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Control of src kinase activity by activators, inhibitors, and substrate chaperones

(synthetic random amino acid polymers/polylysine/calmodulin/ras proteins/heat shock proteins)

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ABSTRACT **The activities of src tyrosine kinases are greatly influenced by substrate modulators (chaperones). In the presence of bovine serum albumin, the phosphorylation of a random polymer of glutamic acid, alanine, and tyrosine (1:1:1) by src kinases is stimulated 20- to 100-fold, but there is little stimulation with a polymer of glutamic acid and tyrosine (4:1) as substrate. This suggests that serum albumin interacts with the substrates rather than with the enzyme. groEL and several other heat shock proteins also stimulate the phosphorylation of a random polymer of glutamic acid, alanine, and tyrosine (1:1:1). In the absence of substrate modulators, the phosphorylation of calmodulin and of several ras proteins by src kinase is barely detectable. In the presence of polylysine or protamine, marked phosphorylation is observed. Another type of control of src kinase activities appears to be directed toward the enzyme rather than the substrate. Triton X-100 extracts of plasma membranes of bovine brain contain a heat-stable factor that stimulates c-src kinase activity with any of the polymers as substrate. The same extract contains a heat-labile factor that preferentially inhibits c-src kinase activity. The two factors are separated by DEAE-Sephacel and phosphocellulose chromatography. The presence of the activator enhances the potency of the inhibitor.**

We have previously reported that synthetic random polymers containing tyrosine residues, but no serine or threonine residues, are excellent substrates for a number of protein tyrosine kinases (1, 2). There are distinct differences, however, between the various kinases. For the insulin receptor tyrosine kinase, the best polymer is a random polymer of glutamic acid and tyrosine in a 4:1 molar ratio [poly(E_4Y_1)]; less active is poly ($E_6A_3Y_1$), whereas most other polymers including poly($E_{36}K_{24}A_{35}Y_5$) are less than 10% as active. Epidermal growth factor (EGF) receptor and Abelson tyrosine kinases prefer poly($E_6A_3Y_1$) over poly(E_4Y_1). For Fujinami tyrosine kinase, poly($E_{36}K_{24}A_{35}Y_5$) is by far the best substrate, whereas poly(E_1Y_1) is about 30% as active and slightly superior to poly(E_4Y_1). It should be noted that poly(E_4Y_1) is a suitable substrate for all tyrosine protein kinases thus far tested, including several partially purified kinases from placenta, brain (3), and Ehrlich ascites tumor cells (4). This fact and the suitability of poly(R_3S_1) as a substrate for protein serine (threonine) kinases (5, 6) raises the question of the role of the so-called consensus sequences for protein kinases when the substrates are large proteins. We proposed (7) that the consensus sequence, well established for small synthetic polypeptides (8, 9), may be less significant in the case of large proteins.

Bovine serum albumin (BSA) (0.5–5 mg/ml) stimulates the phosphorylation of poly($E_1A_1Y_1$) by either c-src or v-src kinase over 100-fold but has little or no effect on poly(E_4Y_1) as substrate. At relatively low concentrations (100 μg/ml), groEL and other heat shock proteins are as effective or more effective than BSA. Stimulation of the phosphorylation of poly(E_1Y_1) by high concentrations of BSA (up to 10 mg/ml) was first reported in the case of the insulin receptor kinase (10). Over a 20-fold stimulation of phosphorylation of poly($E_1A_1Y_1$) by src kinase in the presence of groEL (640 μg/ml) was observed. In contrast, an activator obtained from extracts of bovine membranes stimulates the phosphorylation of both polymers. We suggest that it affects the enzyme directly. We also describe a heat-labile factor present in extracts from bovine membranes that inhibits preferentially c-src activity, an inhibition that is enhanced in the presence of the src kinase activator.

