SOME REGULATORY PROPERTIES OF CYTIDINE DEAMINASE AND URIDINE PHOSPHORYLASE OF *BACILLUS CEREUS*

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1. Introduction

Cytidine deaminase has been identified in a variety of biological sources (see [1] for review); its regulatory properties have first been described by Ipata et al. for the yeast enzyme [1].

In *B. cereus* an enzyme activity, catalyzing the deamination of cytidine was originally found by Lawrence and Tsan [2]. The properties of the enzyme, however, have never been reported.

The kinetic and regulatory properties of *B. cereus* cytidine deaminase and uridine phosphorylase reported here show that the two closely related enzymes, probably involved in the "salvage pathway" of pyrimidine nucleotide biosynthesis, are allosterically inhibited by a number of nucleotides.

2. Experimental

2.1. *Preparation of crude extracts*

B. cereus strain AICB 8122 was grown at 37° on a basal medium of the following composition: peptone 5 g; beef extract 3 g; yeast extract 3 g; $MnSO_4$ 0.1 g; H_2O to 1000 ml.

The vegetative forms were harvested at the end of the exponential phase and washed three times with water by centrifugation at 2°. The cellular sediment was weighed and then ground in the cold with twice its wet weight of aluminium oxide according to Mc Ilwain's technique [3].

The ground vegetative forms were extracted with 0.2 M potassium phosphate buffer pH 7.0 and then centrifuged at 10,000 *g* in the cold. The supernatant fluid was subjected to fractional ammonium sulfate precipitation. The fraction precipitated between 33% and 83% saturation was solubilized with tris-HCl buffer 0.05 M, pH 7.0, and extensively dialyzed against the same buffer. The final protein concentration was 13 mg per ml. Both cytidine deaminase and uridine phosphorylase were almost completely recovered in the ammonium sulfate precipitate.

2.2. *Assay procedures*

Cytidine deaminase was assayed according to Ipata et al. [1] by a spectrophotometric method based on the differential absorption of cytidine and uridine at 286 nm, where the molar differential extinction coefficient is 3,140.

Uridine phosphorylase was assayed according to Carter [4] by following the phosphate dependent decrease in absorbance at 280 nm, where the molar differential extinction between uridine and uracil is 2,100. Assays were made in 0.1 M potassium phosphate buffer, pH 7.2. No decrease in optical density was observed, when 0.1 M tris-HCl buffer, pH 7.2, was substituted for phosphate buffer.

The molarities of all substrate and inhibitor solutions were estimated spectrophotometrically from the extinction coefficients at 260 nm at pH 7 [5].

