Deoxycytidylate Aminohydrolase. II. Kinetic Properties. The Activatory Effect of Deoxycytidine Triphosphate and the Inhibitory Effect of Deoxythymidine Triphosphate*

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ABSTRACT: Studies of the regulation of the activity of deoxycytidylate aminohydrolase have shown that deoxycytidine triphosphate (dCTP), the allosteric activator, and deoxythymidine triphosphate (dTTP), the allosteric inhibitor of the enzyme, are active only in the presence of a divalent cation. Experiments with Ca^{2+}, Mg^{2+}, and Mn^{2+} ions indicate that the true enzyme effectors are the complexes between the deoxynucleoside triphosphates and the metal ions. The dependence of the enzyme reaction on the substrate concentration is of an order higher than one in the buffers studied and the substrate concentration at half-enzyme saturation is dependent on the buffer. In the presence of 3×10^{-7} M dCTP the kinetics becomes of order one, while in the presence of increasing concentration of dTTP the apparent order of the reaction increases up to a limiting value of 4. The addition of dCTP removes the inhibition by dTTP. Likewise, high concentrations of glycerol activate the enzyme and high salt concentrations inhibit the enzyme.

The dependence of the reaction velocity of deoxycytidylate aminohydrolase on the substrate concentration indicates a multimolecular kinetics as has been reported in previous papers (Scarano *et al.*, 1963, 1964). Moreover, in the presence of dCTP[1] the kinetics is in agreement with the Michaelis–Menten theory; while in the presence of dTTP, the apparent order of the reaction with respect to the substrate increases (Scarano, 1964; Scarano *et al.*, 1965a) up to a limiting value of 4. The results suggest the occurrence of multiple catalytic sites and regulatory sites on the enzyme molecule. The substrate sites might interact cooperatively (Monod *et al.*, 1965); the interaction increasing after the binding of dTTP to the enzyme and disappearing after the binding of dCTP.

The availability of a homogeneous preparation of dCMP-aminohydrolase (Scarano *et al.*, 1965b; Geraci *et al.*, 1967) permits the study of the kinetic properties of the enzyme employing spectrophotometric and titrimetric methods under conditions which allow accurate measurements of initial reaction rates.

The kinetic data reported in the present paper are in agreement with a model of the enzyme with four substrate sites and multiple regulatory sites. The effects of salt, pH, and glycerol on the kinetic properties of the enzyme, also described in the present paper, indicate that the regulation of the enzyme activity is based on conformational changes of the molecule.

Materials and Methods

Quartz-redistilled water was used for all the solutions. All reagents were analytical grade and were always recrystallized from 10 mM Versene and then from water. Nucleotides were purchased from Calbiochem and when necessary purified by thin layer chromatography (Grippo *et al.*, 1965). 5-CH_3-dCTP was prepared from 5-CH_3-dCMP according to Smith and Khorana (1958).

The enzyme was prepared as described in the previous paper (Geraci *et al.*, 1967). All the experiments, except where otherwise stated, were carried out with the homogeneous enzyme.

The kinetic measurements were performed by a spectrophotometric and by a titrimetric method. (a) A Beckman DK2A recording spectrophotometer was used to measure the initial rates of the enzyme reaction. The instrument was equipped with a thermostatic chamber and with a device, designed by Terzano, Milano, Italy, which allowed the automatic recording of the absorbancy at a fixed wavelength as a function of time; a velocity of scanning of 10 cm/min was used. This method was used in the range of substrate concentrations from 0.05 to 3 mM. Cuvets of 10- or 1-mm light paths were utilized depending on the substrate concentration. (b) The initial rates of the enzyme reaction were determined titrimetrically with a Radiom-

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[1] Abbreviations used: dCTP, deoxycytidine triphosphate; dTTP, deoxythymidine triphosphate; dUMP, deoxyuridine monophosphate; ATP, adenosine triphosphate.

