Spermine Inhibits the Phosphorylation of the 11,000- and 10,000-Dalton Nuclear Proteins Catalyzed by Nuclear Protein Kinase NI in NB-15 Mouse Neuroblastoma Cells*

(Rceived for publication, August 20, 1985)

Rati Verma and Kuang Yu Chen
From the Department of Biochemistry and Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08893

The nuclear protein kinase NI (NI kinase) was purified from NB-15 mouse neuroblastoma cells by phosphocellulose column and casein affinity column chromatography. The purified NI kinase exhibited (i) an apparent subunit molecular weight of about 37,000, (ii) auto phosphorylation, and (iii) insensitivity to inhibition by heparin. When NI kinase was added to heat treated neuroblastoma nuclei in the presence of [γ-32P] ATP, two proteins with apparent subunit molecular weights of 11,000 and 10,000 were prominently phosphorylated. Other protein kinases tested including the nuclear protein kinase NII, Type I cAMP-dependent protein kinase, and protein kinase C did not catalyze the phosphorylation of these two proteins. The NI kinase-catalyzed phosphorylation of these two proteins was completely inhibited by 1 mM spermine. In contrast, 10 mM putrescine, 2 mM spermidine, 5 mM arginine, and 10 mM NH4Cl, had no inhibitory effect on this phosphorylation reaction. Our study also indicated that the phosphorylation of the 11,000- and 10,000-dalton proteins occurred in the nuclear matrix fraction but not in heterogeneous nuclear ribonucleoproteins, high mobility group proteins, or histone fractions. We have previously reported that spermine specifically inhibits the endogenous phosphorylation of an 11,000-dalton nuclear protein in various mammalian cell lines (Chen, K. Y., and Verma, R. (1984) Biochem. Biophys. Res. Commun. 118, 710–716). The present study suggests that the 11,000- and 10,000-dalton nuclear proteins may be native substrates of nuclear protein kinase NI and that their phosphorylation can be affected by physiological concentrations of spermine.

Polymamines (putrescine, spermidine, and spermine) are naturally occurring organic cations widely distributed in living organisms (for reviews see Refs. 1–3). Abundant evidence has been reported in the literature indicating that polyamines play an important role in growth regulation of eukaryotic and prokaryotic cells (1–3). We previously reported that differentiation of mouse neuroblastoma cells is accompanied by significant changes in polyamine metabolism which include decrease in ornithine decarboxylase activity and polyamine content (4), polyamine transport (5), metabolic labeling of an 18,000-dalton protein (6), and an increase of putrescine to γ-amino butyric acid conversion (6). Moreover, we found that α-monofluoromethyl ornithine, a mechanism-based suicide inhibitor of ornithine decarboxylase, acts synergistically with suboptimal concentrations of dibutyryl cAMP in eliciting a maximal differentiation of mouse neuroblastoma cells (7). However, the precise molecular action of polyamines in the differentiation of mouse neuroblastoma cells remains to be defined.

Phosphorylation-dephosphorylation of nuclear proteins, either histones or nonhistone proteins, occurs ubiquitously in eukaryotic cells and appears to be intimately related to the regulation of gene expression (6–10). That polyamines may exert some of their growth regulatory effects by affecting nuclear protein phosphorylation is suggested by results of the following experiments: (i) polyamines affect the endogenous protein phosphorylation pattern of chromatin fractions isolated from various organisms (11–16), and (ii) polyamines have been shown to stimulate certain protein kinase activities including NII nuclear kinase/casein kinase II (17, 18) and affect other cyclic nucleotide-independent protein kinases (19). We have previously reported that polyamines, particularly spermine, inhibit the endogenous phosphorylation of 11,000- and 10,000-dalton nuclear proteins in NB-15 mouse neuroblastoma cells and other cell lines (20, 21). The presence and apparent ubiquity of the phosphorylation of the 11,000- and 10,000-dalton proteins, together with the specificity of the inhibitory action of polyamines, prompted us to search for the native kinase system(s) which phosphorylate these two proteins. In this paper, we report that purified NII nuclear kinase can phosphorylate these two proteins in nuclear preparations whose endogenous kinase activity has been inactivated by brief heat treatment and that phosphorylation is inhibited by spermine at physiological concentrations. These data suggest that the 11,000- and 10,000-dalton proteins may be native substrate proteins for the endogenous NII nuclear kinase.

