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Regulation of intracellular ribonuclease of *Bacillus subtilis* by ATP and ADP

During the course of an investigation on protein biosynthesis in *Bacillus subtilis* it was found that the activity of intracellular (acid) ribonuclease (ribonucleate nucleotido-2'-transferase (cyclizing), EC 2.7.7.17) was completely inhibited by ATP. Further studies revealed that ADP was another effective inhibitor.

The inhibition of pancreatic ribonuclease by ATP, ADP and GDP has been reported[1]. The inhibition was, however, only partial even if the concentration of ATP or ADP was very high, and the mode of inhibition was uncompetitive. In contrast to the case of pancreatic ribonuclease, the intracellular ribonuclease activity of *B. subtilis* was completely inhibited by low concentrations of ATP or ADP, and the mode of inhibition was competitive.

The activity of ribonuclease was determined by measuring the absorbance of the acid-soluble fraction at 260 mμ using a purified yeast RNA (Sigma Chemical Co., St. Louis, Mo., U.S.A., final 0.3%) and phosphate buffer (final 0.04 M, pH 5.7).

Deoxyribonuclease (deoxyribonucleate oligonucleotido-hydrolase, EC 3.1.4.5) activity was measured similarly using heat-denatured calf-thymus DNA (Sigma Chemical Co., final 0.08%) as substrate. Phosphodiesterase (orthophosphoric diester phosphohydrolase, EC 3.1.4.1) activity was measured by determining the absorbance of liberated *p*-nitrophenol at 420 mμ using bis-(*p*-nitrophenyl)-phosphate (Daiichi Pure Chemical Co., Tokyo, Japan), final concn. 1 mM in 0.04 M phosphate buffer (pH 5.7), as substrate. Ascending paper chromatography of nucleotides was carried out using Solvent I (80% satd. $(NH_4)_2SO_4$–*tert.*-butanol–1 M NH_4OH (200:3:1, v/v/v))[2] or Solvent II (95% ethanol–1 M ammonium acetate (7:3, v/v))[3].

B. subtilis strain K was cultured aerobically in 1 l nutrient broth at 30° for 20 h (stationary phase). Organisms were collected centrifugally, washed 3 times with 0.01

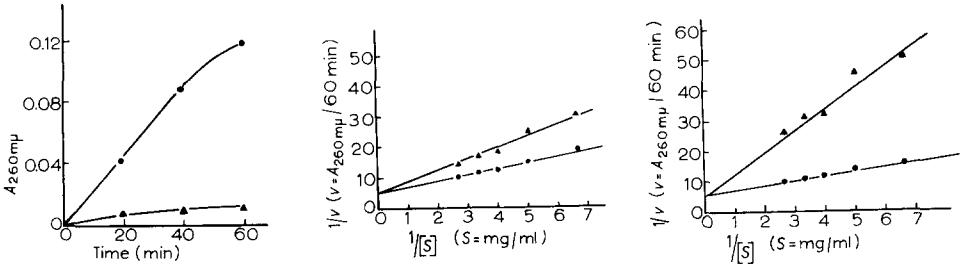

Fig. 1. Time course of RNA degradation by intracellular ribonuclease in the presence or absence of ATP. The total volume of the reaction mixture (pH 5.7), which contained 0.05 ml S-105 fraction and 0.3% yeast RNA, was 1.0 ml. The reaction was carried out at 30°. ●—●, no ATP; ▲—▲, $1 \cdot 10^{-4}$ M ATP.

Fig. 2. Lineweaver–Burk plot of the inhibition of intracellular ribonuclease by ATP. The assay conditions were the same as described in Fig. 1 except that the concentration of yeast RNA was varied and the reaction time was 60 min. ●—●, no ATP; ▲—▲, $2 \cdot 10^{-7}$ M ATP.

Fig. 3. Lineweaver–Burk plot of inhibition of intracellular ribonuclease by ADP. The assay conditions were the same as described in Fig. 2. ●—●, no ADP; ▲—▲, $2 \cdot 10^{-6}$ M ADP.

