ON THE REGULATORY PROPERTIES OF DEOXYCYTIDYLATE AMINOHYDROLASE [+]

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In a previous paper we suggested the occurrence of at least one regulatory site (Scarano et al. 1962a and 1963) on dCMP aminohydrolase. The present paper reports further experiments that support this hypothesis.

Methods

The enzyme was partially purified from the spleen of the ass; the purification procedure will be described elsewhere. In the presence of 4 mM dCMP, at 38°C, pH 7.3, the enzyme deaminated 31 µmoles of the substrate per mg protein per minute, as measured by the titrimetric assay (see below).

Enzyme activity was determined spectrophotometrically as already described (Scarano et al. 1962b) and by a titrimetric assay. A Radiometer titrator type TTTI with titration assembly type SBR2/SBU1 was used for the titrimetric assay. By using the titrimetric assay we became aware that the correction factors which were used in previous work (Scarano 1963 and 1962b) to calculate the initial velocity at high substrate concentrations from spectrophotometric experiments, were too low. As reported in this paper inhibition of dCMP aminohydrolase by excess substrate does not occur.

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Results and Discussion

The data in fig. 1 show the kinetic behavior of dCMP aminohydrolase and the changes of the kinetics properties of the enzyme caused by addition of dCTP, dTTP, and phosphate respectively to the incubation mixtures: 1) In the absence of any regulatory compound, namely in the presence of substrate alone, a straight line is obtained in the Lineweaver and Burk plot. In the presence of phosphate a multimolecular kinetics with respect to the substrate is obtained at low concentrations. At substrate concentrations between 2 mM and 10 mM dCMP a straight line is obtained from which it is possible to calculate a Michaelis-Menten constant. 2) In the presence of dCTP a straight line is obtained throughout the whole range of substrate concentrations used both in the presence and in the absence of phosphate. The K_m calculated in the presence of dCTP is lower than that obtained in the experiments in which only the substrate and the enzyme are present in the incubation mixture. The increase in affinity of the enzyme for the substrate is easy to demonstrate at low substrate concentrations, while at concentrations higher than 3 mM the addition of dCTP has no effect on the

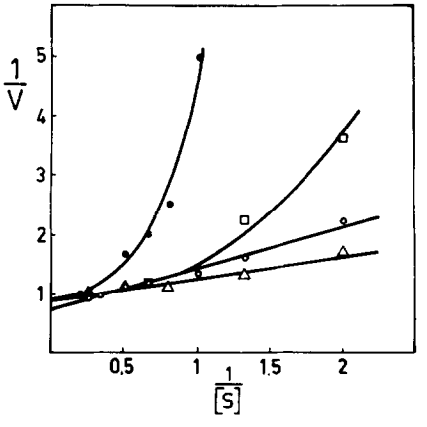


Figure 1

Effect of dCTP and dTTP on deoxycytidylate aminohydrolase.

—o—o— no addition;
—△—△— 0.06 mM dCTP;
—●—●— 0.006 mM dTTP;
□—□ 0.125 mM phosphate.
V is expressed in μmoles of dCMP deaminated per minute per 4 ml incubation mixture;
[S] = mM dCMP.
Titrimetric assay T = 38°; pH = 7.3; Enzyme 40 μg/4 ml; 50 μg/4 ml crystalline seroalbumin;
K_m (no addition) = 0.9 mM
K_m + dCTP = 0.4 mM

reaction. Beside the effect on the dependence of velocity upon substrate concentration, dCTP removes the inhibition of dCMP aminohydrolase caused by allosteric inhibitors (Scarano et al. 1962a and 1963). This and other evidence (Scarano et al. 1962b) excludes a coenzyme effect and indicates that dCTP is an allosteric effector of dCMP aminohydrolase. The difference between the results of Maley and Maley (1963) and ours may be explained by the fact that they use a ten minute assay, while we extrapolate the initial velocity from measurements in the first minute after the addition of the enzyme. It may also be postulated that the chicken embryo enzyme of Maley and Maley (1963) has properties different from those of the spleen enzyme. 3) In the presence of dTTP a curve approximating a parabola is obtained and consequently no K_m can be determined. The V_{max} is unchanged.