EXPERIMENTAL PROCEDURES

Cell Culture—NB-15 mouse neuroblastoma cells were grown as monolayer cultures in Dulbecco’s modified Eagle’s medium (with 4500 mg of glucose/liter) supplemented with 10% fetal calf serum under conditions previously described (21). Cells at 80–90% confluency were used for all studies described.

Preparation of Intact Nuclei—Approximately 3.0 × 10⁸ NB-15 cells were harvested, washed, and freeze-thawed once in 145 ml of a homogenization buffer containing 0.05 M Tris-HCl (pH 7.4), 0.25 M sucrose, 3 mM MgCl₂, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride (Buffer A). The homogenate was centrifuged at 1,000
x g for 5 min, and the pellet was washed once with Buffer A containing 0.25% Triton X-100. The pellet was resuspended in the same buffer, mixed with a 2.2 mM sucrose solution containing 0.01 M MgCl₂ and 0.05 M Tris- HCl (pH 8.0), and centrifuged at 25,000 rpm in a SW 28.1 Beckman rotor for 70 min at 4 °C. The pellet obtained greater than 95% intact nuclei as judged by phase contrast microscopy.

Preparation of Nucleoplasm—The purified nuclei were resealed twice by resuspending in a hypotonic solution containing 75 mM NaCl, 25 mM EDTA (pH 8.0), and 0.5 mM phenylmethylsulfonyl fluoride followed by centrifugation at 8000 × g for 10 min. The pellet obtained was further extracted four times with 10 mM Tris- HCl (pH 8.0) containing 0.5 mM phenylmethylsulfonyl fluoride and centrifuged at 8000 × g for 10 min. The supranatant solutions were combined and dialyzed against Buffer B (20 mM Tris-HCl, 0.1 mM EDTA, 10 mM β-mercaptoethanol, 10% glycerol, and 0.25 mM phenylmethylsulfonyl fluoride) containing 0.3 M NaCl.

Purification of the NI Nuclear Kinase—NI nuclear kinase was purified from NB-15 mouse neuroblastoma cells using nucleasepase as the starting material. Nucleasepase was concentrated by pressure filtration using an Amicon concentrator equipped with a PM 10 membrane. The concentrated nucleasepase was then loaded onto a phosphocellulose column (0.5 × 20 cm) equilibrated with Buffer B containing 0.5 M NaCl. The kinase activity was determined in an assay mixture of 75 mL containing 50 mM Tris- HCl (pH 7.4), 5 mM MgCl₂, 1 mM dithiothreitol, 50 µM [γ-32P]ATP, 100 mM NaCl, and 2 mg/mL of calf thymus casein. The kinase was incubated at 30 °C for 30 min at room temperature and then for 2 h at 4 °C. The digested nuclei were then washed three times with the same buffer and then eluted with the 0.5 M NaCl in Buffer B. The kinase activity was measured in an assay mixture of 50 mL containing 50 mM Tris- HCl (pH 7.4), 5 mM MgCl₂, 1 mM dithiothreitol, 50 µM [γ-32P]ATP, 100 mM NaCl, and 2 mg/mL of calf thymus casein. The kinase was incubated at 30 °C for 20 min. The activity obtained was increased by 2.3 times when the buffered nuclei were gently stirred in a buffer containing 10 mM Tris- HCl (pH 7.0), 100 mM NaCl, 1 mM MgCl₂, and 1 mM dithiothreitol at 4 °C for 20 min. The pellet was then extracted three times with the same buffer (pH 8.0 instead of 7.0) at room temperature. The extracts were pooled, concentrated, and stored at −70 °C. The preparation, though not pure, did exhibit an enrichment in proteins characteristic of the nHNP fraction, particularly the C-proteins with apparent molecular weights of 44,000 and 42,000 (Fig. 7b, lane H).

The HMG proteins were prepared according to the method of Paulson and Taylor (27). The nuclei were extracted with 5% perchloric acid and centrifuged at 10,000 × g for 15 min. HMG proteins were then obtained by precipitation at 25% of trichloroacetic acid. The pellet was washed twice with acetone, dried, resuspended in distilled H₂O, and stored frozen. The gel electrophoresis of HMG proteins was carried out according to the procedure described by Berezney et al. (28) using a 15% polyacrylamide gel as shown in Fig. 8.

Other Procedures—Dephosphorylated a-casein was prepared according to the procedure of Thornburg and Lindell (29). Casein-Sepharose affinity resin was prepared by the method of Farron, Furstenthal, and Lightholler (30). Protein concentrations were determined by either the Lowry method (31) or the method of Bradford (32).

