular weight (HMW; ≥40,000 M_r) and the sarcoma virus-specific LMW (≈15,000 M_r) kinase activities. The LMW activity from ts 110 MSV was 60% inactivated in 30 min at 43°C but not at 22°C. The HMW activity showed no detectable inactivation at either temperature.

The thermal inactivation kinetics for various MSV kinases were measured at 23° ± 1°C and at 42.5° ± 1°C. The results are presented in Fig. 1. At 42.5°C, the wt enzyme preparation incorporated 68%, while the mutant viral enzyme catalyzed 57% of the respective incorporation observed at 22°C. After preincubation at 43°C for up to 90 min, the wt enzyme did not show any inactivation. The mutant enzyme showed a final inactivation of up to 50% within 1 hr of preincubation and no further inactivation was observed after 2 hr of treatment. Assuming this inactivation value to be the total thermal lability at 43°C, the half-time of the inactivation process would be 40 min (Fig. 1). Neither of the enzyme preparations showed any detectable inactivation when treated at 22°C for up to 2.5 hr.

The reversibility of the *in vitro* thermal inactivation was also tested. Enzymes preincubated at 43°C for 30 min or 60 min in the standard assay buffer were cooled on ice and assayed at 22°C after addition of phosvitin and [γ-^{32}P]ATP. The incorporation catalyzed by the wt virion enzyme was unaffected by preincubation at 43°C (Fig. 1). In the case of the enzyme from the mutant virus the incorporation levels were restored to the level observed without preincubation at 43°C (Fig. 1, arrows). The time-dependent thermal inactivation of the labile component of the actin-bound kinase enzyme from ts MSV thus appears to be a reversible process.

The observation that the final extent of thermal inactivation of the ts MSV kinase preparation was 50% suggested that among the MSV kinase molecules that bind to actin there might be subpopulations with different levels of thermolability. We have previously shown, by Sephadex G-75 gel filtration, that the actin-bound kinase preparations contain HMW enzyme activities in addition to the sarcoma virus-specific LMW kinase (1). To identify the thermolability of the different size forms of the actin-bound kinase, Sephadex G-75 column fractions from the actin-binding kinase of ts 110 MSV were incubated for 1 hr at 43°C or 22°C in 0.02 M Tris·HCl (pH 8.0)/0.1 M KCl/5 mM $MgCl_2$. Phosvitin (250 μg/ml) and [γ-^{32}P]ATP (1 μM) were added to initiate the reaction. Assay at either temperature was carried out for 30 min. The results in Fig. 2 show the ratio of incorporated ^{32}P at 22°C/43°C for each of the individual column fractions; the *inset* shows actual ^{32}P incorporated at the two different temperatures. The LMW kinase in column fraction 15 showed a value of 4.5 for the catalytic activity ratio at 22°C/43°C; the HMW species had a ratio of 1.2. Thus, incubation at 43°C for 60 min causes >75% inactivation of the LMW form of the actin-binding kinase from the ts MSV. There might have been a minor thermolabile kinase species present in the high molecular weight region of the column effluent. An examination of representative column fractions of Sephadex G-75 fractionated wt enzyme did not show any thermolability in either the high or the low molecular weight material. The data strongly suggest that the sarcoma virus-specific LMW kinase represents the primary thermolabile activity associated with the ts MSV pseudotype. The small change in the activity ratio at 22°C/43°C observed with the HMW activity from the ts MSV might arise from the presence of a minor subpopulation of kinase molecules that are thermolabile. However, the majority of the molecules do not appear to be thermolabile, and a specific association of the HMW form of actin-binding kinase with sarcoma virus cannot be shown at the present time. This minor HMW activity has, therefore, not been further characterized.

