SOME PROPERTIES OF ATP DEPENDENT DEOXYRIBONUCLEASES FROM NORMAL AND *rec*-MUTANT STRAINS OF *BACILLUS SUBTILIS*

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1. Introduction

DNAase, the activity of which depends on the presence of ATP was discovered in extracts of *Micrococcus lysodeikticus* [1] and later found and studied in a variety of microorganisms [2–5]. Activity of the enzyme was very low in extracts of some mutants of *Escherichia coli* and *Diplococcus pneumoniae* [6–10] deficient in recombination ability. This fact attracts special attention and leads to the suggestion that this DNAase may be a participant in the recombination process. We therefore studied the properties of this enzyme in wild-type and *rec*-mutant strains of *Bacillus subtilis*.

In the present paper the finding of ATP dependent DNAase in extracts of *B. subtilis* SB 25 is described as well as its partial purification and some of its properties. Among the *rec*-mutants of *B. subtilis* one mutant (342) was found which possesses 5–10-fold lower activity of the DNAase.

2. Methods

The strain of *B. subtilis* SB 25 *his, ind* was the source of wild-type enzyme. All *rec*-mutants used were derivatives of this strain, their properties being described previously [11]. The bacteria were grown at 37° with aeration up to the second half of the logarithmic phase in the Spizizen's medium [12] supplemented with lactalbumin hydrolysate (1.2 g/l, GeeLawson), histidine (50 mg/l) and indole (50 mg/l). The cultures were cooled, harvested and the cells washed once with 0.01 M Tris-HCl, pH 8.0–0.15 M NaCl. Packed cells were stored at −10°.

Partial purification of the enzyme [13] was achieved as follows: (I) opening of cells by lysozyme (1 mg of enzyme per 1 g of frozen cells), (II) streptomycin sulfate fractionation, (III) ammonium sulfate fractionation, (IV) gel filtration on Sephadex G-200 and (V) DEAE-cellulose chromatography. At all stages of purification except V the enzyme might be stored as an ammonium sulfate precipitate at −10° for 1 month at least. The enzyme was very unstable in solution but could be stabilized by the presence of 0.01 M dithiothreitol. Stage V corresponded to a 100-fold purification as compared with the crude extract, however, its specific activity increased only 30-fold because of high enzyme instability in the course of the last two stages of purification.

[^{14}C]DNA was isolated by modified [13] Marmur method [14] from prototroph cells of *B. subtilis* SB 25 grown in the same medium without histidine and indole but with [^{14}C]uracil (0.8 mCi/l; Czechoslovakia).

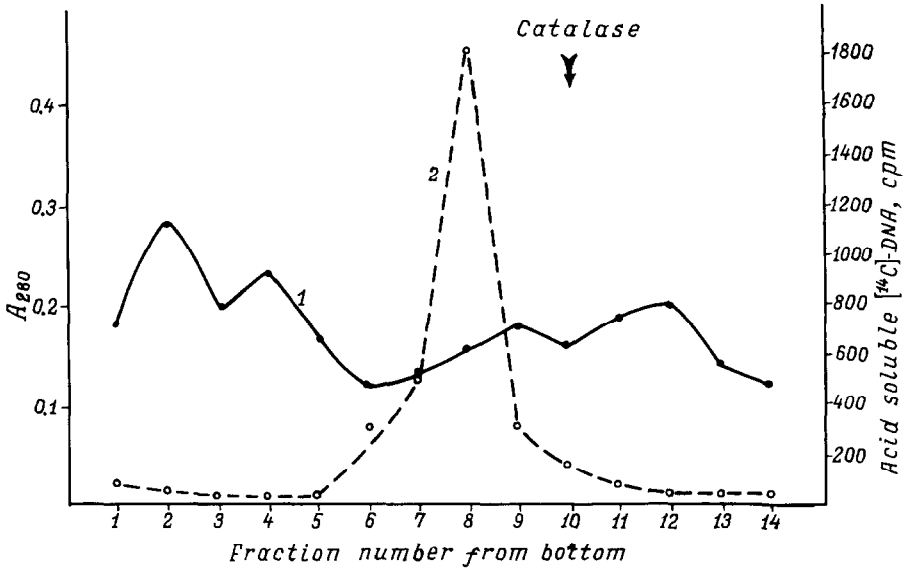

Fig. 1. Sedimentation patterns of ATP-dependent DNAase. 2 mg of the enzyme (stage III) in 0.2 ml of 0.02 M Tris-HCl, pH 7.5–1 mM EDTA were layered on a 10–35% linear glycerol gradient (4.8 ml) in the same buffer and spun for 10 hr at 40 000 rpm in an RKS–50T rotor (ultracentrifuge UCP–60; USSR) at 4°. A_{280}(1) and DNAase activity (2) were determined in each fraction. Catalase was taken as a reference.

Specific radioactivity of the [^{14}C] DNA was 1.2×10^4 cpm/μg.