MATERIALS AND METHODS

Samples of chaperonins groEL and groES were gifts from G. Lorimer of E. I. du Pont de Nemours. The heat shock proteins Hsp70 and Hsp78 from calf liver and a mixture of heat shock proteins from HeLa cells, all purified by ATP agarose chromatography, were donated by H. Weissbach of Roche Institute of Molecular Biology. Other reagents and inhibitors were obtained from Sigma or as described previously (5, 6). Pigeon cytochrome *c* and cytochrome *c* peptides containing residues 1–80 or 1–65, obtained by cyanogen bromide cleavage, were gifts from A. Schejter and E. Margoliash of Northwestern University. Frozen insect Sf9 cells infected with recombinant baculovirus containing c-*src* or v-*src* genes were generously donated by R. Clark and F. McCormick of Cetus. Monoclonal antibody 327 and hybridoma cells expressing the antibody were gifts from J. Brugge of The University of Pennsylvania. Two c-Ha-ras proteins were donated by J. B. Gibbs of Merck Sharp & Dohme. The T24 oncogenic ras and the yeast RAS2 were prepared by R. J. Resnick as described (11).

Protein Kinase Assay. Src kinases were assayed in a final volume of 50 μl containing 50 mM Tris Cl (pH 7.4), 10 mM $MgCl_2$, 1 mM dithiothreitol, 5 μg of the synthetic random amino acid polymers, and indicated amounts of src kinase preparation. The reaction was initiated by addition of 10 μM [γ-^{32}P]ATP (4000–6000 cpm/pmol) and analyzed on filter paper squares and by SDS/PAGE as described (2, 5).

Purification of src Kinases from Baculovirus-Infected Insect Cells. Frozen transformed insect cells (≈0.3 ml) were suspended in 1 ml of buffer A [20 mM Tris Cl (pH 7.3), 0.1 mM EDTA, 10 mM Mg^{2+}, 4 μg of leupeptin, Trasylol at 17 μg/ml, and 2 mM phenylmethylsulfonyl fluoride] and centrifuged in an Eppendorf microfuge for 15 min at maximum speed. The

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Abbreviations: PK, protein kinase; BSA, bovine serum albumin; EGF, epidermal growth factor. The synthetic polymers are designated as follows: the single-letter code is used for the amino acids and subscripts indicate the molar ratios.

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supernatant or 1% Triton X-100 cell extracts were purified by affinity chromatography using synthetic polymers as will be described elsewhere.

Purification of Activator and Inhibitor of Poly(E_4Y_1) Phosphorylation by c-src Kinase. *Isolation of bovine brain membranes.* Frozen bovine brains (500 g) were homogenized in 2 liters of 0.25 M sucrose, 20 mM Tris Cl (pH 7.4), and 2 mM EDTA in a Waring blender at maximum speed for 30 sec in three equal 10-sec bursts and then disrupted in a Polytron at high speed by two equal 30-sec bursts. The suspension was centrifuged for 20 min at 1500 × *g*. The supernatant was collected through four layers of cheesecloth and centrifuged at 4°C at 11,000 × *g* for 45 min. The supernatant was collected as described above and centrifuged for 45 min at 150,000 × *g*. The pellet was collected and suspended in 150 ml of 0.25 M sucrose and 20 mM Tris Cl (pH 7.4) and centrifuged as described above, and the pellet was suspended in 60 ml of the same buffer. The suspension was frozen in liquid nitrogen and stored at −70°C (protein concentration = 17–20 mg/ml).

Solubilization of bovine brain membranes. Fifteen milliliters of bovine membranes (≈250 mg) was suspended in 50 ml of buffer B [20 mM Tris Cl (pH 7.4), 10 mM thioglycerol, and 10% (vol/vol) glycerol] containing 1% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride (final concentration). The mixture was incubated for 20 min at 4°C and then centrifuged for 60 min at 200,000 × *g* at 4°C.