Immunologic characterization of the LMW kinases from MSV pseudotypes

The m1 variant of MSV has been shown to specify phosphorylated 60,000 M_r polypeptides containing the NH_2-terminal determinants of the Moloney MuLV *gag* gene (7). The

Table 1. Effect of temperature on the extents of substrate phosphorylation catalyzed by actin-binding kinase

Preincubation and reaction temperature, °C	Preincubation time, min	% of control activity* of actin-binding kinase from: m3MSV (wt)	MSVcl349 (wt)	Clone 110 (ts)	Clone 110 Sephadex G-75 fraction: HMW	LMW
22	0	100	100	100	100	100
	30	99	102	100	96	98
33	0	100 (118)	NT	100 (119)	NT	NT
	30	98 (115)	NT	96 (114)	NT	NT
43	0	100 (71)	100 (69)	100 (60)	100	100
	30	100 (70)	100 (70)	73 (43)	92	38
47	0	100 (54)	100 (56)	100 (49)	NT	NT
	30	23 (11)	21 (11)	18 (9)	NT	NT
50	0	100 (46)	NT	100 (44)	NT	NT
	30	18 (8)	NT	15 (6)	NT	NT

NT, not tested.

* For each enzyme preparation, 100% control activity represents the amount of ^{32}P radioactivity transferred to phosphoprotein in 30 min at each indicated temperature without any preincubation. Values within parentheses indicate % of activity at 22°C for each enzyme.

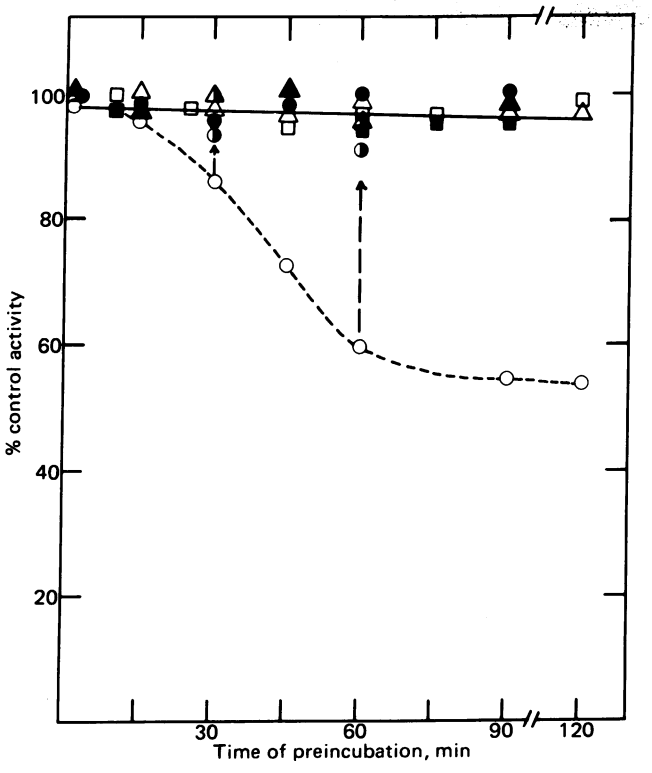

FIG. 1. Heat inactivation of actin-binding kinase from MSV pseudotypes. Aliquots of actin-binding kinases were preincubated at 22°C or 43°C for the indicated lengths of time and then assayed at the preincubation temperature. The activity values were calculated by using the incorporation at the respective temperatures, without any preincubation, as 100%. To test the reversibility of heat inactivation, the enzyme preparations, after preincubation at 43°C, were cooled and assayed at 22°C. The activity in this case was normalized to the activity at 43°C assay as 100%, because even with the wt virion enzyme there was an irreversible reduction in the amount of final incorporation to the level observed at the 43°C assay condition. ▲, wt m3MSV enzyme at 22°C; △, wt m3MSV enzyme at 43°C; ■, wt MSVc1349 enzyme at 22°C; □, wt MSVc1349 enzyme at 43°C; ●, ts 110 MSV enzyme at 22°C; ○, ts 110 MSV enzyme at 43°C; ▲, wt m3MSV enzyme preincubated at 43°C and assayed at 22°C; ◑, ts 110 MSV enzyme preincubated at 43°C and assayed at 22°C.

