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BACTERIOPHAGE T7 PROTEIN KINASE IS MAGNESIUM-DEPENDENT AND SULFATE-ACTIVATED

by

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SUMMARY

The cyclic-AMP-independent protein kinase induced by bacteriophage T7 in Escherichia coli was optimally activated by 60 mM magnesium. Substitution of magnesium sulfate for magnesium chloride produced approximately a two-fold increase in protein kinase activity, while magnesium acetate was least effective in potentiating T7 protein kinase activity. Also, T7 protein kinase was demonstrated to be ionic strength dependent with maximal activity occurring at ionic strengths of 260 to 300 millimolar.

INTRODUCTION

Previous studies have shown bacteriophage T7 protein kinase (T7PK) to be magnesium dependent (1); require 15 mM magnesium chloride; and require ATP as phosphate donor--GTP did not serve as phosphate donor. T7PK is also cyclic AMP-independent with a molecular weight of about 37,000 daltons (2). In E. coli, T7PK phosphorylates DNA-dependent RNA polymerase at threonine residues in a site-specific manner thereby inhibiting host RNA synthesis within 3 minutes after infection (3). Initiation factor IF-3 of E. coli is phosphorylated at both serine and threonine residues (2-2.5:1) at specific sites but this phosphorylation has no effect on the ribosomal binding function of IF-3 (4).

Cyclic nucleotide-independent protein kinases of human and rabbit red blood cells are magnesium-dependent, inhibited by calcium and able to

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[1] Abbreviations used: AMP, adenosinemonophosphate; ATP, adenosinetriphosphate; GTP, guanosinetriphosphate; (γ-^{32}P)ATP, gamma-^{32}P-ATP; TMA buffer, 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 22 mM NH_4Cl, 5% glycerol and 1 mM dithiothreitol; EDTA, ethylenediaminetetraacetic acid, tetrasodium salt; T7PK, T7 bacteriophage-induced protein kinase.

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phosphorylate casein and phosvitin much better than histone or protamine (5). Cyclic nucleotide-independent protein kinases of avian sarcoma virus (6,7) hepatitis B virus (8) equine herpesvirus 1 (9), vaccinia virus (10), avian myeloblastosis virus (11,12), sendai virus (13), adenovirus (14), murine sarcoma virus (15) and rous sarcoma virus, prague c strain (16) were stimulated to maximal activity by magnesium chloride at concentrations of 1 mM to 25 mM. In the cases of frog polyhedral cytoplasmic deoxyribovirus (17), african swine fever virus (18) and chilo iridescent virus type 6 (19), manganese was either required or substituted for magnesium in the protein kinase reaction.

Because of the variations in cation requirements of various protein kinases, it seemed of interest to determine the cation and/or anion requirements of T7PK, while examining the ionic strength dependency in concomitant studies. This paper reports that sulfate anion and magnesium cation produce synergistic activation of T7PK.

MATERIALS AND METHODS

Escherichia coli Bs-1 (ATCC # 23224) was obtained from American Type Culture Collection (Rockville, MD.). Phage strain T7 am 193 was kindly provided by Dr. F. William Studier (Upton, NY).

Protein kinase enzyme extracts were prepared according to the method of Rahmsdorf and coworkers (1) with minor changes in procedure; namely, Escherichia coli Bs-1 was infected with $T7_{am193}$ at a multiplicity of infection of 10 to 20. After 6 minutes at 30 °C, the infection was stopped by addition of frozen and crushed TMA buffer (10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 22mM NH_4Cl, 5% glycerol and 1 mM dithiothreitol) and the infected cells collected by centrifugation at 5,000 rpm for 10 minutes at 4 °C. The supernatant was discarded and the cells washed once in TMA buffer. The pellet was resuspended in 5 ml of 10mM Tris-HCl, pH 8.1 containing 25% sucrose. To this suspension was added 0.5 ml of 0.64 milligram percent lysozyme in 0.25 M Tris-HCl, pH 8.1 and 0.5 ml of 0.054 mM EDTA. The cell suspension was lysed by incubation at 30 °C for 90 seconds, then 0.75 ml of 5% Brij-58 in 10 mM Tris-HCl, pH 7.2 and 1.5 ml of 0.1 M $MgSO_4$ were added and the preparation was incubated at 30 °C for 5 minutes. The lysate was then sonicated at full power for 1 minute in melting ice. The sonicate was centrifuged at 5,000 rpm for 10 minutes at 4 °C and the pellet discarded. The supernatant contained 233 micrograms protein per ml and was stored at -70 °C in 0.5 ml aliquots. Prior to use the material was diluted ten-fold in 10 mM Tris-HCl, pH 8.0, and added to the reaction tubes in 30 l amounts.