Materials—Putrescine, spermidine, spermine, phenylmethylsulfonyl fluoride, dithiothreitol, and other biochemicals were purchased from Sigma. All tissue culture media and sera were obtained from Gibco Laboratories. [γ-32P]ATP (3000 Ci/mmol) was purchased from Amersham Corp. Protein kinase C and Type I cAMP-dependent protein kinase were generous gifts of Dr. A. Cheng (National Cancer Institute, NIH) and Dr. A. Y. C. Liu (Rutgers University), respectively. Monospecific antiserum against calf thymus casein kinase I was kindly given to us by Dr. M. S. Darmus (University of California, Davis). NI nuclear kinase was purified from NB-15 mouse neuroblastoma cells.

RESULTS

Purification and Characterization of the NI Nuclear Kinase—Nuclear protein kinase NI was purified 986-fold over the crude casein kinase activity present in nucleoplasm by phosphocellulose and casein-Sepharose affinity column chromatography (Table I). The increase of the total activity in nucleoplasm (6,659 units) over that of the nuclei (1,743 units) was probably due to removal of some inhibitory substances. The NI kinase purified from NB-15 mouse neuroblastoma cells was used for subnuclear fractionation at all times.

TABLE I

<table>
<thead>
<tr>
<th>Purification of nuclear protein kinase NI from NB-15 mouse neuroblastoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinase activity was measured in the standard assay mixture containing 25 µL of each fraction as described under &quot;Experimental Procedures&quot; using a-casein (2 mg/ml) as a substrate. One unit is defined as the amount of enzyme which catalyzed the incorporation of 1 pmol of phosphate into dephosphorylated a-casein/min at 30 °C and at pH 7.4.</td>
</tr>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Nuclei</td>
</tr>
<tr>
<td>Nucleoplasm</td>
</tr>
<tr>
<td>Casein-Sepharose</td>
</tr>
</tbody>
</table>

*Numbers in the parentheses indicate values that would be obtained if purification were calculated starting from the nuclei instead of the nucleoplasm.
cells shared the following characteristics with NI kinase and casein kinase I purified from other sources (33, 34). (i) It was eluted at 0.5–0.6 M NaCl on a phosphocellulose column (Fig. 1A). Many protein kinases bind to the phosphocellulose column, but nearly all of them except casein kinases I and II (or NI and NII nuclear kinases) can be eluted by the 0.3 M NaCl wash (35, 36). (ii) It had an apparent subunit molecular weight of 37,000 on sodium dodecyl sulfate-polyacrylamide gel (Fig. 2, lane A) as determined by immunoblotting procedure using a monospecific antiserum against calf thymus casein kinase I (a gift of Dr. M. E. Dahmus). This result indicates that our nuclear kinase preparation and calf thymus casein kinase I are immunologically related. Since our enzyme was isolated from purified nuclei, we designated it as nuclear protein kinase NI using the nomenclature of Desjardins et al. (37). (iii) It underwent autophosphorylation (Fig. 2, lane B). (iv) Heparin, a potent inhibitor of NII nuclear kinase/casein kinase I1 (38), was not effective in inhibiting its activity (Fig. 3). We have also tested the effects of cAMP, GMP, Ca2+/calmodulin, and Ca2+/phosphatidylserine on NI kinase activity and found that none was stimulatory (data not shown), suggesting that our NI kinase preparation was free of other kinase contamination.

Nuclear Protein Kinase NZ Catalyzed the Phosphorylation of 11,000- and 10,000-dalton proteins with apparent subunit molecular weights of 11,000- and 10,000-daltons were prominently phosphorylated in an endogenous protein phosphorylation reaction of isolated nuclei (20, 21). The phosphorylation of both nuclear proteins can be inhibited by 1 mM spermine (20, 21). To identify the kinase system(s) which may phosphorylate

**Fig. 1.** A, phosphocellulose chromatography of NB-15 mouse neuroblastoma nuclear protein kinase activities. Nucleoplasm, prepared as described under “Experimental Procedures,” was applied to a column (1.5 x 20 cm) of phosphocellulose equilibrated with Buffer B containing 0.3 M NaCl. After adsorption, the column was washed with Buffer B and eluted with a linear gradient of 0.3–1.0 M NaCl in Buffer B at a flow rate of 0.5 ml/min. Aliquots (25 µl) of each fraction were assayed for protein kinase activity as described under “Experimental Procedures.” Fractions from peak I were pooled, concentrated, and designated as NI kinase. ---, kinase activity; -- - , NaCl gradient.