The activity of ATP dependent DNAase was determined by the release of acid-soluble label after 30 min incubation of [^{14}C] DNA with the enzyme at 37°. The standard reaction mixture (1 ml) contained 0.05 M Tris-HCl, pH 9.5; 0.025 M $MgCl_2$; 0.5 mM EDTA and 0.01 M 2-mercaptoethanol. The amounts of added [^{14}C] DNA, ATP and enzyme are given in the tables. The reaction was stopped by cooling and 0.1 ml cold solution of bovine serum albumin (10 mg per ml) and 0.2 ml 18% trichloroacetic acid were immediately added. The precipitate was removed by centrifuging and 0.5 ml of the supernatant was pipetted onto a planchet and dried. The radioactivity was measured in a gas flow counter (LAG-βM, USSR) with effectivity for ^{14}C of about 70%.

Protein was determined after Lowry et al. [15] and DNA after Spirin [16].

3. Results and discussion

3.1. *Properties of ATP-dependent DNAase*

The partially purified *B.subtilis* DNAase had an absolute dependence on the presence of Mg^{2+} for its activity. The optimal Mg^{2+} concentrations were found to be 5 to 30 mM. DNAase had maximal activity at pH 9 to 10. The enzyme had pronounced specificity to double stranded DNA, which it degraded 6–7 times faster than thermally denatured DNA. As much as 40–50% of DNA might be hydrolyzed with a sufficient amount of the enzyme (0.4 mg/ml). No lag-period was found with native DNA indicating an exonucleolytic mode of hydrolysis.

According to the sedimentation coefficient (16–17 S), which was determined by glycerol density gradient (10–35%) centrifugation with catalase as a reference, (11 S) molecules of *B.subtilis* DNAase are of a large size (fig. 1);

These properties of *B.subtilis* ATP dependent DNAase were similar to those of the analogous enzyme of other microorganisms [5,7,17,18]. On the other hand *B.subtilis* DNAase differs from the majority of ATP dependent DNAases in its ability to be activated by nucleotides, it is only active in the presence of ATP or dATP (table 1). In this respect *B.subtilis* DNAase differs markedly from the analogous *Mycobacterium smegmatis* [17] and *E.coli* [7] enzymes and is similar to *D.pneumoniae* DNAase [5]. DNAases of the first

Table 1

Effect of different nucleotides on the activity of ATP dependent DNAase.

Exp. 1		Exp. 2	
Nucleotide	Acid-soluble [^{14}C] DNA, cpm	Nucleotide	Acid-soluble [^{14}C] DNA, cpm
ATP	2409	ATP	1549
AMP	0	dATP	1417
GTP	58	ADP	0
CTP	108	dGTP	0
UTP	69	dCTP	0
NAD	44	TTP	0

The test tube contained 5 μg of [^{14}C] DNA, 3×10^{-5} M of each nucleotide and 0.2 mg enzyme of stage III (exp.1) or of stage IV (exp.2).

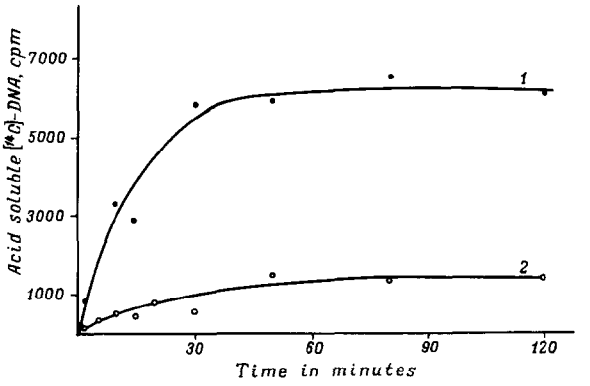

Fig. 3. Activity of ATP dependent DNAase of the normal (1) and *rec*-mutant (2) strains of *B.subtilis*. Test tubes contained 15 μg of [^{14}C] DNA; 8×10^{-5} M ATP and 0.4 mg of enzyme preparations (stage III).

two bacteria were activated by most natural ribonucleoside- and deoxyribonucleoside-5′ triphosphates, almost as effectively as by ATP. Thus *B.subtilis* DNAase possessed a greater specificity, since it was activated only by ATP and dATP.

The dependence of *B.subtilis* DNAase activity on the ATP concentration is rather peculiar. Fig. 2 shows that at all concentrations used there are relatively narrow limits of ATP concentrations for maximal activity. At optimal ATP concentration the enzyme activity was not proportional to the quantity of the enzyme preparation, but it was at higher ATP concentrations. There was also a proportionality between enzyme quantity and optimal ATP concentration.