Separation of activator and inhibitor. DEAE-Sephacel (Sigma) was loaded into a 10-ml disposable plastic syringe. After equilibration with buffer B containing 0.1% Triton X-100, 23 ml (≈68 mg) of solubilized membrane was loaded onto the column, and the column was washed with 2 column volumes of buffer B and then eluted with 2 column volumes of buffer B containing 0.1, 0.3, 0.5, and 1.0 M NaCl. Fractions containing salt were dialyzed for 15 hr against 2 liters of buffer B containing 0.1% Triton X-100 and tested for activation or inhibition of c-src and v-src. Fractions containing 0.5 and 1.0 M NaCl were heated for 5 min at 90°C and centrifuged, and the supernatant was dialyzed for 46 hr against 4 liters of distilled water (with two changes) and lyophilized.

RESULTS

Stimulation of Phosphorylation of Poly(E_1Y_1) and Poly($E_1A_1Y_1$) by src Tyrosine Kinases by BSA and Intracellular Chaperones. The phosphorylation of poly(E_1Y_1) or poly($E_1A_1Y_1$) by either c-src or v-src kinase was very slow compared to the phosphorylation of poly(E_4Y_1) (Table 1). Addition of BSA, up to 8 mg/ml, enhanced the phosphorylation of these polymers by either c-src or v-src kinase by 20- to 100-fold. Poly($E_1A_1Y_1$) was more rapidly phosphorylated than poly(E_1Y_1). The phosphorylation of poly($E_6A_3Y_1$) was stimulated only 2-fold or less, whereas little or no enhancement was observed with poly(E_4Y_1) as substrate. Since high concentrations of BSA were required for maximal stimulation of poly(E_1Y_1) and poly($E_1A_1Y_1$) phosphorylation, we considered the possibility that a contaminant in BSA was responsible, similar to our observations with PK-C (6). However, the purified protein kinase C (PK-C) activator did not stimulate the src kinase (data not shown). Although four samples of BSA from different sources showed one major band when 10 μg was analyzed by SDS/PAGE and gave the same stimulation, the presence of a very potent chaperone contaminant has not been ruled out. Several intracellular unidentified components in the cytosol of fibroblasts and yeast (data not shown) effectively substituted for BSA as well as several identified proteins (Table 2). At 0.5 mg/ml, some cytochrome *c* preparations activated the phosphorylation of poly($E_1A_1Y_1$) by c-src, sometimes as well as or better than BSA, whereas ovalbumin and gamma globulin were virtually inactive. Cytochrome *c* and myoglobin (as well as polylysine) are basic proteins that stimulated poly($E_1A_1Y_1$) phosphorylation markedly. A curious effect was encountered with cleavage products of pigeon cytochrome *c*. At 0.5 mg/ml, they had little effect, whereas at 0.1 mg/ml (which was optimal) they stimulated better than BSA at 0.1 mg/ml, though still considerably less than BSA at 0.5 mg/ml. Microperoxidase (peptide containing residues 11–21 of cytochrome *c*) was inactive.

Several heat shock proteins (groEL, Hsp70, Hsp78, a mixture of heat shock proteins from HeLa cells, and DnaK) were tested at 0.1 mg/ml in this system. All except DnaK were as good or better than BSA at 0.1 mg/ml. At 0.5 mg/ml, however, BSA stimulated 100-fold, and groEL stimulated about 15- to 20-fold. Of particular interest is that groEL at 1 mg/ml stimulated after 60 min the phosphorylation of poly($E_1A_1Y_1$) 37-fold, whereas poly(E_4Y_1) phosphorylation was inhibited 60% (Table 3). These observations are consistent with the proposal that the effect of heat shock proteins is on the substrate rather than on the enzyme.

Effect of Polymer Concentrations on the Rate of Phosphorylation by src Kinase. A clue to the difference in response between poly(E_4Y_1) and poly($E_1A_1Y_1$) was obtained by titration of the polymer substrates. The rate of poly(E_4Y_1) phosphorylation by src kinase increased up to 2 mg of poly(E_4Y_1) per ml. The phosphorylation of poly($E_1A_1Y_1$) in the presence of BSA increased with concentration only up to about 100 μg of polymer per ml and then declined (Fig. 1*A*).