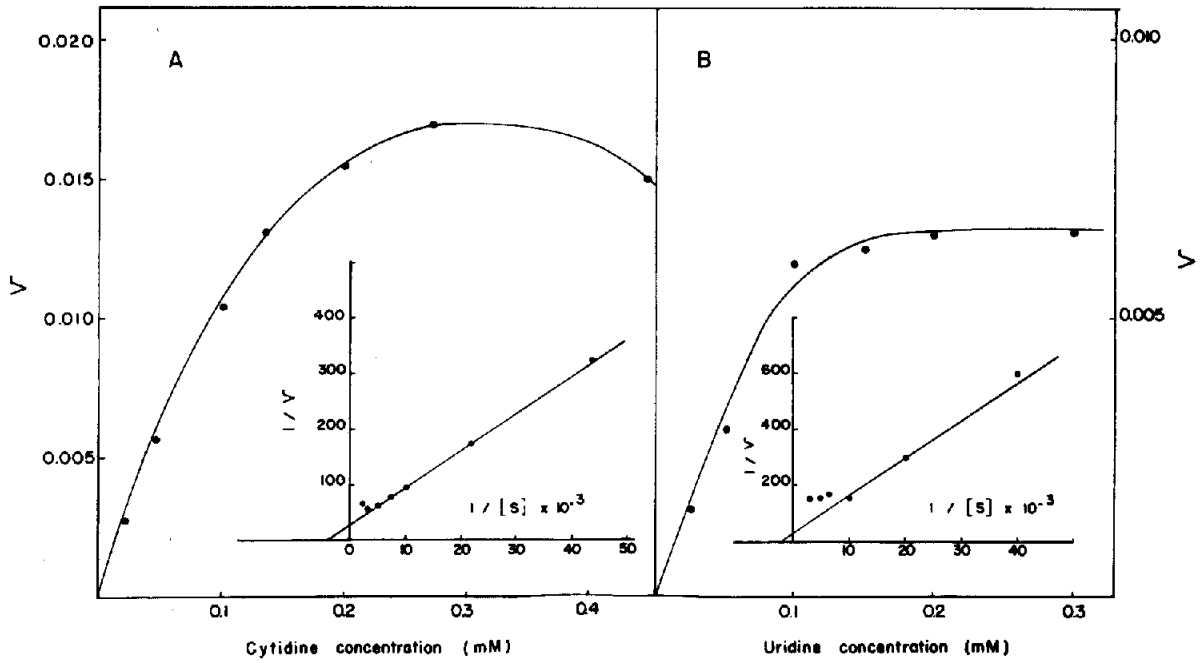

Fig. 1. Effect of cytidine concentration on initial reaction velocity of cytidine deaminase (A) and of uridine concentration on initial reaction velocity of uridine phosphorylase (B). The velocity is expressed as Δ absorbance per minute at 286 nm for cytidine deaminase and at 280 nm for uridine phosphorylase. The insets show the plots of $1/v$ versus $1/[S]$ for the evaluation of the K_m values. The K_m values for cytidine deaminase and uridine phosphorylase were 0.25 mM and 0.5 mM respectively.

3. Results

The products of the enzymic degradation of cytidine and uridine were identified by paper chromatography in the butanol-ethanol-water system described by Carter [4]. Incubation mixtures consisted of 1 ml of *B. cereus* extract, 1 ml of a solution containing 500 μg of uridine in 0.1 M potassium phosphate buffer pH 7.0, or 500 μg of cytidine in tris-HCl buffer 0.2 M pH 7.0. The reaction temperature was 37°. At various time intervals, 0.2 ml aliquots were withdrawn and the reaction was halted by addition of 0.050 ml of 50% (w/v) trichloroacetic acid. Tubes were centrifuged and 0.040 ml of the supernatant fluid were chromatographed on Whatmann no 1 filter paper. Uracil was the only product formed with uridine as substrate, even after 60 min incubation. With cytidine as substrate, uridine was the only product formed after 30 min incubation. After a longer incubation period, a small amount of uracil was detectable, probably formed from uridine and some endogenous orthophosphate, through the action of uridine phosphorylase. The presence of a contaminating uridine hydrolase might also account for the latter result.

Both cytidine deaminase and uridine phosphorylase display uncomplicated reaction kinetics (fig. 1). In the experimental conditions employed the time course of both reactions is linear for about 15 min.

Cytidine deaminase is inhibited by a number of nucleotides, including XMP, GTP, CMP, GMP and CTP; the concentrations required for 50% inhibition range between 0.25 and 0.35 mM (see also fig. 2). AMP, ATP, UMP, UTP are without effect, even at 0.5 mM, the maximal concentration tested.

Uridine phosphorylase is inhibited by GTP, CMP, CTP, XMP, GMP; the concentrations required for 50% inhibition range between 0.15 and 0.3 mM. With UMP, maximal inhibition of about 27% was observed (see also fig. 2). UTP, ATP and AMP are without effect, even at 0.5 mM.

As shown in fig. 2, for both cytidine deaminase and uridine phosphorylase sigmoidal forms of inhibi-