Protein kinase assays were performed according to the methods of Kleiman and Moss (20). Membrane filters were obtained from Millipore Corporation (Bedford, MA). (γ-^{32}P)ATP was obtained from New England Nuclear (Boston, MA). Lysozyme and chemicals used in the assays were from Sigma Chemical Company (St. Louis, MO).

Table 1

Effects of Magnesium Salt Concentration Upon T7 Protein Kinase Activity

Salt	Amount Added	Ionic Strength	Picomoles ^{32}P Incorporated	Percentage of Maximal Reaction
$MgCl_2$	81mM*	268	27.0	52
	85mM	280	27.7	53
	88mM	289	28.8	55
	91mM	299	30.7	59
	95mM	310	29.0	55
	98mM	319	26.4	50
$MgSO_4$	61mM	269	50.0	95
	63mM	277	50.5	96
	66mM	297	52.4	100
	71mM	309	49.6	95
	73mM	317	48.6	93

* All tubes received 1.22 nanomoles [γ-^{32}P]ATP, 30 microliters of enzymes, 600 micrograms lysozyme, 25mM Tris-HCl, pH 8.5 and the indicated amounts of magnesium in a total volume of 0.1 ml. Incubation was at 24°C for 15 minutes.

RESULTS AND DISCUSSION

Previous studies have demonstrated that 60mM magnesium chloride is optimal for T7PK activity; neither manganese, copper, zinc nor calcium substituted for magnesium in the reaction (21). Eight millimolar calcium was shown to inhibit the kinase reaction by 93% but addition of 8mM EDTA prevented this inhibition. When magnesium sulfate is substituted for magnesium chloride, the optimal concentration is also 60 mM, but the reaction is stimulated almost two-fold over that detected with magnesium chloride .

Adjustment of the ionic strength of the reaction mixture by addition of increasing amounts of magnesium chloride or magnesium sulfate revealed the optimal ionic strength for both magnesium chloride and magnesium sulfate to be near ionic strength 300 (Table 1). As noted earlier, sulfate ions stimulated

Table 2
Determination of the Optimal Ionic Strength
for T7 Protein Kinase in the Presence of $MgSO_4$

Ionic Strength	Addition	Picomoles ^{32}P Incorporated	Percentage of Maximal Reaction
265	None*	51.0	97
290	25mM NaCl	52.5	100
315	50mM NaCl	47.1	90
340	75mM NaCl	45.2	86
340	25mM Na_2SO_4	42.7	81
365	100mM NaCl	45.0	86
415	150mM NaCl	35.9	68
415	50mM Na_2SO_4	41.8	80
465	200mM NaCl	30.0	57
565	300mM NaCl	25.0	48
565	100mM Na_2SO_4	29.6	56

*All tubes contained 60mM $MgSO_4$, 25mM Tris-HCl, 30 microliters of enzyme, 600 micrograms lysozyme, 1.22 nanomoles [γ-^{32}P]ATP and the indicated additions in a volume of 0.1 ml.