Table 1. Effect of BSA on the phosphorylation of synthetic random polymers by c-src and v-src tyrosine kinases

	Phosphorylation, cpm			
	c-src		v-src	
Polypeptide	− BSA	+ BSA	− BSA	+ BSA
Poly(E_4Y_1)	34,700	35,600	30,400	46,900
Poly(E_1Y_1)	600	18,600	700	12,700
Poly($E_6A_3Y_1$)	2,500	4,100	13,900	21,600
Poly($E_1A_1Y_1$)	1,000	61,400	700	35,200
None	200	300	1,700	1,200

The experimental conditions were as described in *Materials and Methods* with 5 μg of the polymers, 100 ng of affinity column-purified src kinases, and where indicated 4 mg of BSA. After a 5-min incubation at room temperature, 20-μl samples were placed on filter paper squares, dried, and assayed for radioactivity. The activity of the [γ-^{32}P]ATP was 3000 cpm/pmol.

Table 2. Stimulation of phosphorylation of poly($E_1A_1Y_1$) by various proteins

		Phosphorylation, cpm		
Addition	Conc., mg/ml	15 min	30 min	60 min
None		240	370	300
BSA	0.5	36,000	67,000	78,000
Myoglobin	0.5	23,000	30,000	37,000
Ovalbumin	0.5	2,600	3,000	2,800
Gamma globulin	0.5	2,600	3,200	2,200
Horse cytochrome *c*	0.5	19,000	23,000	30,000
Pigeon cytochrome *c*				
Full-length peptide	0.5	52,000	73,000	85,000
Residues 1–80	0.5	1,400	3,000	6,600
Residues 1–64	0.5	350	660	1,200
Residues 1–80	0.1	17,000	26,000	29,000
Residues 1–64	0.1	12,000	17,000	24,000

Experimental conditions were as described in *Materials and Methods* with poly($E_1A_1Y_1$) as substrate in the presence of 100 ng of c-src and the indicated amounts of proteins. Samples (10 μl) were taken at the indicated times of incubation at room temperature. The activity of the [γ-^{32}P]ATP was 4000 cpm/pmol.

Table 3. Effect of groEL on the phosphorylation of poly($E_1A_1Y_1$) and poly(E_4Y_1) by c-src kinases

Substrate	c-src kinase, ng	groEL, mg/ml	Phosphorylation, cpm 15 min	30 min
Poly($E_1A_1Y_1$)	200	0	1,700	2,000
	200	0.32	16,000	19,000
	200	0.64	26,000	45,000
	200	1.03	42,000	74,000
Poly(E_4Y_1)	100	0	29,000	64,000
	100	0.32	36,000	64,000
	100	0.64	28,000	49,000
	100	1.03	13,000	25,000

Experimental conditions were as described in *Materials and Methods* with the indicated amounts of c-src kinase and groEL. Substrates were used at 100 μg/ml. Incubation was at room temperature for 15 and 30 min.

In the absence of BSA, there was significant activity at 5 and 10 μg of polymer per ml, which decreased rapidly with increasing concentration. There was negligible activity at 100 or 200 μg/ml (Fig. 1*B*). It seems likely therefore that at the higher concentrations these lesser charged polymers interact with each other, thereby preventing access to the enzyme, whereas the more negatively charged poly(E_4Y_1) remains susceptible to phosphorylation.