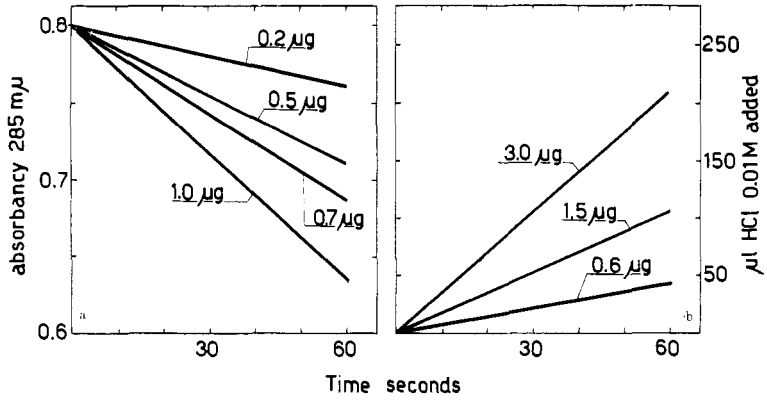

FIGURE 1: Spectrophotometric (a) and titrimetric (b) assays. (a) Incubation mixture (0.3 ml) containing 3 mM dCMP-50 mM Tris, pH 7.5, 38°. The recording of absorbancy starts at 15 sec after the addition of the enzyme. (b) Incubation mixture (2 ml) containing 5 mM dCMP, pH 7.5, 38°. The recording of HCl addition starts 10 sec after the addition of the enzyme. The amount of enzyme used in each assay is indicated in the figures.

eter titrator type TTTI with titration assembly type SBR2-SBU1, in the range of substrate from 0.5 to 30 mM. The titrating solution was HCl from 1 to 10 mM.

With both methods it is easy to measure the initial rate of the enzyme reaction. In every instance the reaction was started by addition of the enzyme to the incubation mixture equilibrated at 38°. The automatic recording was started at 10–15 sec after the addition of the enzyme and the reaction was followed for 30 sec. The initial rate of the reaction was measured from the linear part of the graph. Typical spectrophotometric and titrimetric assays are reported in Figures 1a and b. All the assays, except where otherwise indicated, were made with the spectrophotometric method.

Results

The activation of dCMP-aminohydrolase by dCTP and by 5-CH_3-dCTP is demonstrated in Figure 2. In the absence of magnesium ions there is no activation of the enzyme by the two deoxynucleoside triphosphates. The same maximal activation is obtained with dCTP and with 5-CH_3-dCTP, and is observed at a concentration of 0.2 and 0.7 μM, respectively. A further increase in the concentration of the regulatory nucleotides does not produce any further increase of the velocity of the reaction.

The effect of the substitution of Mg^{2+} with Mn^{2+} and Ca^{2+} on the activation of the enzyme by dCTP is shown in Figure 3. Mn^{2+} is the most and Mg^{2+} is the least effective ion.

The inhibition of the enzyme by dTTP and its dependence on Mg^{2+} is demonstrated in Figure 4. It is possible to obtain 100% inhibition. In the absence of magnesium ions, dTTP has no effect.

The activatory effect of dCTP and the inhibitory effect of dTTP in the presence of Mg^{2+} as a function of the substrate concentration were studied with dCMP and with 5-CH_3-dCMP as substrates. Tris, phosphate, and imidazole buffers were used in the experiments

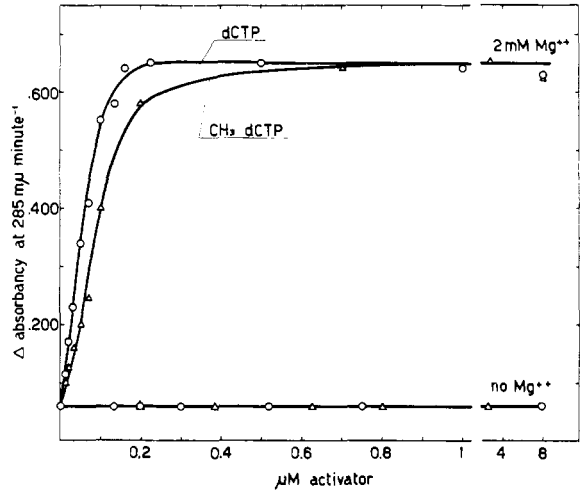

FIGURE 2: Effect of dCTP and 5-CH_3-dCTP on the activity of dCMP-aminohydrolase. Substrate, 0.1 mM dCMP in 0.1 M Tris, pH 7.5. Enzyme (0.4 unit)/ml of incubation mixture.

