Biochimica et Biophysica Acta, 293 (1973) 443–448

BBA 66954

THE EFFECT OF pH ON THE ALLOSTERIC PROPERTIES OF BAKER'S YEAST CYTOSINE DEAMINASE

ETTORE BALESTRERI[a], ROMANO ANGELO FELICIOLI[a] AND PIER LUIGI IPATA[b]

[a]*Laboratory for the Study of Physical Properties of Biomolecules and Cells, C.N.R., 56100 Pisa and,* [b]*Institute of Biological Chemistry University of Perugia, Faculty of Pharmacy, 06100 Perugia (Italy)*

(Received March 8th, 1973)

SUMMARY

Baker's yeast cytosine deaminase is inhibited by nucleoside mono-, di- and triphosphates. When CMP and CDP are used as inhibitors the shapes and the interaction coefficients (n') of the inhibition curve strictly depend on both the H^+ concentration and the number of negative charges on the inhibitor molecule.

The inhibition exerted on yeast cytosine deaminase (EC 3.5.4.1) by cytidine nucleotides has been reported before[1] and was found to be of the allosteric nature, as shown by the sigmoidal shape of the inhibition curves obtained when the enzyme was assayed in the presence of increasing concentrations of inhibitory nucleotides.

In this paper the effect of pH on the inhibition of baker's yeast cytosine deaminase by nucleoside mono-, di- and triphosphates is reported. The data show that

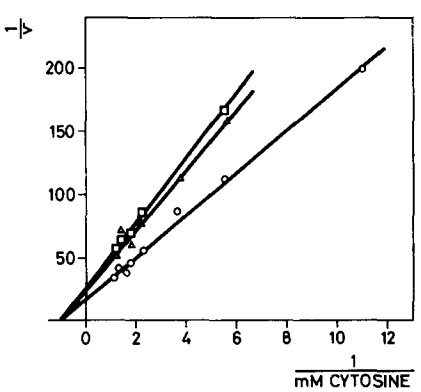


Fig. 1. Double-reciprocal plots for cytosine concentration and initial reaction velocities in the absence of inhibitors (○—○) and in the presence of 0.26 mM CMP (□—□) and 0.700 mM CTP (△—△). v is expressed as ΔE/min at 286 nm × 10^3. The assays were conducted at pH 7.

TABLE I

EFFECT OF pH ON THE INTERACTION COEFFICIENT (n') FOR NUCLEOTIDES

n' values were determined as shown in the insets of Figs 2 and 3.

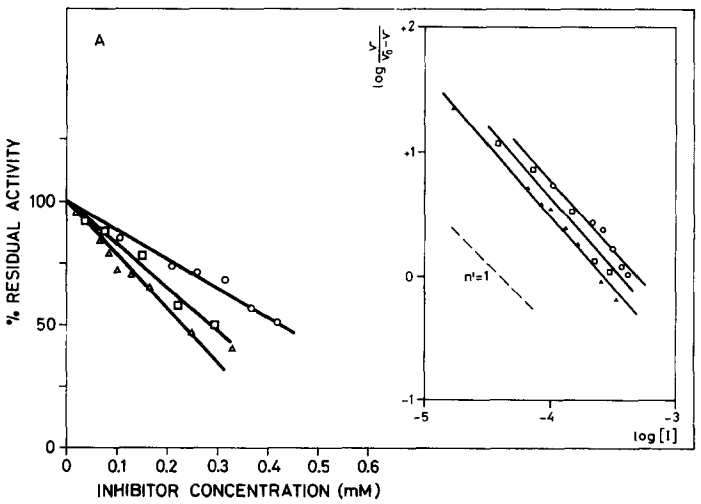
Inhibitor	*pH*		
	5	*7*	*8*
CMP	1.1	2.5	6*
CDP	1.1	1.5	3.0*
CTP	1.1	1.3	1.0
GMP	1.8	2.1	2.3*
GTP	1.5	1.3	1.8
UMP	—	1.2	1.4
UDP	—	0.8	1.3
UTP	—	1.2	1.4

* These values were obtained from the steepest slopes of the biphasic Hill plots of Fig. 2C.

the values of the interaction coefficients, n', for the cytidyl compounds used as inhibitors are remarkably pH dependent. Furthermore, it has been shown that the enzyme is inhibited to less than 50% by uridyl compounds.

