A DNA-DEPENDENT ATPase FROM E.COLI INFECTED WITH BACTERIOPHAGE T4

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Summary: A DNA-dependent ATPase has been purified from E.coli infected with bacteriophage T4. The enzyme has a low molecular weight and is not detectable in uninfected E.coli. In the presence of DNA, ATP is cleaved to ADP and inorganic phosphate. Calf thymus DNA, E.coli DNA and heat-denatured T4 and T7 bacteriophage DNA's stimulate the ATPase. However, native T4 and T7 DNA's do not activate the enzyme. In addition to ATP breakdown, the enzyme catalyzes dATP and, to a lesser extent, CTP degradation to their respective diphosphates. No exo- or endonucleolytic activity has been detected. The role of DNA in the reaction is currently under investigation.

In this communication we report our studies on the purification and some of the properties of a DNA-dependent ATPase found in extracts of T4 bacteriophage-infected E.coli.

Material and Methods

E.coli ER22 (endonuclease I$^-$) was infected with bacteriophage T4 am E605 (an amber, temperature-sensitive ligase mutant) at a multiplicity of 4-5. The infected cells were incubated for 25 minutes, centrifuged, and stored at -20°C. A cell extract, prepared by rupturing the cells in a French pressure cell, was treated with streptomycin sulfate, and the supernatant was recovered. Solid ammonium sulfate to 55% saturation was added to the supernatant, and the precipitate, resuspended in 0.01M tris pH 7.5 + 0.01M mercaptoethanol, was chromatographed, after dialysis, on DEAE cellulose. The enzyme, which was eluted by 0.05 M NaCl, was then chromatographed on Sephadex G50, followed by chromatography on hydroxylapatite. The ATPase was eluted from hydroxylapatite by 0.14 M potassium phosphate buffer, pH 6.85, and was stabilized by the addition of bovine plasma albumin (final concentration, 1 mg/ml).

Details of the enzyme purification will appear in a subsequent communication.

The standard assay mix (0.15 ml) contained tris-HCl, pH 7.6, 67 mM; $MgCl_2$, 3.3 mM; dithiothreitol, 11 mM; ATP, 0.013 mM; ^{14}C-ATP, 3.5 μM (specific activity 29.6 mc/mmole); DNA, 0.073 mM; and 0.05 - 3.0 units of enzyme. One unit is the amount of enzyme necessary to produce 0.5 mμmoles of ADP in 30 minutes at 37°C. ADP formation varies linearly with enzyme concentration over the range of 0.05 to 3.0 units. The hydroxylapatite fraction was used in all experiments.

ADP formation was analyzed by thin layer chromatography on polyethyleneimine cellulose developed with 1 M LiCl (1a). ADP spots visualized by UV were cut out and the amount of ^{14}C-ADP was determined using a Nuclear Chicago gas flow counter.

γ^{32}P-ATP and ^{32}Pi were resolved by thin layer chromatography on polyethyleneimine cellulose using solvent system 1 (1b).

Results and Discussion

A DNA-dependent ADP-forming activity was eluted at 0.05 M NaCl on DEAE-cellulose chromatography of extracts of bacteriophage-infected E.coli; this activity was absent in extracts of uninfected E. coli (Figure 1). A molecular weight near 15,000 can be assigned to the enzyme from its behaviour on Sephadex G-100. Complete dependence of the reaction on the presence of DNA and Mg^{++} is shown by the data in Table I. The optimum pH for activity in tris-HCl buffer lies between 7.3 and 8.5.

Other nucleoside triphosphates were tested for their ability to participate in the reaction (Table II). ATP and dATP are cleaved to similar extents under standard assay conditions. When CTP is used as substrate a forty-fold reduction occurs in the amount of nucleoside diphosphate formed. No detectable production of diphosphates were found for the other nucleoside triphosphates tested. Inorganic phosphate is released in equimolar quantities to the ADP produced (Table III). No AMP is formed. DNA is not labelled by γ^{32}P-ATP or 8-^{14}C-ATP.