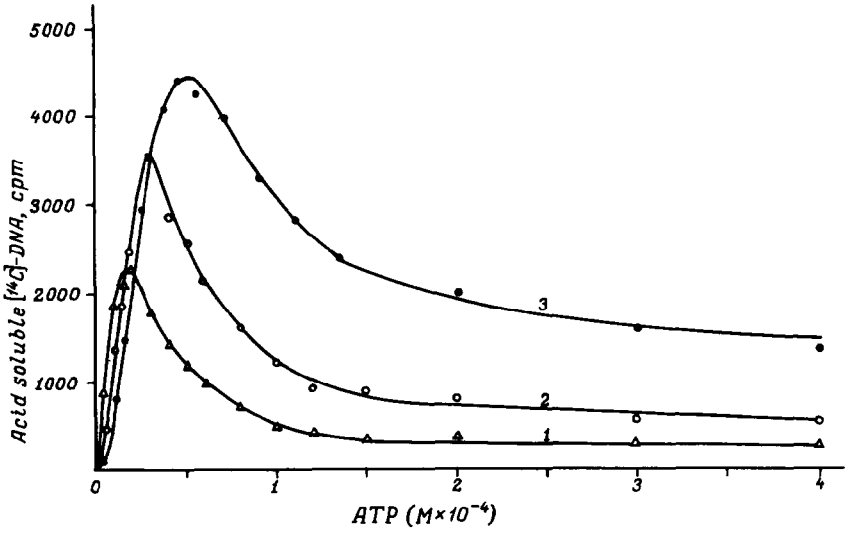

Fig. 2. Dependence of DNAase activity on the ATP concentration. Test tubes contained [^{14}C] DNA, 7μg; enzyme of stage IV: 0.05 mg (curve 1); 0.1 mg (curve 2) and 0.2 mg (curve 3).

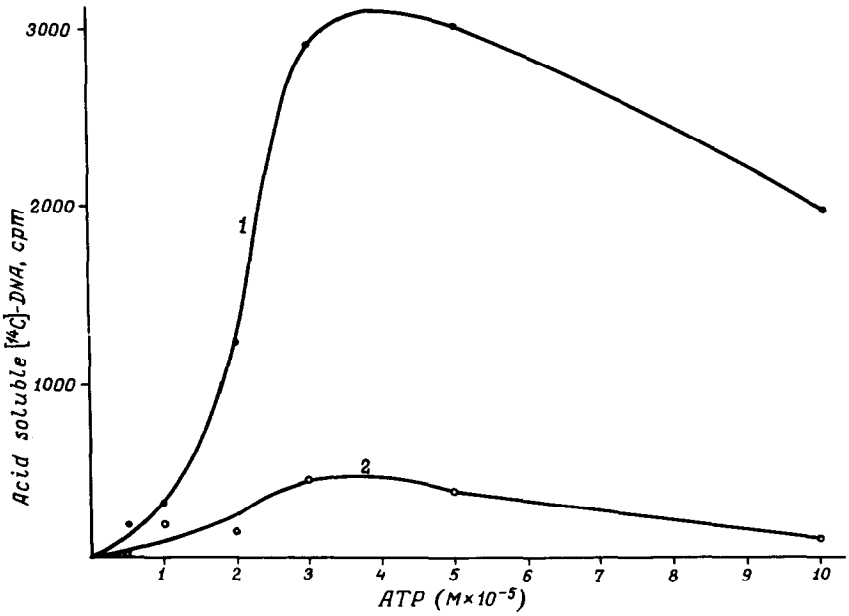

Fig. 4. Dependence of DNAase activity of normal (1) and mutant (2) enzymes on ATP concentration. Test tubes contained 10 μg of [^{14}C] DNA and 0.2 mg of enzyme (stage III).

Similar although not identical results have been described for *E.coli* [7] and *D.pneumoniae* [5] DNAases.

3.2. *Rec-mutant 342 ATP dependent DNAase*

Among *rec*-mutants of *B.subtilis* SB 25 [11] one (SB 342) was found with ATP dependent DNAase activity 5–10 times lower than that of the parent strain (fig. 3). The same ratio of activities was retained at stages II and III of purification. The low activity of *rec*-mutant DNAase was not connected with the appearance of an inhibitor since in the mixture of normal and mutant extracts the enzyme activity was not lower than the total activity of the same extracts measured separately (table 2). The mutant preparations seemed to contain approximately the same number of enzyme molecules as the normal preparation, since maximal activity of equal amounts of both enzymes was observed at the same ATP concentrations (fig. 4).

Table 2
Activity of mixtures of normal and mutant ATP dependent DNAases.

Enzyme (mg)		Acid-soluble [^{14}C] DNA (cpm)
B.subtilis SB 25	*rec*-mutant 342	
0.10	–	3997
–	0.10	517
0.05	0.05	2444
0.10	0.10	4974

The test tube contained 5 μg [^{14}C] DNA; 3×10^{-5} M ATP and enzyme (stage III).

Mutant 342 was characterized by the disturbance of the DNA recombination process, because it was a very bad recipient in genetic transformation and transduction experiments [11]. Thus our results served to support the opinion of wide occurrence of ATP dependent DNAases in different groups of bacteria and that the enzyme is involved in the DNA recombination process.

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