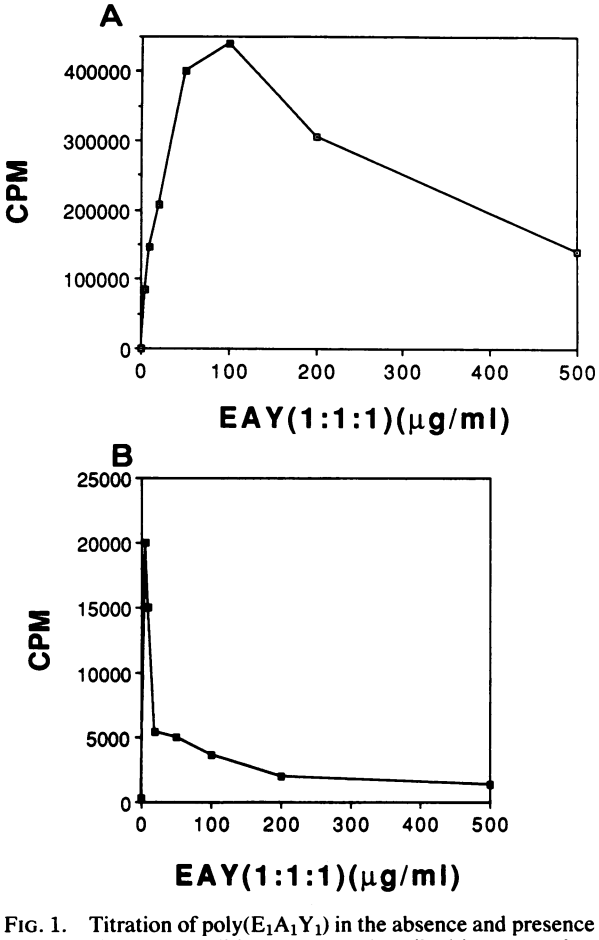

FIG. 1. Titration of poly($E_1A_1Y_1$) in the absence and presence of BSA. Experimental conditions were as described in *Materials and Methods* except that 20 μM [γ-^{32}P]ATP (4800 cpm/pmol), 100 ng of c-src kinase, and the indicated concentrations of poly($E_1A_1Y_1$) were used. After 30 min at room temperature, 20-μl samples were assayed on filter paper squares. (*A*) In the presence of BSA (4 mg/ml). (*B*) In the absence of BSA.

Inhibitors and Stimulators of c-src Kinase with Poly(E_4Y_1) as Substrate. c-src kinase activity is controlled by phosphorylation of the tyrosine residue at 527 at the C terminus (12, 13). Some evidence suggests that this reaction is not catalyzed by autophosphorylation, but by an unidentified protein tyrosine kinase (14, 15). A purified enzyme from neonatal rat brain, which differs from c-src kinase, has been reported to phosphorylate Tyr-527 of c-src (15). On the other hand, experiments with c-src mutants expressed in yeast strongly suggest that c-src can phosphorylate an inactive c-src mutant at Tyr-527 (16). Our observation that phosphorylation rates by c-src kinase are linear up to 60 min suggests that either another enzyme for Tyr-527 has been separated or that the autophosphorylation of Tyr-527 by src requires a chaperone. Accordingly, we undertook a search for a factor that preferentially inhibits the c-src kinase using poly(E_4Y_1) as substrate. Indeed, membrane extracts from mouse or bovine brain or from Rat1 cells were found to contain a heat-labile factor that inhibited c-src kinase preferentially to v-src kinase. The crude extract, however, also contained a heat-stable activator that stimulated the phosphorylation of poly(E_4Y_1). A separation of the activator from the inhibitor from bovine brain was achieved by DEAE chromatography. The inhibitor was eluted between 0.1 and 0.3 M NaCl; the activator was eluted between 0.5 and 1 M NaCl.

The effect of the partially purified inhibitor from bovine brain on the phosphorylation of poly(E_4Y_1) by src kinases is shown in Table 4. In the presence of 90 ng of the inhibitor (DEAE, 0.1 to 0.3 M NaCl eluate), the activity of c-src was reduced by 68%, while the activity of v-src was only reduced by 15%. With some preparations of inhibitor, the difference between c- and v-src kinase was more pronounced, with some, less. As shown in Table 5, there was a significant increase in the percent inhibition when the inhibitor was tested in the presence of the activator.

