Baker's Yeast Cytidine Deaminase: Substrate and Inhibitor Specificity, and a Hypothesis on Metabolic and Regulatory Significance

Partially purified cytidine deaminase from baker's yeast was shown to be allosterically inhibited by both cytidine and 5'-CMP[1]. Cytidine deaminase is usually thought to play a role in pyrimidine catabolism[2]; however, WISDOM and ORSI[3] have reported interesting kinetic data on the sheep liver enzyme, suggesting that it may have a role in a salvage pathway for converting deoxycytidine to thymine nucleotides. The latter was presented as an alternative to the deoxycytidylate route.

This report presents some data on substrate and inhibitor specificity of the yeast enzyme, together with a tentative hypothesis on its metabolic role. It is shown that deoxycytidine is also deaminated by cytidine deaminase and that sigmoidal inhibition curves with finite asymptotes are obtained as a function of dTTP concentration.

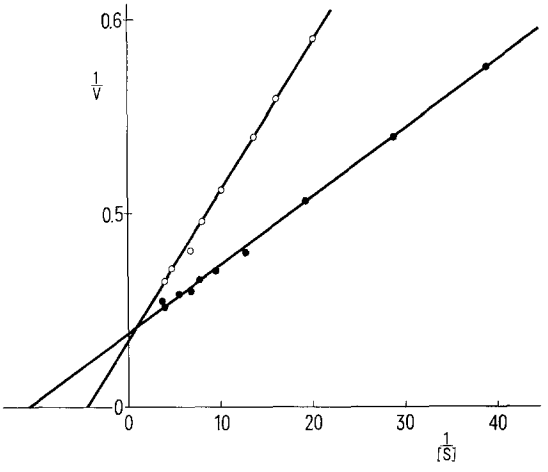


Fig. 1. Lineweaver-Burk plots for cytidine deaminase using deoxycytidine (●–●–●) and cytidine (○–○–○) as substrates [S] = mM. The velocity is expressed as nanomoles of uridine formed per minute.

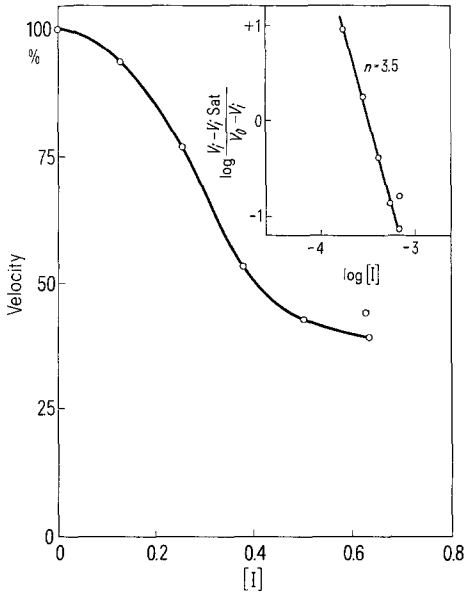


Fig. 2. Effect of varying dTTP concentration on cytidine deaminase activity. [I] = dTTP. Deoxycytidine was used as substrate at 0.125 mM concentration. The velocity V_i is expressed as % of the velocity in the absence of the inhibitor (v_o). The inset shows the Hill plot modified according to JENSEN and NESTER[7]; v_{sat} was evaluated by plotting the reciprocal of % inhibition as a function of the reciprocal of dTTP concentration; the value obtained was 35%.

Materials and methods. Cytidine deaminase was partially purified about 45-fold from commercial yeast as previously reported[1]. Deamination of cytosine derivatives was followed spectrophotometrically using the same assay procedure as for cytidine[1]. Different molar extinction coefficients at 286 nm of 3,140 and 2,650 for the conversion of cytidine to uridine derivatives and deoxycytidine to deoxyuridine derivatives respectively, were used.