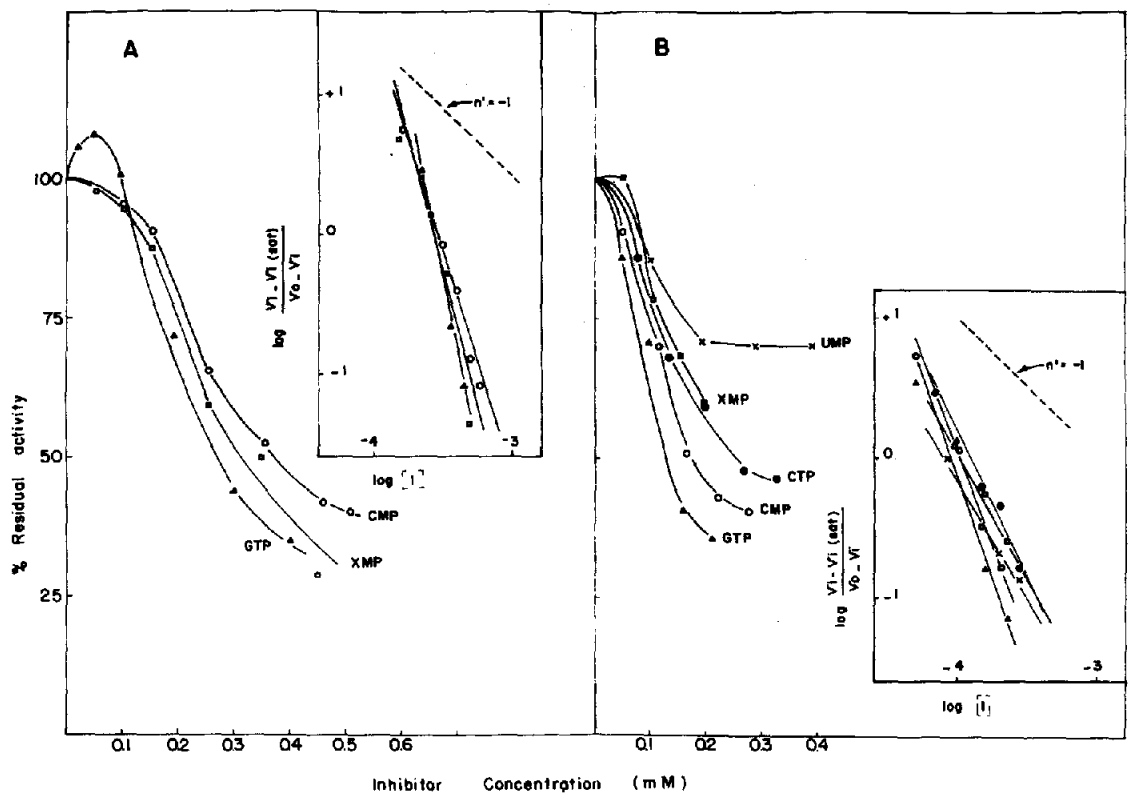

Fig. 2. Effect of varying concentrations of nucleotides on the initial velocities of cytidine deaminase (A) and uridine phosphorylase (B). *Insets.* Determination of interaction coefficient, n', between inhibitor binding sites. The calculated values for n' were − 3.23, − 3.18 and − 2.85 for GTP, XMP and CMP respectively (cytidine deaminase) and − 2.1, − 2.66, − 1.33, − 1.46 and − 1.66 for GTP, XMP, CMP, UMP and CTP respectively (uridine phosphorylase).

tion curves are observed when the enzymes are assayed in the presence of increasing concentrations of most of the inhibitors. When the Hill system of coordinates is applied to kinetic measurements by plotting $\log v_i - v_i(\text{sat})/v_0 - v_i$ (where v_i is the reaction velocity in the presence of inhibitors, $v_i(\text{sat})$ is the reaction velocity at saturating concentration of inhibitors and v_0 is the reaction velocity in the absence of inhibitors) against log of inhibitors concentration [6], a straight line of slope, n', more negative than − 1 is obtained (fig. 2), suggesting that one enzyme molecule interacts with more than one inhibitor molecule.

The data reported in table 1 show that the effect of two nucleotides acting together on uridine phosphorylase activity led to a residual activity equal to the product of the separate residual activities. A cooperative inhibition, however, is suggested between CMP and CTP molecules by the fact that a residual activity significantly lower than the product of the separate residual activities was observed in the presence of the two nucleotides.

We have observed (unpublished results) that uracil, formed by the combined action of cytidine deaminase and uridine phosphorylase, is readily converted to UMP, in the presence of 5-phosphorybosyl-1-pyrophosphate. Since the only known pathway for the formation of cytidine nucleotides involves the amination of UTP formed from UMP by successive kinase reactions [7], the observed cooperative inhibition by CMP and CTP on uridine phosphorylase may play a key role in the regulation of the "salvage pathway" of pyrimidine nucleotides biosynthesis in *B. cereus*.

Table 1
Inhibition of uridine phosphorylase by pairs of nucleotides.

Inhibitors 1	Inhibitors 2	Concn. (μM)	% Residual activity	% Residual activity with inhibitors 1 + 2	Predicted residual activity, (%)[2]
UMP		92	89	57	55
	CMP	160	62		
UMP		185	87	75	73
	CMP	107	84		
CMP		160	63	24	40
	CTP	197	64		
CMP		107	83	60	81
	CTP	130	97		
UMP		185	70	49	45
	CTP	197	64		
UMP		92	89	35	33
	CTP	262	37		
GTP		104	57	38	33
	CTP	200	58		
GTP		157	63	54	59
	CTP	130	94		
GTP		104	57	49	36
	CMP	160	63		
GTP		104	57	62	50
	CMP	107	89		

Uridine phosphorylase activity was measured in the presence of either each nucleotide alone or of a mixture of two nucleotides in terms of total nucleotide concentration. The predicted residual activity is obtained from the product of the residual activities in the presence of each inhibitor alone.

Acknowledgement

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