Effect of Polylysine on the Phosphorylation of ras Proteins and Calmodulin by c-src Kinase. Four different preparations of ras proteins (1 μg) were used. Two were different samples of c-Ha-ras, one was yeast RAS2, and one was the oncogenic ras T-24. As shown in Fig. 2*A*, with all ras proteins there was little or no phosphorylation by src kinase in the absence of polylysine. Optimal stimulations were observed at about 0.2 μM polylysine (41,000 Da); 2 μM resulted in much less phosphorylation. The phosphorylated bands of ras were alkali-stable. The preparation of src kinase used in this experiment (Fig. 2*A*) contained an unidentified impurity at a

Table 4. Phosphorylation of poly(E_4Y_1) by c- and v-src kinase in the presence of an inhibitor from brain plasma membranes

Kinase	Inhibitor, ng	Phosphorylation, cpm 30 min − BSA	30 min + BSA	60 min − BSA	60 min + BSA
c-src	0	36,800	38,000	68,000	62,000
	90	12,200	14,400	22,400	35,800
	180	7,300	12,300	15,000	21,000
	270	5,300	7,600	8,200	13,500
	360	3,800	6,600	5,300	12,000
v-src	0	32,500	33,600	41,900	40,700
	90	27,500	28,700	32,700	33,600
	180	17,800	22,300	24,700	29,500
	270	14,800	17,700	24,700	27,200
	360	10,200	15,300	16,500	20,500

Experimental conditions were as described in *Materials and Methods*. Poly(E_4Y_1) was used as substrate with 250 ng of c-src or 600 ng of v-src in the absence and presence of 25 μg of BSA and the indicated amounts of inhibitor purified by DEAE chromatography. After 30 and 60 min at room temperature, 15-μl samples were assayed by the filter paper method.

Table 5. Effect of activator and inhibitor from bovine brain on src kinase activity

Addition	30 min		60 min	
	cpm	% inhibition	cpm	% inhibition
None	38,000		75,000	
Activator	61,000		102,000	
Inhibitor	24,000	37	31,000	59
Both	16,000	74	21,000	78

In a final volume of 50 μl containing 5 μg of poly(E_4Y_1), 20 mM Tris Cl (pH 7.4), 10 mM $MgCl_2$, 1 mM dithiothreitol, and [γ-^{32}P]ATP (4000 cpm/pmol), 30 ng of c-src kinase was added in the absence or presence of 300 ng of activator (0.5–1 M NaCl eluate from the DEAE column) or 4.4 μg of inhibitor (0.1–0.3 M NaCl eluate from the DEAE column) or both together. After a 30- or 60-min incubation at room temperature, 20-μl samples were placed on filter paper squares and assayed for radioactivity.

M_r of about 58,000 (just below src kinase) that was also phosphorylated in the presence of polylysine. Some impurities present in the ras preparations were also phosphorylated at 0.2 μM polylysine. It therefore appears that this phenomenon is not infrequent. In this experiment, 41,000-Da polylysine was used but several larger and smaller preparations were equally effective. As can be seen from Fig. 2*B*, calmodulin was also phosphorylated by src kinase in the presence of polylysine, but in contrast to ras, there was only a minor decrease of phosphorylation at 2 μM, which became more apparent at 5 μM. The autophosphorylation of src kinase at a M_r of 60,000 was diminished at the higher polylysine concentrations. Impurities in calmodulin that appeared below a M_r of 14,000 were optimally phosphorylated at 1 μM polylysine. This points to the repeatedly observed fact (17) that the effect of polylysine and of other modulators varies greatly with different substrates.