Yeast cytosine deaminase was prepared and assayed spectrophotometrically according to Ipata *et al.*[1-2]. The assay was conducted in microcuvettes with a 1-cm light path and was monitored at 286 nm with a Saitron absorbance recording spectrophotometer at 37 °C. The standard reaction mixture contained in a final volume of 1 ml: 0.05 M Tris acetate buffer at the desired pH, varying concentrations of substrate, and about 70 μg of enzyme preparation. The reaction was started by addition of enzyme preparation, and the decrease in absorbance was recorded against a reference cuvette, in which substrate was substituted by water. Under the experimental conditions used, the velocity (as defined in the legend to Fig. 1) for cytosine deamination was 11, 35 and 26 at pH 5, 7 and 8, respectively.

Fig. 1 shows the double-reciprocal plots for cytosine concentration and initial



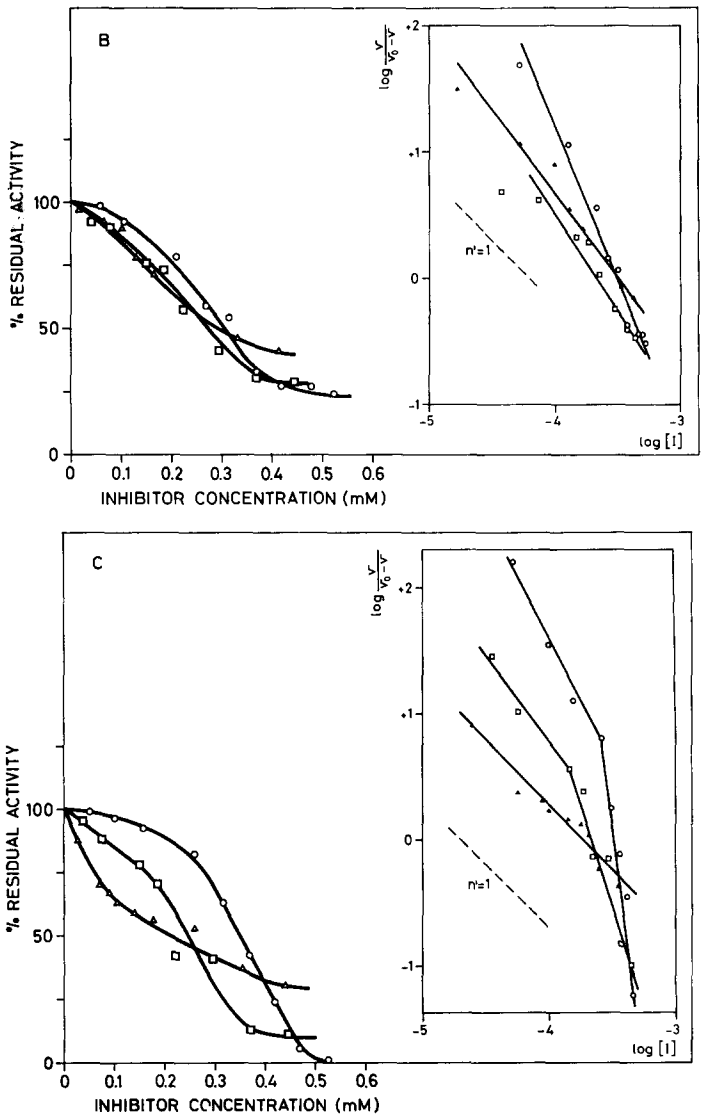


Fig. 2. Effect of varying concentrations of CMP (○—○), CDP (□—□), and CTP (△—△) at pH 5 (A), pH 7 (B) and pH 8 (C).

reaction velocities in the absence and in the presence of cytidyl compounds at pH 7. The inhibition exerted by cytidyl compounds appears to be of the non-competitive type. Similar results were obtained with uridyl and guanyl compounds listed in Table I. The apparent K_m value for cytosine was about $1 \cdot 10^{-3}$ M at pH 5, 7 and 8.