M Tris–HCl buffer (pH 7.5, containing 0.01 M β-mercaptoethanol and 0.002 M $MgCl_2$), suspended in the same buffer and lyzed by lysozyme treatment and osmotic shock. The lysate was centrifuged at 105 000 $\times$ *g* for 90 min and the supernatant (S-105) was dialyzed against the Tris–HCl buffer. The dialyzed S-105 (50 ml) was used as the enzyme preparation.

As illustrated in Fig. 1, 0.1 mM ATP strongly inhibited the activity of intracellular ribonuclease. To determine the effect of other nucleotides, various nucleoside triphosphates, nucleoside diphosphates, and nucleoside monophosphates were examined. The results are summarized in Table I. ATP and ADP were highly inhibitory, *e.g.* 1 mM of either nucleotide was sufficient for complete inhibition at pH 5.7. The pH value inside *B. subtilis* cells was concluded to be around 7.0 from the pH value of a cell extract which was prepared by lyzing the organisms in a minimum amount of distilled water. At pH 7.0, the inhibition by ATP and ADP was a little stronger than at pH 5.7, *e.g.* 0.1 mM of ATP inhibited the activity of intracellular ribonuclease by 90% and 95% at pH 5.7 and 7.0, respectively. GDP and UTP were also inhibitory.

TABLE I

THE INHIBITORY EFFECT OF VARIOUS NUCLEOTIDES ON THE ACTIVITY OF INTRACELLULAR RIBONUCLEASE OF *B. subtilis* AS EXPRESSED IN INHIBITION PER CENT

The assay conditions were the same as described in Fig. 1. The reaction time was 60 min.

	% Inhibition			
	$1 \cdot 10^{-3}$ *M*	$5 \cdot 10^{-4}$ *M*	$1 \cdot 10^{-4}$ *M*	$1 \cdot 10^{-5}$ *M*
ATP	100	97	90	74
GTP	43	43	6	
CTP		7		
UTP		54	30	
ADP	110		90	53
GDP	83		15	
CDP	10			
UDP	4			
Ado-5′-*P*	30		10	
Guo-5′-*P*			6	
Cyd-5′-*P*			2	
Urd-5′-*P*			7	
Ado-3′-*P*	13		0	
Guo-3′-*P*			8	
Cyd-3′-*P*			6	
Urd-3′-*P*			11	

The modes of inhibition of ATP and ADP were competitive as shown in Figs. 2 and 3, respectively.

K_i values for ATP and ADP were calculated as 0.24 μM and 0.53 μM, respectively, from Figs. 2 and 3. The K_m value was 0.024%.

The intracellular ribonuclease activity was not derived from polynucleotide phosphorylase (nucleoside diphosphate:polynucleotide nucleotidyltransferase, EC 2.7.7.8) because the acid-soluble fraction contained mainly 3′-nucleotides as tested by paper chromatography using Solvent I and II. The possibility of participation of

phosphodiesterase in the RNA degradation was also excluded by the fact that the activity of phosphodiesterase was completely inhibited by phosphate ion under the assay conditions employed. Recently, the presence of nucleases which preferentially degrade single-stranded nucleic acids in *B. subtilis* has been reported[4-7]. The S-105 fraction, however, contained little or no deoxyribonuclease activity. These observations strongly suggest that the intracellular ribonuclease activity observed was really that of ribonuclease[8].

Extracellular (alkaline) ribonuclease of *B. subtilis* K was not influenced by ATP and ADP at either pH 5.7 or 7.5.

An intracellular inhibitor against extracellular ribonuclease has been reported[9], but no inhibitor of intracellular ribonuclease is known.

In conclusion, ATP and ADP probably act as natural regulators or inhibitors of intracellular ribonuclease in *B. subtilis*. The purification of the intracellular ribonuclease is now in progress.

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Localization of lysozyme activity in a Paneth cell granule fraction

The identity of the content of the Paneth cell granules is still largely unknown[1]. Histochemical studies indicate that these granules are rich in protein[2,3], tryptophan[4] and arginine[5], that disulfide bridges are present[6] and that the overall isoelectric point exceeds 10.3 pH units[5]. Administration of pilocarpine is known to produce a discharge of granules into the intestinal lumen[7]. Following the administration of pilocarpine we observed a rapid increase of lysozyme (mucopeptide *N*-acetylmuramylhydrolase, EC 3.2.1.17) in the small intestinal fluid. As there is some analogy between