The inhibition by dTTP is removed by dCTP, while the inhibition by dTMP, a typical competitive inhibitor of the enzyme, is not removed (Scarano et al. 1963).

In the presence of a chelating agent for divalent cations such as versene, citrate or pyrophosphate dTTP has no inhibitory effect on the enzyme, as shown in table I.

TABLE I. Effect of Versene, Citrate and Pyrophosphate on the Inhibition of Deoxycytidylate Aminohydrolase by dTTP.

Temperature 38°C; substrate 1 mM dCMP; pH 7.3; Titrimetric assay

Addition	Activity μmoles min^{-1} ml^{-1}
None	175
Mg^{++}	174
Chelating Agent 1 mM	174
dTTP 13 μM	15
dTTP 13 μM plus chelating agent 1 mM	165

The addition of Mg^{++}, Ca^{++} or Mn^{++} restores the inhibition by dTTP, as demonstrated in fig. 2. On the contrary, chelating agents do not interfere with the dTMP inhibition.

Maley and Maley (1962 and 1963) have described Mg^{++} dependence of the activation of dCMP aminohydrolase by dCTP. We have not been able to obtain clear-cut experiments on the relationships between dCTP and divalent cations. In our experiments dCTP is active on the enzyme also in the presence of 0.01 M versene. It is pertinent to mention that the monkey liver enzyme, which deaminates 60 μMoles of dCMP per mg protein per minute, at pH 7.1 and 38°C, is fully active without any addition of Mg^{++} and dCTP at 2 mM dCMP.

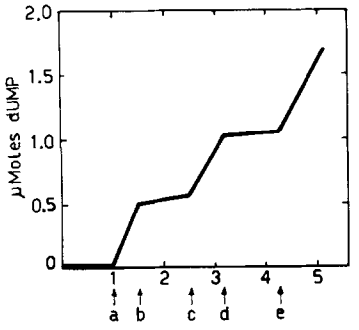


Figure 2

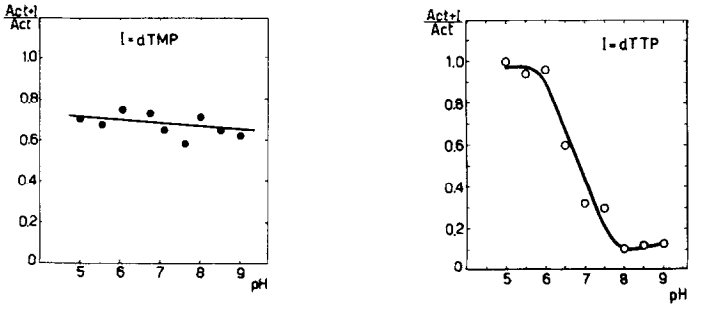
Effect of chelating agents and divalent cations on the inhibition of deoxycytidylate aminohydrolase by dTTP.

The arrows indicate successive addition into the same incubation mixture. The volumes added are, in total, 1/50 of the volume of the incubation mixture. dCMP = 4 mM; T = 38°C; pH 7.3; Titrimetric assay: a) 31 μg enzyme per 4 ml b) dTTP 12 μM c) versene, citrate, or pyrophosphate 2 mM d) Mg^{++}, Ca^{++}, Mn^{++} 0.5 mM e) dCTP 60 μM. Absissa: time in minutes.

A different pH dependence of the inhibition of dCMP aminohydrolase by dTTP and dTMP is evident from the experiments reported in fig. 3.

The different action of chelating agents and divalent cations on the inhibition of dCMP aminohydrolase by dTTP and dTMP supports the hypothesis that different groups on the enzyme are responsible for the action of the two inhibitory nucleotides. The same conclusion is strengthened also by the different dependence upon pH of the inhibitions of the enzyme by dTTP and dTMP. The present evidence does not permit to distinguish, whether dCMP aminohydrolase has one regulatory site for both allosteric inhibitors and allo-

Figure 3



pH Dependence of the Inhibition of dCMP Aminohydrolase by:

A: 0.6 mM dTMP B: 0.05 mM dTTP

The enzyme activity was determined using dCMP as substrate by assay 1 (Scarano et al. 1960).

steric activators or whether the enzyme has two distinct sites for the two classes of regulatory compounds.

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