Sephadex G-100 (Pharmacia) chromatography was carried out according to Andrews with a 60 × 2 cm column[4]. Nucleosides and nucleotides were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

Results and discussion. Table I shows the relative rates of deamination of some cytidine analogues. As can be seen, deoxycytidine is deaminated at a rate similar to that of cytidine, whereas the presence of a methyl group at position 5 of the pyrimidine ring or a phosphate at the 5'-position prevents deamination of both the nucleoside and the deoxynucleoside.

Figure 1 shows a double reciprocal plot of initial velocity vs substrate concentration from which the apparent K_m and V_{max} were calculated; the values are reported in Table I. Deoxycytidine seems to be a better substrate than cytidine for the yeast enzyme. The same behaviour was found with the corresponding enzyme from *E. coli*[5] whereas enzymes from mammalian sources show a higher affinity for cytidine[3,4,6]. Substrate inhibition was not observed (as was the case with cytidine[1]) using deoxycytidine at concentrations nearly 10 times greater than the K_m. Owing to the non-homogeneity of enzyme preparation, the presence of two different proteins carrying the catalytic activities towards cytidine and deoxycytidine could not be excluded a priori. However, our data are not in contrast with the hypothesis that the same enzyme protein is responsible for the deamination of the two compounds. The ratio of the specific activities towards cytidine and deoxycytidine is constant at every step during the purification procedure.

Table II shows the inhibitory effect of some nucleotides on cytidine deaminase; the same values were obtained using cytidine or deoxycytidine as substrate at concentrations nearly equal to their K_m. These results are also in favour of the hypothesis that a single enzyme deaminates both nucleosides. The following nucleotides, at concentrations from 0.25 to 0.75 mM, did not inhibit either substrate; AMP, GMP, UMP, GDP, dCMP, dCTP and those already listed in reference[1]. It seems an apparent inconsistency that UMP, a more direct end product of the pathway than cytidine nucleotides, does not inhibit the

[1] P. L. IPATA, G. CERCIGNANI and E. BALESTRIERI, Biochemistry *9*, 3390 (1970).
[2] B. S. ACHAR, R. K. MALLER and C. S. VAIDYANATHAN, Indian J. Biochem. *3*, 356 (1966).
[3] G. B. WISDOM and B. A. ORSI, Eur. J. Biochem. *7*, 223 (1969).
[4] P. ANDREWS, Biochem. J. *91*, 222 (1964).
[5] T. P. WANG, Meth. Enzym. *2*, 478 (1955).
[6] R. TOMCHICK, L. D. SASLAW and V. S. WARAVDEKAR, J. biol. Chem. *243*, 2534 (1968).
[7] R. A. JENSEN and E. W. NESTER, J. biol. Chem. *241*, 3373 (1966).

Table I. Rates of deamination of some cytidine analogues by cytidine deaminase

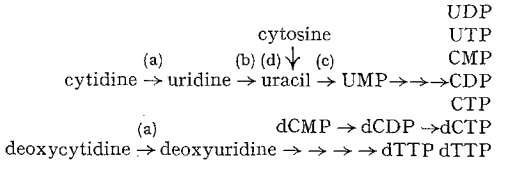
Substrate	Concentration (mM)	Rate[a]	K_m (M)	V_{max} (nmoles/min)
Cytidine	0.250	85	25×10^{-5}	5.28
Deoxycytidine	0.250	100	9.1×10^{-5}	5.00
5-methyl-cytidine	0.250	0	—	—
5-methyl-deoxycytidine	0.250	0	—	—
CM-5′-P[b]	0.250	0	—	—
dCM-5′-P	0.250	0	—	—

[a] Referred to that of deoxycytidine, taken as 100. [b] Rate is zero even at 0.75 mM concentration.

enzyme, in contrast to the very pronounced inhibition exerted by CMP. This problem might be solved by obtaining a clearer picture of the alternative pathways for the pyrimidine nucleotide synthesis.