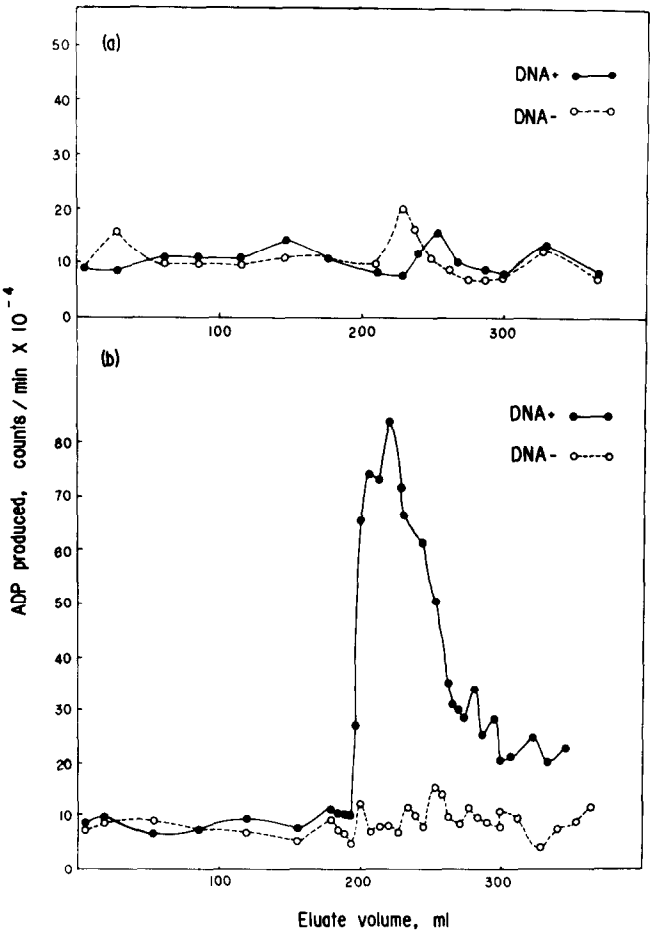

Fig. 1. DEAE-cellulose chromatography of extracts of (a) uninfected, and (b) T4amE605-infected E.coli ER22. The ammonium sulfate preparations were chromatographed on 1.5 x 10 cm. columns of DEAE-cellulose, with 1 1, 3-chambered concave gradients of 0 - 0.8 M NaCl, in 0.01 M tris-HCl, 0.01M mercaptoethanol (pH 7.6). 6 ml fractions were collected every 10 minutes, and assayed for ADP formation in the presence and absence of calf thymus DNA.

Different DNA substrates vary in their ability to stimulate the reaction, as shown in Table IV. In every case, heat-denatured DNA was more active than native DNA; native T7 DNA and native T4 DNA were inactive. When E.coli sRNA was substituted for DNA no enzyme activity was noted.

No exonuclease or endonuclease activity was detected. Enzyme treatment produced no change in the sedimentation of ^{14}C-labelled heat-denatured T7 DNA in alkaline sucrose gradient.

		mμmoles ADP produced	
		-DNA	+DNA
1. DNA	Expt. 1	<0.04	1.00
	Expt. 2	<0.04	0.66

	$-Mg^{++}$	$+Mg^{++}$	$+Mg^{++}$ + EDTA
2. Mg^{++}	<0.04	0.57	<0.04

Table I. Dependence of ATPase activity on DNA and Mg^{++}. Standard assay conditions were used with denatured calf thymus DNA. A 2.5 fold excess of EDTA was used in the reaction mixture.

	mμmoles nucleoside diphosphate produced	
	Expt. 1	Expt. 2
ATP	0.76	0.39
dATP	0.76	0.33
GTP	<0.08	<0.08
dGTP	<0.5	<0.5
CTP	0.02	0.01
dCTP	<0.14	<0.14
UTP	<0.01	<0.01
TTP	<0.08	<0.08

Table II. Participation of Different Nucleoside Triphosphates in the Reaction. The appropriate nucleoside triphosphates were substituted for ATP in the standard assay using denatured calf thymus DNA. Many of the labelled nucleoside triphosphates used here were contaminated with the corresponding diphosphates and the < values are equivalent to the level of this contamination.