DISCUSSION

There are numerous examples for the modulation of substrates that make them more susceptible to phosphorylation by protein kinases. The first examples for such substrate modulators are spermidine and spermine, which enhance the phosphorylation of casein by casein kinase 2 (18, 19). PK-P, a membranous protein serine (threonine) kinase (20) related to casein kinase 2, is markedly stimulated by histone 1 or polylysine or by a natural modulator isolated from human platelets (17). PK-P phosphorylates some substrates in the absence of a modulator, whereas other substrates may show partial or even complete dependence on the presence of the modulator (17). Another example is the stimulation of the phosphorylation by PK-C of some substrates (e.g., lamin B) by an acidic contaminant of the BSA preparation or by some intracellular acidic components (6), including RNA or DNA (S.Z. and E.R, unpublished observation). Modulators can either stimulate or inhibit the phosphorylation of substrates. We have shown (17) that the phosphorylation by EGF receptor of lipocortin 1, which has an isoelectric point of 6.8, is strongly inhibited by polylysine, which serves as a chaperone, preventing a fertile interaction. On the other hand, acetyl lipocortin, which has an isoelectric point of 4.9, is very poorly phosphorylated by the EGF receptor but becomes a good substrate at high concentrations of polylysine, which now serves as a facilitator of the interaction. The autophosphorylation of the EGF receptor and the phosphorylation of several endogenous membrane proteins is greatly stimulated by polylysine. Polylysine and other basic proteins also markedly stimulate phosphorylation of membrane proteins and of calmodulin catalyzed by the insulin receptor (21–24). It was proposed (22, 23) that the basic proteins interact with an acidic region of the insulin receptor, thereby stimulating its