T7PK activity. With 60 mM magnesium sulfate as the magnesium source, the addition of either sodium chloride or sodium sulfate above ionic strength 290 resulted in a linear reduction of T7PK activity (Table 2), a finding which demonstrates inhibition by high ionic strength. Evidence that the chloride anion does not inhibit T7PK and that the optimal ionic strength lies between 270 and 300 is presented in Table 3. However, when one reaches very high ionic strengths, sulfate inhibits the reaction less on the basis of ionic strength than does chloride. The experiments testing effects of various combinations of salts and buffers on T7PK activity demonstrate that 60 mM $MgSO_4$ plus 20 mM Tris-H_2SO_4 buffer gives the maximum T7PK activity (Table 4); furthermore, chloride gives less stimulation, while acetate results in the lowest activity observed. In addition, phosphate seems unable to replace

Table 3

Effects of Chloride and Sulfate Anions Upon the T7 Protein Kinase Reaction.

Source of Magnesium	Additive	Ionic Strength	Picomoles ^{32}P Incorporated	Percentage of Maximal Reaction
60mM $MgCl_2$	+ 21mM Na_2SO_4*	268	40.8	79%
	+ 35mM Na_2SO_4	310	37.8	73%
	+ 48mM Na_2SO_4	349	36.0	70%
81mM $MgCl_2$	None	268	27.0	52%
60mM $MgSO_4$	+ 4mM NaCl	270	51.8	100%
	+ 44mM NaCl	309	47.7	92%
	+ 84mM NaCl	349	38.0	73%
	+ 150mM NaCl	415	37.8	73%
	+ 300mM NaCl	565	17.8	34%
	+ 50mM Na_2SO_4	415	47.1	91%
	+ 100mM Na_2SO_4	565	23.0	44%

* All tubes received 1.22 nanomoles [γ-^{32}P]ATP, 30 microliters of enzyme, 25mM Tris-HCl, pH 8.5, 600 micrograms lysozyme plus the noted additions in a total reaction volume of 0.1 ml. Incubation was at 24°C for 15 minutes.

chloride or sulfate in the activation of T7PK.

These studies reveal T7PK to be a magnesium-dependent, sulfate-activated enzyme with optimal activity occurring at pH 8.5 and ionic strength of 260 to 300 millimolar. The best acceptor protein discovered to date for T7PK is histone IIIs[2], which is rich in threonine and lysine. T7PK resembles casein kinase from swine polymorphonuclear leucocytes in that it is unaffected by cyclic AMP and inhibited completely in the presence of calcium (22). The only role for T7PK demonstrated to date is that of "turning off" host cell transcription as an early step in T7 virus replication; in this case threonine residues were phosphorylated to a greater degree than serine (3).

The finding of a sulfate-activated protein kinase raises questions about

[2] H. W. Rogers and C. L. Phelps, Unpublished Observations.

Table 4
Effects of Anion and Buffer Combination
Upon T7 Protein Kinase Activity

Source of Magnesium	Buffer			
	20mM $(Tris)_2$ SO_4	Tris HCl 40mM	Tris Acetate 40mM	Phosphate
60mM $MgSO_4$	104.0* (100%)	97.5 (94%)	86.6 (83%)	85.2† (82%)
60mM $MgCl_2$	81.3 (78%)	54.8 (53%)	37.8 (36%)	41.1 (39%)
60mM Mg $(Acetate)_2$	49 (47%)	36.4 (35%)	31.7 (30%)	17.8 (17%)

*Picomoles ^{32}P incorporated per 600 mg lysozyme. The ionic strength of each buffer was adjusted by addition of the corresponding buffer's sodium salt to bring the ionic strength to 300. Numbers in parenthesis express the percentage of the maximal protein kinase activity. All tubes received 30 microliters enzyme, 12.21 nanomoles [γ-^{32}P]ATP, 600 micrograms lysozyme, and the indicated additions in a total volume of 0.1 ml. Incubation was at 24°C for 15 minutes.

†The tubes containing $MgSO_4$ received 20mM phosphate buffer and those containing MgCl and magnesium acetate received 40mM phosphate buffer.

the occurrence of similarly-activated kinases in other virus systems. It would be quite interesting to determine if RNA tumor virus or other animal virus protein kinases were activated by sulfate and if they could phosphorylate histone IIIs to the same degree that they phosphorylated IgG molecules of various species. Future studies will investigate the effects of various enzyme inhibitors upon T7PK to further characterize this virion-induced protein kinase.

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