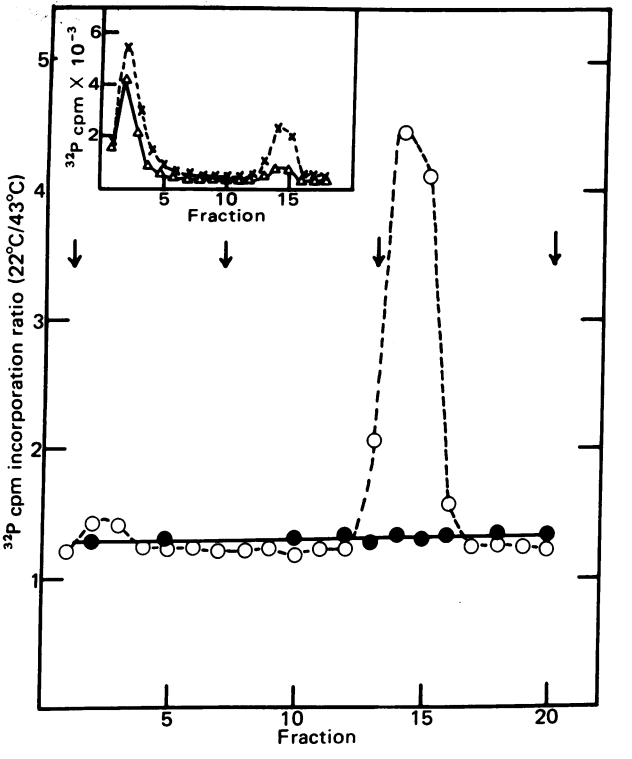

FIG. 2. Thermolability of the different forms of actin-bound kinase from wt m3MSV and ts 110 MSV pseudotypes. The actin-bound kinases prepared from the pseudotypes were subjected to Sephadex G-75 gel filtration in 20 mM Tris·HCl (pH 8.0)/0.3 M KCl/5 mM $MgCl_2$. Individual column fractions, after 1-hr preincubation at 22°C or 43°C, were assayed at the temperature of preincubation. The ratios of ^{32}P cpm incorporated at 22°C/43°C are plotted. (*Inset*) Actual incorporated ^{32}P cpm at 22°C (x) and 43°C (○) with the ts 110 MSV-associated enzyme. The arrows from right to left indicate the positions of included volume ([^{3}H]leucine), ribonuclease (14,000 M_r), chymotrypsin (25,000 M_r), and the void volume. ●, wt m3MSV-associated enzyme; ○, ts 110 MSV-associated enzyme.

m1MSV p60 is packaged in pseudotypes with FeLV (8). Tryptic peptide analysis of the m1MSV p60 indicated the presence of an as yet unidentified peptide ($\leq$20,000 M_r) at the COOH terminal of the molecule (9). Antisera to the m1MSV p60 have been raised to protein purified from the m1MSV(FeLV) pseudotype (2). Two approaches were used to test whether the MSV-coded 60,000 M_r protein molecules contain antigenic determinants for the MSV-associated protein kinase. First, in analogy with the specific immune complex-associated kinase activity reported in the avian sarcoma virus (10, 11), anti-p60 sera were compared with control sera for the ability to provide immunoglobulin substrate molecules. Second, because phosvitin is a more efficient phosphate acceptor than immunoglobulin, the enzyme was first incubated with various amounts of the antibody to allow formation of immune complexes. The ability of the antibody to combine with the enzyme would then be reflected in a net reduction of ^{32}P incorporation into subsequently added phosvitin due to the inability of enzyme molecules to interact with the phosvitin substrate. The data presented in Table 2 show that the goat anti-p60 (*i*) provides substrate for the actin-binding kinase from m3MSV(IC-MuLV) (column 5) and (*ii*) reduces the net ^{32}P incorporation into phosvitin molecules as the amount of anti-p60 serum is increased (columns 6 and 7). The goat anti-p60 at a dilution of 1:50 caused greater than a 2-fold increase in ^{32}P incorporation. In contrast, even at a dilution of 1:10, normal goat serum failed to show any stimulation in the phosphorylation catalyzed by the actin-bound enzyme from any of the MSV pseudotypes tested. The actin-bound kinase from the pseudotypes containing ts MSV or MSVcl349 also used goat anti-m1MSVp60 as substrate and were susceptible to inhibition of phosvitin phosphorylation by the antibody. Other batches of anti-p60 sera also showed similar effects. Thus the actin-binding kinases from different MSV isolates are immunologically related. The kinases from m3MSV and MSVcl349 virions grown in mouse cells and from ts 110 MSV virions grown in rat cells were equally sensitive to the antibody against the p60 protein of m1MSV grown in feline cells (Table 1). The immunological data, then, further suggest that the actin-binding kinase is specified, at least in part, by the MSV genome.