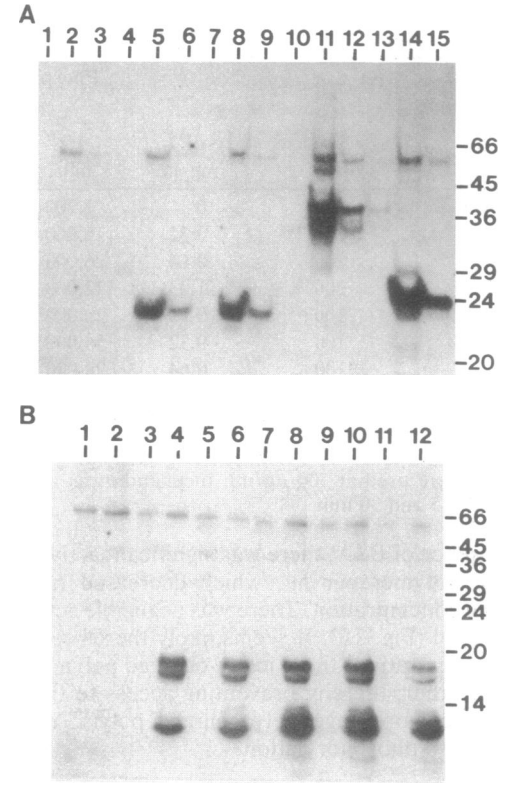

FIG. 2. Effect of polylysine on the phosphorylation of ras proteins and calmodulin by c-src kinase. (*A*) Phosphorylation of three different ras proteins. Affinity column-purified c-src kinase (60 ng), 10 μM ATP (3200 cpm/pmol), 1 μg of ras protein, and various amounts of polylysine were added. The samples (50 μl) were incubated for 60 min at room temperature and analyzed by SDS/PAGE followed by autoradiography. Lanes 1–12 contain c-src kinase (40 ng) with the following additions. Lane 1, none; lane 2, 0.2 μM polylysine; lane 3, 2 μM polylysine; lane 4, Ha-ras; lane 5, Ha-ras plus 0.2 μM polylysine; lane 6, Ha-ras plus 2 μM polylysine; lane 7, Ha-ras (a second sample); lane 8, Ha-ras plus 0.2 μM polylysine; lane 9, Ha-ras plus 2 μM polylysine; lane 10, yeast ras2; lane 11, yeast ras2 plus 0.2 μM polylysine; lane 12, yeast ras2 plus 2 μM polylysine; lane 13, T24 ras; lane 14, T24 ras plus 0.2 μM polylysine; lane 15, T24 ras plus 2 μM polylysine. (*B*) Phosphorylation of calmodulin by src kinase at various concentrations of polylysine. Incubations were as described for *A*. Lanes 1–12 contain c-src kinase (80 ng) with the following additions. Lane 1, none; lane 2, calmodulin (5 μg); lane 3, 0.2 μM polylysine; lane 4, calmodulin plus 0.2 μM polylysine; lane 5, 0.5 μM polylysine; lane 6, calmodulin plus 0.5 μM polylysine; lane 7, 1 μM polylysine; lane 8, calmodulin plus 1 μM polylysine; lane 9, 2 μM polylysine; lane 10, calmodulin plus 2 μM polylysine; lane 11, 5 μM polylysine; lane 12, calmodulin plus 5 μM polylysine. The sizes of the bands ($M_r \times 10^{-3}$) are indicated at right.

catalytic activity. This differs from our proposal (17) that the basic proteins interact with the substrates (including the receptors themselves) and induce a conformational change that makes them more susceptible to phosphorylation. Thus, they act as substrate modulators rather than as enzyme activators. This view is supported by our studies with acidic activators of PK-C (6) and the data reported in this paper. It has been known for many years that denaturation of proteins makes them often more susceptible to proteolysis and phosphorylation by making new sites available for enzymatic attack. We propose that cells contain substrate chaperones that fulfill a similar function by inducing conformational changes in native proteins that render them either more or less susceptible to posttranslational modifications. At this state of the purification of the c-src inhibitor from bovine brain, we cannot conclude whether it is a modulator that enhances the phosphorylation of Tyr-527 by c-src or a

specific Tyr-527 kinase. It is conceivable that both mechanisms operate in intact cells as indicated by published data (15, 16).

There has been an increasing awareness of the role of chaperones in the folding and assembly of proteins and in protein transport (25–31). The first examples of chaperones were two genes (*groEL* and *groES*) that are required for the assembly of the tail of bacteriophages λ and T4 (27, 32, 33). It was shown that nucleoplasmin prevents the random aggregation between histones and DNA and facilitates their formation of nucleosomes, and the name molecular chaperone was coined (31). The name chaperonin was used (25) to describe a group of homologous chaperones in prokaryotes, mitochondria, and chloroplasts. At the biochemical level, the most penetrating studies were performed with bacterial ribulose-bisphosphate carboxylase (Rubisco), a key enzyme in photosynthesis (25, 26). The product of the *groEL* gene keeps Rubisco protein that has been denatured (e.g., with urea) in the proper conformational state, preventing random aggregation—a chaperone in the true sense of the word. Addition of the groES protein in the presence of ATP and Mg^{2+} facilitates, in subsequent steps, the formation of properly folded monomer and then of a catalytically active dimer (26). groEL functions as a chaperonin; groES functions as a match-maker. Based on our studies mentioned above, we propose that the concept of chaperones be extended to the activation of substrates for phosphorylation by a mechanism akin to the action of groEL, which keeps Rubisco in the unfolded form.

It is therefore of special interest that groEL and several other heat shock proteins increase the susceptibility of substrates to phosphorylation. It may be possible to increase the intracellular concentrations of certain chaperones by heat shock or genetic manipulations and establish a physiological significance to the phenomenon of substrate activation for phosphorylation described here. Of particular interest is the phosphorylation of ras proteins by src kinase in the presence of polylysine.

The observations that polylysine, chaperones, and intracellular activators and inhibitors profoundly alter the patterns of phosphorylation, and that differences were observed in the response by c-src and v-src kinases, raise the question of a participation of chaperone activators and inhibitors in the control of kinase activities in normal and transformed cells.

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