**Fig. 2.** A, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting of NI kinase. Electrophoresis of casein-Sepharose purified NI kinase was carried out on a linear 7–15% polyacrylamide gel as described under “Experimental Procedures.” The subunits from the gel were transferred to nitrocellulose paper essentially by the method of Towbin et al. (44) except that 0.1% sodium dodecyl sulfate was present in the transfer buffer. The paper was incubated with antiserum to calf thymus casein kinase I overnight, and the bound antibody was visualized by using peroxidase-linked anti-rabbit IgG antibody. The molecular weight of the subunit was determined by using prestained standards from Bethesda Research Laboratories which were run on a parallel lane in the gel and transferred to nitrocellulose simultaneously with the enzyme. B, autophosphorylation of NI protein kinase. NI kinase, purified by casein-Sepharose chromatography, was added to a 75-µl solution containing 80 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 50 µCi [γ-32P] ATP, 1 mM dithiothreitol, and the assay mixture was incubated at 30° C for 30 min. The reaction was stopped by the addition of ½ volume of Laemmli’s stop solution. Electrophoresis under denaturing conditions and autoradiography were carried out as described under “Experimental Procedures.”

**Fig. 3.** Effect of heparin on nuclear protein kinases NI and NII purified from NB-15 mouse neuroblastoma cells. Protein kinase activity was assayed in the standard assay mixture (described under “Experimental Procedures”) containing either 2 units of casein-Sepharose purified NI kinase (O—O) or 4 units of purified NI kinase (O—O), 2 mg/ml of dephosphorylated a-casein, and various concentrations of heparin.
these two proteins, we examined the pattern of protein phosphorylation upon addition of purified protein kinases to heat-treated nuclei. Brief heat treatment of ruptured nuclei at 60 °C for 5 min was sufficient to inactivate all endogenous kinase activities (Fig. 4, lane A) but did not affect the ability of nuclear proteins to serve as substrates for exogenously added protein kinases (Fig. 4, lanes B, D, F, and H). Among all the protein kinases that we have examined, only NI nuclear kinase phosphorylated the 11,000- and 10,000-dalton proteins (Fig. 4, lane B). Moreover, this phosphorylation was sensitive to inhibition by spermine (Fig. 4, lane C). Cyclic AMP-dependent protein kinase (Type I) and the NI nuclear kinase phosphorylated many nuclear proteins but not the 11,000- and 10,000-dalton proteins (Fig. 4, lanes D and F). Spermine at 1 mM inhibited the cAMP-dependent protein kinase-catalyzed phosphorylation but generally stimulated the NI kinase-catalyzed phosphorylation of various nuclear proteins. In a separate experiment, we found that the phosphatidyserine/Ca²⁺-activated protein kinase C did not phosphorylate the 11,000- and 10,000-dalton proteins (Fig. 4, lane H). The results shown in Fig. 4 clearly demonstrate that these protein kinases have different substrate specificity, and only the NI kinase can phosphorylate the 11,000- and 10,000-dalton proteins. This result, together with the observation that 1 mM spermine completely inhibited the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins in a fashion similar to what we had observed in an endogenous nuclear protein phosphorylation reaction (21), suggests that the 11,000- and 10,000-dalton proteins might be the native substrates of NI kinase. It should be noted that the apparent molecular weights of these two proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing condition. Thus the value is that of the subunit and may not be that of the native protein.

**Specificity of the Inhibitory Effect of Spermine**—We have examined the specificity of the inhibitory effect of spermine on the phosphorylation of the 11,000- and 10,000-dalton proteins. As shown in Fig. 5, spermine at 1 mM (lane D) almost completely abolished the phosphorylation of the 11,000- and 10,000-dalton proteins. In contrast, arginine at 5 mM (lane H), NH₄Cl at 10 mM (lane I) and putrescine at 10 mM (lane J) had no significant inhibitory effect. Spermidine was effective, but a much higher concentration (>4 mM, lane G) was needed to inhibit the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins. These data suggest that both the charge distribution and flexible hydrocarbon backbone of spermine may be needed for its inhibitory action.