When the antibody was preincubated with purified *gag* proteins—namely p30, p15, p12, and p10 of Moloney MuLV—there was no detectable reduction in the ability of the antibody to interact with the kinase. This kinase activity thus appears to reside in a domain beyond the *gag*-related portion of the p60, or else the kinase might be part of a different population of 60,000 M_r protein molecules that does not contain the *gag* polypeptide sequences. Attempts to examine the effect of preadsorption of the antibody with the p60 protein of m1MSV(FeLV) were complicated by the low solubility of the protein.

We further analyzed the effects of the different antisera on the ability of the MSV-specific LMW kinase activity to phos-

Table 2. Immunological relatedness among actin-binding kinases from different MSV virions

Enzyme source, virus (cell line)	Serum dilution	Phosvitin, ng	^{32}P cpm × 10^{-3} Incorporated	Net in antibody	Net in phosvitin	% of control phosvitin phosphorylation
m3MSV(IC-MuLV)	—	—	2.1	—	—	—
(mouse)	—	10	25.2	—	23.1	100
	Gαp60 (1:50)	—	4.7	2.6	—	—
	Gαp60 (1:50)	10	26.2	—	21.5	93
	Gαp60 (1:25)	—	6.4	4.3	—	—
	Gαp60 (1:25)	10	23.2	—	16.8	73
	Gαp60 (1:20)	—	6.3	4.2	—	—
	Gαp60 (1:20)	10	21.6	—	15.3	66
	Gαp60 (1:10)	—	6.6	4.5	—	—
	Gαp60 (1:10)	10	12.8	—	6.2	27
	NGS (1:50)	—	2.2	0.5	—	—
	NGS (1:50)	10	25.7	—	23.5	102
	NGS (1:10)	—	2.0	0.5	—	—
	NGS (1:10)	10	24.9	—	22.9	99
	NGS (1:10)	—	1.8	—	—	—
MSVcl349-MuLV	—	10	14.7	—	12.9	100
(mouse)	Gαp60 (1:20)	—	4.2	2.4	—	—
	Gαp60 (1:20)	10	13.2	—	9.0	71
	NGS (1:10)	—	1.6	0.5	—	—
	NGS (1:10)	10	14.9	—	13.3	103
	NGS (1:10)	—	2.6	—	—	—
ts110MSV-MuLV	—	10	24.4	—	21.8	100
(rat)	Gαp60 (1:20)	—	6.7	4.1	—	—
	Gαp60 (1:20)	10	19.8	—	13.1	60
	NGS (1:10)	—	2.3	0.5	—	—
	NGS (1:10)	10	24.5	—	22.2	102

All sera were heat inactivated (56°C for 30 min) and dialyzed against 20 mM Tris·HCl (pH 8.0)/0.15 M KCl prior to use in the inhibition studies. Various dilutions of test sera when incubated with or without added phosvitin substrate gave <300 cpm of ^{32}P incorporation into phosphoproteins under standard assay conditions. The test sera presented in the table did not show any inhibition of a commercially available protein kinase purified from beef heart (Sigma). Various dilutions of test sera were incubated with the enzyme preparations at room temperature for 10–15 min in 20 mM Tris·HCl (pH 8.0)/100 mM KCl. Phosphorylation of immunoglobulins was tested by the addition of [γ-^{32}P]ATP at 1 μM final concentration and 5 mM $MgCl_2$. To study substrate phosphorylation, phosvitin (Sigma) was added to a final concentration of 250 μg/ml. The reaction mixtures were incubated at 22°C for 30 min before the trichloroacetic acid-precipitable ^{32}P radioactivity was measured. Gαp60, goat anti-m1MSV p60; NGS, normal goat serum.

