SHORT COMMUNICATIONS

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Effect of dipicolinic acid on bacterial cyclic 3',5'-nucleotide phosphodiesterase

In a separate paper we reported that some compounds inhibited cyclic 3',5'-nucleotide phosphodiesterase of *Serratia marcescens*[1]. In this communication we report that dipicolinic acid strongly inhibits the phosphodiesterase of *Brevibacterium liquefaciens*, a bacterium which makes a large amount of adenosine cyclic 3',5'-monophosphate (cyclic AMP)[2], and that of *Escherichia coli*.

For the preparation of enzyme, *B. liquefaciens* ATCC 14929 was cultured overnight in 1 l of a nutrient broth. The cells (40 g wet wt.) were suspended in 80 ml of 50 mM Tris–HCl buffer (pH 8.0) containing 3 μg/ml of deoxyribonuclease and were disrupted in a French pressure cell. The 30 000 $\times$ *g* supernatant of cell extract was treated with protamine sulfate (45% to protein) and the supernatant obtained after centrifugation was further treated with $(NH_4)_2SO_4$ (57.5–65% satn.). The precipitate was collected and was dissolved in 10 ml of 50 mM Tris–HCl (pH 8.0). The purification achieved was 5.4-fold on a protein basis and the yield was 57%. When the phosphodiesterase assay (see below) was performed with 82 μg of enzyme, the reaction proceeded linearly over 60 min and the conversion of cyclic AMP to 5'-AMP was stoichiometric. The enzyme has a broad pH optimum over the range 6–9. K_m for cyclic AMP was 0.55 mM.

E. coli 1895 (kindly donated by Dr. Lederberg, Stanford University, Palo Alto, Calif., U.S.A.) was cultured as described above, and the 30 000 $\times$ *g* supernatant of cell extract was used as enzyme (K_m for cyclic AMP 0.12 mM).

The reaction mixture for phosphodiesterase (100 μl) contained 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 1.2 mM cyclic [8-^{14}C] AMP and enzyme. The reaction proceeded for 40 min (*B. liquefaciens* enzyme) or 20 min (*E. coli* enzyme) at 30° and was processed according to Method 2 described in a previous paper[1].

TABLE I

EFFECT OF DIPICOLINIC ACID ON CYLIC 3',5'-NUCLEOTIDE PHOSPHODIESTERASE

Preincubation with or without dipicolinic acid was performed for 30 min at 30° in 200 μl of 50 mM Tris–HCl (pH 8.0) using 236 μg of *B. liquefaciens* enzyme or 1.7 mg of *E. coli* enzyme. The amount of protein in the assay tube of phosphodiesterase was 82 μg (*B. liquefaciens*) and 170 μg (*E. coli*).

Enzyme	*Dipicolinic acid (mM):*	*Without preincubation*				*Preincubated with dipicolinic acid*			
		0	*1*	*3*	*10*	*0*	*1*	*3*	*10*
B. liquefaciens	5'-AMP (nmoles)	14.8	14.2	13.8	10.7	10.6	1.92	1.05	0.93
	Inhibition (%)	0	4	8	27.6	0	81.9	90.9	91.2
E. coli	5'-AMP (nmoles)	20.8	20.5	17.2	13.0	22.3	7.18	5.25	4.80
	Inhibition (%)	0	1.5	17	37.5	0	67.8	76.5	78.5

Table I shows that when dipicolinic acid was added to the reaction mixture only slight inhibition was observed. On the other hand, when the enzyme was preincubated with dipicolinic acid, the reaction rate was lowered by about 90%. The lowering of enzyme activity is due to the inactivation of enzyme rather than to the inhibition of the reaction (see below). Partial inactivation was also seen with rabbit brain enzyme[3], but phosphodiesterase from *S. marcescens*[1] was completely resistant to dipicolinic acid.

One explanation of the action of dipicolinic acid is that phosphodiesterases of *B. liquefaciens* and *E. coli* are metalloproteins, and dipicolinic acid, which is known to be a physiologically important metal-chelating agent in spore-forming bacteria[4], deprives the enzymes of their metal atoms. This explanation was verified by the following observations: (1) Inactivation of the enzyme by dipicolinic acid was protected by Mn^{2+} or Ca^{2+} but not by Mg^{2+}. (2) When the enzyme was incubated with dipicolinic acid in metal-free buffer and then dipicolinic acid was removed by gel filtration, no enzymic activity could be seen. However, when this inactivated enzyme was again incubated with 10 mM Mn^{2+} or Ca^{2+} for 30 min at 30°, partial reactivation (70% with Mn^{2+} and 40% with Ca^{2+} to water-incubated control) occurred. Mg^{2+} did not have this effect.

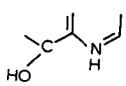
TABLE II

EFFECT OF PREINCUBATION OF CYCLIC 3′,5′-NUCLEOTIDE PHOSPHODIESTERASE WITH METAL-CHELATING AGENTS

B. liquefaciens enzyme was used. Conditions for preincubation and assay are the same as Table I. All acidic reagents were neutralized with $NaHCO_3$.

Agent	*Concn. (mM)*	*Inhibition (%)*
2,3-Pyridine dicarboxylic acid	10	16.0
2,4-Pyridine dicarboxylic acid	10	38.5
2,5-Pyridine dicarboxylic acid	0	21.5
Dipicolinic acid	2.5	82.3
	10	94.8
8-Hydroxyquinoline	2.5	23.3
	10	59.7
EDTA	10	15.5
Sodium citrate	10	5.5
α,α'-Dipyridyl	10	8.6
Preincubated with water	—	0

Table II shows the effect of preincubation of enzyme with several metal-chelating agents. While the effect of EDTA, sodium citrate or α,α'-dipyridyl was, if any, small, pyridine dicarboxylic acids and 8-hydroxyquinoline, which have a portion of their structure in common, as dipected below, showed a more or less similar effect.



As described above, dipicolinic acid inactivates the phosphodiesterase of some bacteria probably by deprivation of metal(s) other than Mg^{2+}. The treatment does

not cause damage of adenyl cyclase of *B. liquefaciens*. Thus, dipicolinic acid may be a useful reagent for the study of microbial adenyl cyclase and phosphodiesterase.

Shionogi Research Laboratory TADASHI OKABAYASHI
Shionogi and Co., Ltd., MISAO IDE
Fukushima-ku, Osaka (Japan)

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