**The Effect of Spermine on NI-catalyzed Phosphorylation of Nonphysiological Substrates**—Among the three commonly used nonphysiological substrates for kinase assay, NI kinase phosphorylated both α-casein and phosphovitin but not histone (Fig. 6). The phosphorylation of α-casein and phosphovitin at 0.2 mg/ml was inhibited by spermine with I₅₀ values of 5.8 mM and 1.1 mM, respectively. A similar inhibitory effect of spermine was also observed at higher substrate concentrations. Thus at saturating substrate concentrations of α-casein and phosphovitin (1 mg/ml), the I₅₀ values were 8.4 mM and 1.2 mM, respectively, (data not shown). These experiments were done at a kinetically optimal NaCl concentration of 130 mM. At a suboptimal concentration of NaCl (30 mM), the inhibitory effect of spermine on the phosphorylation of α-casein, but not phosphovitin, was less pronounced (data not shown). In contrast, the inhibition of the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins by spermine was not

**Fig. 5.** Specificity of the inhibitory effect of spermine on the phosphorylation of the 11,000- and 10,000-dalton nuclear proteins catalyzed by NI kinase. Nuclei, purified as described under “Experimental Procedures” were briefly sonicated and heated at 60 °C for 5 min. The heat-treated nuclei were then used as substrates for casein-Sepharose column purified NI kinase. The phosphorylation assay mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 30 μg/ml of bovine serum albumin, and 20 μl of either casein-Sepharose purified NI kinase (lanes B and C) or 20 μl of NII kinase (lanes F and G). In another assay, 10 μM cAMP and 20 μl of Type I cAMP-dependent protein kinase was added to the above phosphorylation mixture (lanes D and E). Spermine was added to a final concentration of 2 mM (lanes E, and G). In a separate experiment (lane H), protein kinase C (5 μl) was added to the phosphorylation mixture in the presence of 2 μg of phosphatidylserine and 0.1 mM CaCl₂. In each case, incubation was at 30 °C for 15 min. The reaction was terminated by the addition of 1/5 volume of sodium dodecyl sulfate stop solution. Electrophoresis and autoradiography were done as described under “Experimental Procedures.”
Spermine and NI Nuclear Kinase

Fig. 6. Effects of spermine on NI kinase activity using either \( \alpha \)-casein, phosvitin, or histones as substrates. Protein kinase activity was determined in the standard reaction mixture containing casein-Sepharose purified NI kinase using either 0.2 mg/ml of dephosphorylated \( \alpha \)-casein (\( \bullet \bullet \bullet \)), or 0.2 mg/ml of dephosphorylated phosvitin (\( \Delta \Delta \Delta \)), or 0.2 mg/ml of dephosphorylated histone (\( \bullet \bullet \bullet \bullet \)) as substrate proteins in the presence of various concentrations of spermine.

Sensitive to the concentration of NaCl (e.g. compare Fig. 5, lane D versus Fig. 7a, lane C).

Subnuclear Localization of the 11,000- and 10,000-Dalton Proteins—In an attempt to localize the 11,000- and 10,000-dalton proteins in the nucleus, we prepared subnuclear fractions and examined the phosphorylation patterns of these fractions in the presence of exogenously added NI kinase. Phosphorylation of the 11,000- and 10,000-dalton proteins was only observed in nuclear matrix (Fig. 7a, lane E). No phosphorylated protein bands were apparent in the 10,000-dalton region in either the hnRNP (Fig. 7a, lane I) or HMG fractions (Fig. 8a, lane B). Phosphorylation of the 11,000- and 10,000-dalton proteins in nuclear matrix was reproducibly observed if the DNase I concentration and digestion times were kept constant at 5 pg/ml and 130 min, respectively. However, increasing the DNase I concentration or the digestion time led to a loss of the 11,000- and 10,000-dalton proteins from the nuclear matrix.

DISCUSSION

The evidence that the 11,000- and 10,000-dalton proteins might serve as native substrates for NI kinase includes the following: (i) both the NI kinase and the 11,000- and 10,000-dalton proteins are localized in the nucleus; (ii) exogenously added NI kinase prominently phosphorylated these two proteins in heat-treated nuclei (Fig. 4, lane B); all other kinases tested including NII nuclear kinase, CAMP-dependent protein kinase, and protein kinase C did not phosphorylate these two proteins under similar experimental conditions (Fig. 4); and (iii) the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins was sensitive to the inhibitory action of spermine (Fig. 5) at a comparable concentration range as we have observed in endogenous protein phosphorylation (21).