phorylate the phosvitin substrate and immunoglobulin molecules. The kinase was incubated with various dilutions of the goat anti-p60 antiserum. After the incubation either only [γ-^{32}P]ATP was added or phosvitin was added together with [γ-^{32}P]ATP. The reaction was terminated by the addition of 1% sodium dodecyl sulfate and 50 mM dithiothreitol, followed by heating at 90°C for 10 min. The reaction products were then analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The results are presented in Fig. 3. In the presence of phosvitin substrate, increasing amounts of the goat anti-p60 serum caused a gradual reduction in the radioactivity in the phosvitin band with simultaneous increase in the phosphorylation of IgG heavy chain (lanes 2–5, Fig. 3 *upper*). Even in the absence of the preferred substrate, phosvitin, no relative enhancement of IgG phosphorylation could be observed (lanes 6–8, Fig. 3 *upper*). An antibody raised in rabbits against the m1MSV p60 protein (lane 8, Fig. 3 *lower*) as well as a different batch of goat anti-p60 (lane 9, Fig. 3 *upper*), also inhibited the phosphorylation of phosvitin and the immunoglobulin heavy chains were once again phosphorylated. A variety of high-titer sera raised against different leukemia viruses [Rauscher MuLV, FeLV, endogenous feline virus (RD-114)] failed either to inhibit phosvitin phosphorylation or to show heavy chain phosphorylation (Fig. 3 *lower*). In a control experiment, 1 ng of actin added to the reaction mixture caused an inhibition of phosvitin phosphorylation (lane 3, Fig. 3 *lower*) and the radioactivity did not appear to be incorporated into actin molecules. There were other phosphorylated proteins in the anti-p60 sera incubated with the LMW kinase. The levels of phosphorylation of at least two such species, one ≈70,000 M_r and another at ≈40,000 M_r, appear to bear a direct correlation with the extent of heavy chain phosphorylation. The origin of these bands is not clear at the present time. The data, then, clearly suggest that goat anti-p60 sera do contain antibody that can specifically recognize the LMW kinase of MSV and can act as a substrate phosphate acceptor in the immune complex.

DISCUSSION

Viral transformation of eukaryotic cells is characteristically accompanied by an appreciable disruption of the organization of cytoskeletal filaments (12–15). The use of mutant DNA and RNA tumor viruses, temperature sensitive in their ability to transform cells, allowed the observation of rapid temperature-dependent changes in the cytoskeletal organization (14, 16). This prompted the suggestion that the product of the transforming gene of each of these viruses possessed the ability to affect the organization of the cytoskeleton of the target cells. Biochemical studies using a ts mutant of an avian sarcoma virus led to the identification of a thermolabile protein kinase activity in immune complexes formed with specific tumor antisera (10, 11). Phosphorylation of proteins regulates the activity of a variety of cellular effector molecules (17). In the case of retroviruses the ability of the major viral phosphoprotein to bind to the homologous viral RNA genome is regulated by the level of phosphorylation (18).