A strict dependence on H^+ concentration of the shape of the inhibition curves for cytosine deaminase was found when the enzyme was assayed in the presence of increasing concentratoins of CMP and CDP. Fig. 2 reports the inhibition curves for cytosine deaminase at pH 5, 7 and 8. It can be seen that at pH 5, n' values close to

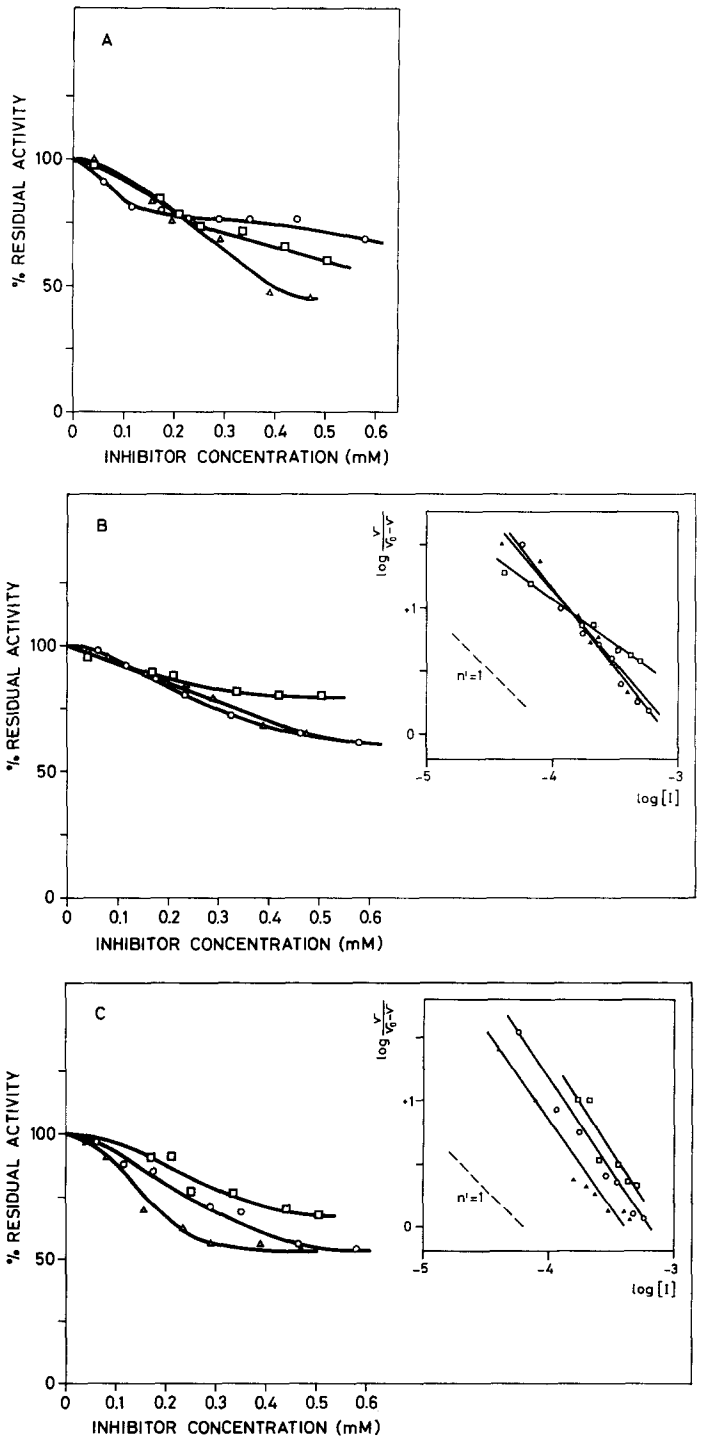


Fig. 3. Effect of varying concentrations of UMP (○—○), UDP (□—□) and UTP (△—△) at pH 5 (A), pH 7 (B) and pH 8 (C).