In order to screen for exonuclease activity the ^{14}C-labelled DNA was precipitated with $Cl_3CHCOOH$ after undergoing the assay procedure; no acid-soluble radioactivity was found. It is noteworthy that in these experiments the amount of ADP produced was in excess of the amount of DNA-P present (Table V, Expt. 1). This fact together with the lack of nuclease activity

	ADP, mμmoles	Pi, mμmoles
Expt. 1	0.36	0.37
Expt. 2	0.16	0.18

Table III. Products of the Reaction. Pi was measured by the release of ^{32}Pi from γ-^{32}P-ATP and ADP by the production of ^{14}C-ADP from ^{14}C-ATP, as described in Materials and Methods.

		mμmoles ADP produced	
Type of DNA		Native	Heat denatured
Calf thymus			
	Expt. 1	0.32	0.63
	Expt. 2	0.18	0.33
E.coli	Expt. 1	0.33	0.62
	Expt. 2	0.26	0.70
T7	Expt. 1	< 0.04	0.35
	Expt. 2	< 0.04	0.82
T4	Expt. 1	0.02	0.65
	Expt. 2	0.05	0.97

Table IV. Effect of the Type of DNA on the Reaction. Equivalent concentrations of the different types of DNA were used under standard assay conditions. DNA samples were heat-denatured by heating at 100°C for 5 minutes followed by rapid cooling to 0°C.

	DNA mμmoles P	ADP mμmoles
Expt. 1	0.48	0.64
Expt. 2	0.11	0.28

Table V. Relationship between concentration of DNA present and ADP produced. In Expt. 1 ^{14}C-labelled, heat-denatured T_7 DNA was used. The DNA concentration was determined by optical density, using an extinction coefficient of 6750 (9).

suggests that the enzyme has an unusual mechanism of action. Since the type of DNA present has a marked effect on the extent of the reaction, the ability of DNA to stimulate the reaction cannot simply be due to its polyanionic character. That denatured T4 DNA functions well in the enzyme reaction is remarkable because few enzymes are known to attack the glucosylated T4 DNA (2). Therefore the enzyme may have a unique role in the metabolism of T4 DNA.

The properties of the enzyme do not correspond to those of the known T4-induced cofactor-requiring enzymes involved in DNA metabolism, such as polynuclectide kinase (3) and polynucleotide ligase (4). The Micrococcus lysodeikticus endonuclease (5) degrades 3 molecules of ATP for every phosphodiester bond broken. Similarly, our enzyme degrades an excess of ATP but in contrast, has no endonuclease activity. The ATP-dependent exonuclease described by Buttin & Wright (6), Oishi (7) and Barbour & Clark (8) which may have a role in genetic recombination is active on T4 DNA as is our DNA-dependent ATPase; however, their enzyme differs from ours in its exonuclease activity and in its preference for native DNA. Thus far all bacteriophage mutants investigated for the presence of the ATPase show normal levels of enzyme activity; these include mutants in genes 30, 33, 39, 41, 42, 43, 44, 45, 46, 47, 52, 55, 58, 59, 60, 62, V and X and polynucleotide kinase.

The ability of the T4 phage-induced, DNA-dependent ATPase to degrade large amounts of ATP and dATP in the presence of DNA, without detectable nuclease activity, suggests that the enzyme has an unusual function. The physiological role of the enzyme in DNA metabolism and the participation of DNA in the reaction are currently under investigation.

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References

1. a) Randerath, K. and Randerath, E., J. of Chromatog. 16, 111 (1964).
b) Randerath, K. and Randerath, E., Anal. Biochem. 13, 575 (1965).

2. Richardson, C.C., Ann. Rev. Biochem. 38, 795 (1969).

3. Richardson, C.C., Proc. Natl. Acad. Sci. U.S. 54, 158 (1965).

4. Weiss, B., and Richardson, C.C., Proc. Natl. Acad. Sci. U.S. 57, 1021 (1967).

5. a) Anai, M., Hirahashi, T., and Takagi, Y., J. Biol. Chem. 245, 767 (1970)
b) Anai, M., Hirahashi, T., Yamanaka, M., and Takagi, Y., J. Biol. Chem. 245, 775 (1970).

6. Buttin, G. and Wright, M.R., Cold Spring Harbor Symp. Quant. Biol., 33, 259 (1968).

7. Oishi, M., Proc. Natl. Acad. Sci. U.S. 64, 1292 (1969).

8. Barbour, S.D. and Clark, A.J., Proc. Nat. Acad. Sci. U.S., 66, 955 (1970).

9. Richardson, C.C., J. Mol. Biol. 15, 49 (1966).

10. Sadowski, P.D. and Hurwitz, J., J. Biol. Chem. 244, 6182 (1969).