We reported (1) the isolation of a sarcoma virion-associated

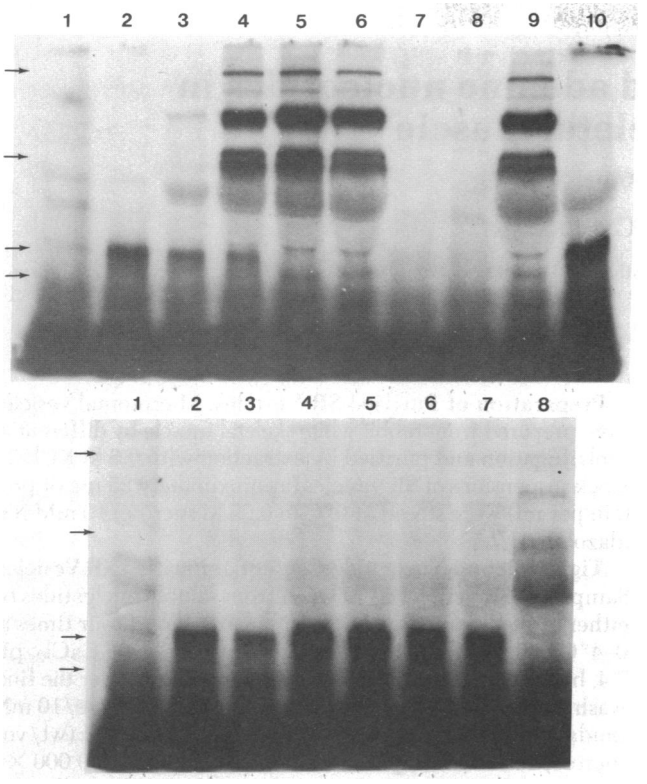

FIG. 3. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis of LMW protein kinase assay products in the presence of antisera and phosvitin. Enzyme preparations [in 0.02 M Tris·HCl (pH 8.0)/0.1 M KCl/5 mM $MgCl_2$/bovine serum albumin at 100 μg/ml] incubated first with different dilutions of various antisera followed by the addition of [γ-^{32}P]ATP and phosvitin were incubated at room temperature for 30 min. The products were analyzed on a 9% polyacrylamide slab gel with 3% stacking gel (6). Autoradiography of the dried slab gels was performed on Kodak No-screen (NS-5T) x-ray film. Abbreviations: Gαp60, goat anti-p60, no. 340 indicates serum from a different goat; Rαp60, rabbit anti-p60; NGS, normal goat serum; NRS, normal rabbit serum; RαMuLV, rabbit anti-MuLV; RαRD-114, rabbit anti-RD-114 feline virus; RαFeLV, rabbit anti-FeLV. (*Upper*) Lane 1, enzyme alone; 2, enzyme + phosvitin; 3, enzyme + Gαp60 (1:80) + phosvitin; 4, enzyme + Gαp60 (1:40) + phosvitin; 5, enzyme + Gαp60 (1:20) + phosvitin; 6, enzyme + Gαp60 (1:40); 7, enzyme + Gαp60 (1:120); 8, enzyme + Gαp60 (1:160); 9, enzyme + Gαp60 no. 340 (1:25) + phosvitin; 10, enzyme + NGS (1:10) + phosvitin. (*Lower*) Lane 1, enzyme alone; 2, enzyme + phosvitin; 3, enzyme + actin (1 ng) + phosvitin; 4, enzyme + NRS (1:10) + phosvitin; 5, enzyme + RαMuLV (1:20) + phosvitin; 6, enzyme + RαFeLV (1:20) + phosvitin; 7, enzyme + RαRD-114 (1:20) + phosvitin; 8, enzyme + Rαp60 no. 340 (1:80). Arrows indicate, from top to bottom, the positions of bovine serum albumin (68,000 M_r); carbonic anhydrase (30,000 M_r); lysozyme (17,000 M_r), and ribonuclease (14,000 M_r).

protein kinase activity on the basis of the affinity for a major cytoskeletal component, actin, which is also a candidate cellular target for the viral transforming gene product. The present examination of the actin-binding protein kinase preparations from pseudotype virions containing a ts transformation mutant of MSV shows the presence of thermolabile protein kinase molecules in these virions. The LMW sarcoma virus-specific protein kinase activity found in these particles is seen to be the major thermolabile species. The nature of the actin-binding HMW kinase and its possible relationship to the transforming activity of the virus stock is not clear. A minor subpopulation of these molecules from ts MSV virions does appear to show thermolability, suggesting a possible relationship to the transforming activity of the ts virus. However, the presence of other kinase activities of uncharacterized nature in the HMW region complicates a functional analysis of this activity.

The LMW kinase from MSV virions is sarcoma virus-specific, possesses actin-binding property, and also represents the major thermolabile population in the ts MSV virion particles. This component was therefore chosen for further analysis. Immunological studies using an antibody raised against the m1MSV-specific p60 protein molecules [packaged in pseudotype virions m1MSV(FeLV)] show that the LMW actin-binding kinase molecules in virions containing different MSV isolates are related with respect to (*i*) their ability to be recognized by anti-p60 immunoglobulins and not preimmune or anti-viral (anti-*gag* and anti-*env*) sera and (*ii*) their loss of ability to phosphorylate added phosvitin molecules when different anti-p60 sera are added prior to the addition of the phosvitin substrate.

The strategy of using actin molecules to construct an affinity matrix reported here is an efficient approach to isolate a biologically active sarcoma virus-associated enzyme whose activity parallels the conditional nature of the transforming ability of the virus. This will now enhance the search for similar cytoskeleton-directed viral and cellular activities that can influence the growth and morphologic behavior of target cells.

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