1 were obtained for the three compounds. At pH 7 and 8, n' values close to 1 were also obtained for CTP, whereas the inhibition curves by CMP and CDP show sigmoidal shapes, the sigmoidicity being more pronounced for CMP than CDP.

When uridyl compounds were used as inhibitors of cytosine deaminase at pH 5, 7 and 8, inhibition curves asymptotic to finite values lower than 50% were observed (Fig. 3).

Table I shows the n' values for cytidyl, guanyl, and uridyl compounds at the three pH values tested. It can be seen that for CMP and CDP the n' values are markedly dependent on the H^+ concentration and on the number of negative charges on the inhibitor molecules. Thus the n' values for CMP were 1.1, 2.5 and 6 at pH 5, 7 and 8, respectively, while those for CTP were constantly about 1. For uridyl compounds n' values slightly higher than 1 were obtained at pH 7 and 8, without appearent change in cooperativity when the mono-, di- or triphosphate was used (at pH 5 the Hill curves were difficult to draw from the experimental points of Fig. 3A).

The dependence of the allosteric properties of regulated enzymes on H^+ concentration has been observed for other regulated enzymes[3–6], and has been ascribed either to a conformational alteration of the enzyme molecules causing a variation in the accessibility of the binding sites to effector molecules, or to a change in protonation of the ligands[6–8]. The increasing cooperativity with nucleoside monophosphates which parallels the increase of pH might be tentatively ascribed to a conformational change of the enzyme molecule, since, at least between pH 7 and 8 where maximal shifts of n' values occur, nucleoside monophosphates are at least 90% deprotonated. However, it cannot be excluded *a priori* that the change in protonation of the inhibitory nucleotides might be even greater in the enzyme–nucleotide complex. The pH effect is more pronounced with with CMP, whose n' values vary from 1.1 at pH 5 to 6 at pH 8.

Changes in n' values with pH were also observed with CDP, but not with the other nucleotides listed in Table I. Thus, with CTP, GTP and UTP, the n' values did not change with pH, indicating that, despite the possible conformational change of the enzyme molecule brought about by change of pH, the cooperativity for nucleoside triphosphates remains unchanged.

The finding that, in addition to cytidyl compounds, also uridyl derivatives partially inhibit baker's yeast cytosine deaminase may be of some relevance in the regulation of the overall "pyrimidine salvage pathway" previously postulated[1]:

$$\text{Cytosine} \xrightarrow{\text{Cytosine deaminase}} \text{uracil} \xrightarrow{\text{Pyrophosphorylase}} \text{UMP} \dashrightarrow \text{CTP} \dashrightarrow \text{CMP}$$

CMP is considered the last product of the "pyrimidine salvage pathway" since CTP is the first cytidine nucleotide formed by amination of UTP[9]. It is therefore not surprising that CMP acts as the finest modulator of cytosine deaminase among the nucleotides tested, and that the inhibition exerted by UMP and other uridyl derivatives, which are intermediates of the pathway, is asymptotic to a value of about 50%.

REFERENCES

1 Ipata, P. L., Marmocchi, F., Magni, G., Felicioli, R. A. and Polidoro, G. (1971) *Biochemistry* 10, 4270–4275

2 Ipata, P. L., Cercignani, G. and Balestreri, E. (1970) *Biochemistry* 9, 3390–3394
3 Maley, F. and Maley, G. F. (1962) *J. Biol. Chem.* 237, PC3311–PC3312
4 Scarano, E., Bonaduce, L. and De Petrocellis, B. (1960) *J. Biol. Chem.* 235, 3556–3561
5 Prusoff, W. H. and Chang, P. K. (1968) *J. Biol. Chem.* 243, 223–230
6 Ipata, P. L. and Cercignani, G. (1970) *FEBS Lett.* 7, 129–131
7 Mansour, T. E. (1963) *J. Biol. Chem.* 238, 2285–2292
8 Mansour, T. E. and Ahlfors, C. E. (1968) *J. Biol. Chem.* 243, 2523–2533
9 Mahler, H. R. and Cordes, E. H. (1967) *Biological Chemistry* p. 203, Harper and Row, New York