It can be noted that NI kinase also phosphorylated other nuclear proteins in various subnuclear fractions (Figs. 7 and 8). The phosphorylation of these other nuclear proteins was also sensitive to the inhibitory action of spermine (Figs. 7 and 8), although to a lesser degree than what we have observed for the 11,000- and 10,000-dalton proteins. Spermine also inhibited the phosphorylation of \( \alpha \)-casein and phosvitin catalyzed by NI kinase (Fig. 6). This is to be contrasted with the finding that polyamines stimulate the casein phosphorylation but inhibit the phosvitin phosphorylation catalyzed by NII kinase (35). At this stage, we do not know whether the interactions of spermine with NI kinase, with substrate proteins, or with both accounted for the inhibitory action of spermine on NI kinase. Polyamines have been reported to inhibit a phosphorylase kinase (39), a phospholipid/calmodulin sensitive Ca\(^{2+}\)-dependent protein kinase (40), a wheat germ protein kinase (41), and the protein kinase-380 isolated from bovine adrenocorticotid (42). The detailed mechanism of inhibition in these cases, however, is not known.

The inhibitory action of spermine on the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins (Fig. 5) could not be solely due to charge or counter-ion effect. Other organic cations such as arginine, putrescine, and inorganic cations such as NH\(_4\)Cl were ineffective even at much higher concentrations. We have also examined the effects of \( N^1 \)-acetyl spermine and Co(NH\(_4\))\(_2\)\(^{++}\) on the NI ki-
In summary, our studies have shown that: (i) the 11,000- and 10,000-dalton proteins are prominently phosphorylated by NI nuclear kinase, (ii) their phosphorylation can be specifically inhibited by spermine and to a lesser degree, spermidine, and (iii) they are localized in the nuclear matrix fraction. Although the functions and physiological significance of these two proteins remain to be studied, our results together with the consideration of importance of polyamines as growth regulatory agents suggest that these two proteins may have an important role in gene regulation.

Acknowledgments—We thank Drs. A. Y. Cheng, M. E. Darmus, and A. Y.-C. Liu for kindly providing us with purified protein kinase C, nonspecific antiserum against calf thymus casein kinase I, and Type I cAMP-dependent protein kinase, respectively.

REFERENCES


Fig. 8. The protein phosphorylation pattern in HMG catalyzed by NI kinase. The HMG fraction was purified as described under "Experimental Procedures." It was heated at 60 °C for 5 min to inactivate any endogenous kinases that might be associated with HMG fraction (lane A). Twenty μl of the preparation (0.8 mg of protein/ml) was added to the phosphorylation mixture as described in the legend of Fig. 7 except that 6 units of NI kinase was used in this experiment (lanes B–D). Spermine was used at 2 mM (lane C) and 4 mM (lane D) as indicated. Incubation was at 30 °C for 15 min and terminated by the addition of ½ volume of sodium dodecyl sulfate sample solution. Electrophoresis and autoradiography were carried out as described under "Experimental Procedures." Panel a, autoradiogram; panel b, Coomassie Blue-stained gel pattern.

Although we have demonstrated that phosphorylation of the 11,000- and 10,000-dalton proteins and found both were ineffective at 1 mM (data not shown). When spermine was added to the assay mixture, no precipitation was observed as judged by high speed centrifugation. In addition, under similar experimental conditions, spermine did not inhibit phosphorylation of various nuclear fractions catalyzed by NII kinase (e.g., Fig. 4, lane G) suggesting that the inhibitory effect of spermine on NI kinase-catalyzed phosphorylation could not be due to precipitation of substrate proteins. Neither could the inhibitory effect be due to an increase of ionic strength by the addition of 1 mM spermine to the assay mixture. The ionic strength of the assay mixture could be increased by varying the concentrations of NaCl from 30 to 200 mM without affecting the NI kinase-catalyzed phosphorylation of the 11,000- and 10,000-dalton proteins in heat-treated nuclei (Fig. 5, lane D versus Fig. 7a, lane C).

In summary, our studies have shown that: (i) the 11,000- and 10,000-dalton proteins are prominently phosphorylated by NI nuclear kinase, (ii) their phosphorylation can be specifically inhibited by spermine and to a lesser degree, spermidine, and (iii) they are localized in the nuclear matrix fraction. Although the functions and physiological significance of these two proteins remain to be studied, our results together with the consideration of importance of polyamines as growth regulatory agents suggest that these two proteins may have an important role in gene regulation.
Spermine and NI Nuclear Kinase

(1951) J. Biol. Chem. 193, 265-275