The effect of varying concentration of dTTP on the initial rate at constant substrate concentration was also studied: Figure 2 shows a typical inhibition curve obtained with deoxycytidine as substrate. As can be seen, inhibition is a sigmoidal function of dTTP concentration, the Hill coefficient being 3.5 (inset of Figure 2); asymptotic inhibition is finite, with a value of nearly 65%; 23% activation was observed, on the other hand, in the presence of 0,3 mM dCTP. A complex inhibition pattern of cytidine deaminase by dTTP has been observed by WISDOM and ORSI[3] with the sheep liver enzyme. The results presented here show that the yeast enzyme is endowed with similar regulatory properties.

The following metabolic scheme may be thought to be operative in yeast:



Uridine hydrolase (b) was shown to be present in yeast by CARTER[8]; UMP pyrophosphorylase (c), whose activity has been found by incubation of uracil and PRPP with yeast extracts (IPATA et al., unpublished results), is under investigation in our laboratories. Cytosine deaminase (d), as a regulatory enzyme, has been characterized by IPATA et al.[9] in yeast. According to this scheme, dTTP and pyrimidine ribonucleotides are the final products of the pyrimidine salvage pathway depicted: inhibition of cytidine deaminase (a) by these compounds may then have a role as a feedback control of this alternative route; activation by dCTP would have the effect of balancing the production of pyrimidine deoxynucleotides. Finally, the role of GTP inhibition is not clear, but it may be recalled here that a similar effect of GMP is observed with deoxycytidylate deaminase[10]. The hypothesis presented here on the metabolic role of cytidine deaminase and uridine hydrolase is in contrast to the suggestion that these enzymes are exclusively involved in pyrimidine catabolism[2], our hypothesis is mainly based on the observation of the enzyme reported here. It may be recalled that a similar scheme has been proposed by IPATA et al.[11] for *B. cereus*.

Table II. Inhibitory effect of some nucleotides on cytidine deaminase[a]

Inhibitor	Concentration (mM)	Inhibition (%)
CMP	0.750	89.1
CDP	0.750	31
CTP	0.750	26
GTP	0.750	86.3
UTP	0.750	33
dTMP	0.400	44.2
dTTP	0.400	48.2

[a] Values of % inhibition coincide for both substrates. These were present in the assay mixture each at a concentration nearly equal to its own K^m.

Riassunto. La citidina deaminasi, parzialmente purificata da lievito di pane, è capace di deaminare sia la citidina che la deossicitidina. I valori delle K_m per ambedue i 2 substrati sono 25×10^{-5} M e 9.1×10^{-5} M rispettivamente. Inoltre l'enzima è inibito da numerosi nucleosidi monofosfati, difosfati e trifosfati. È molto significativa il tipo di inibizione allosterica esercitata dal dTTP. Si riporta una ipotesi sul ruolo metabolico della citidina deaminasi.

G. MAGNI[12], P. IPATA[12], P. NATALINI[12], R. A. FELICIOLI[13] and G. CERCIGNANI[13]

Laboratory of Applied Biochemistry, Faculty of Pharmacy, University of Camerino, I-62032 Camerino (Italy); and Laboratory for the Study of Physical Properties of Biomolecules and Cells, C.N.R., I-56100 Pisa (Italy),
27 December 1973.

[8] E. C. CARTER, J. Am. chem. Soc. *73*, 1508 (1951).
[9] P. L. IPATA, F. MARMOCCHI, G. MAGNI, R. A. FELICIOLI and G. POLIDORO, Biochemistry *10*, 4270 (1971).
[10] G. F. MALEY and F. MALEY, J. biol. Chem. *237*, 3311 (1962).
[11] P. L. IPATA, G. FALCONE and M. C. SERRA, FEBS Lett. *10*, 67 (1970).
[12] Laboratory of Applied Biochemistry, Faculty of Pharmacy, University of Camerino, 62032 Camerino (Italy).
[13] Laboratory for the Study of Physical Properties of Biomolecules and Cells, C.N.R., 56100 Pisa (Italy).