with dCMP as substrate, while only Tris buffer was used in experiments with 5-CH_3-dCMP as substrate (Figure 5). At low substrate concentrations and in the absence of allosteric effectors, the dependence of the velocity upon substrate concentration is of order higher than one. In the double-reciprocal plot a linear relationship is obtained at high substrate concentrations, while at low substrate concentrations the curve approximates a parabola indicating kinetics of order higher than one with respect to the substrate. dTTP, the inhibitory nucleotide, increases the order of the parabola describing the relationship V *vs.* [S]. In the presence of 0.3 μM dCTP, the activatory nucleotide, and 1 mM $MgCl_2$, the kinetics is of first order at all substrate concentrations. The concentration of dCTP was chosen from the data reported in Figure 2. The same general pat-

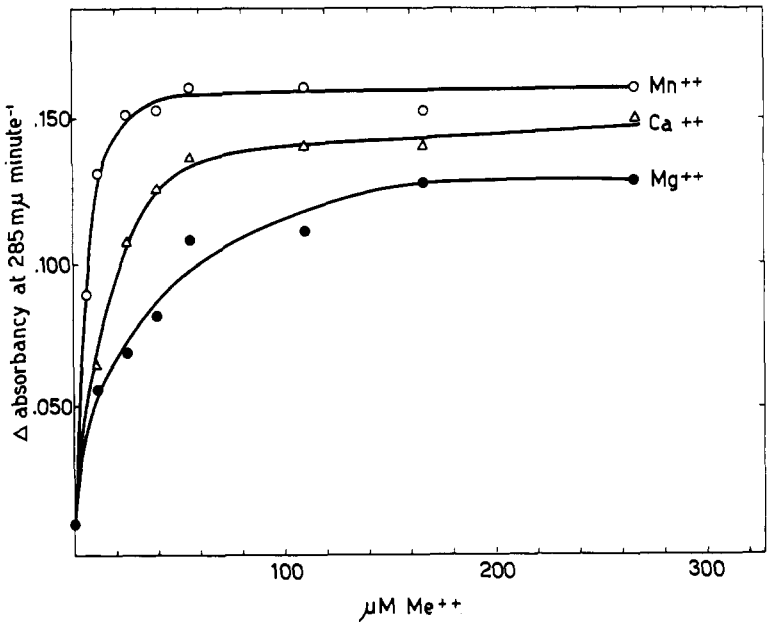

FIGURE 3: Requirement of a divalent cation for the activation of the enzyme by dCTP. 65 μM dCMP–2.2 μM dCTP in 0.1 M Tris, pH 7.5. Enzyme (0.2 unit)/ml of incubation mixture.

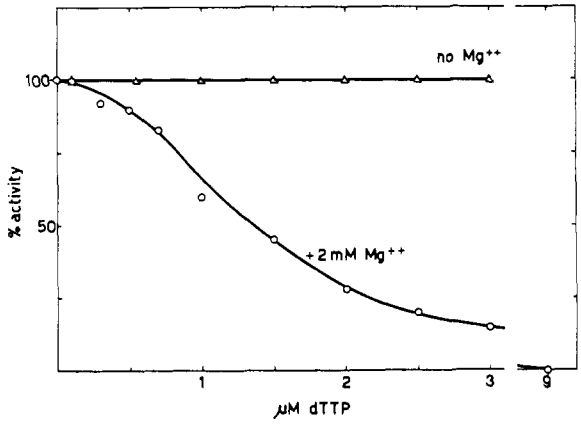

FIGURE 4: Effect of dTTP on the activity of dCMP-aminohydrolase. dCMP (2 mM) in 0.05 M Tris, pH 7.5. Enzyme (0.65 unit)/ml of incubation mixture.

TABLE I: Effect of Buffers and of dCTP and dTTP at pH 7.5 upon the Value of the Substrate Concentration at One-Half the Maximum Velocity.[a]

Addition (μM)	Imidazole–HCl (mM dCMP)	Tris–HCl (mM dCMP)	Phosphate (mM dCMP)
None	0.2	0.48	1.2
dCTP (0.3)	0.09	0.15	0.5
dTTP (0.6)	1.95	1.05	1.95

[a] The incubation mixture contained 0.05 M buffer, 1 mM $MgCl_2$, and 0.7 unit of enzyme/ml.

tern of effects is observed in the buffers used, but the maximum velocity and the value of the concentration of the substrate at half-maximum velocity are different (Table I).

Using 5-CH_3-dCMP as substrate (Figure 5d) the kinetics is analogous to that obtained with dCMP, but higher concentrations of dCTP are required to obtain enzyme activation. The maximum velocity in the presence of 2.5 μM dCTP is lower than that observed with the substrate only. The extent of the inhibition by dCTP at high 5-CH_3-dCMP concentrations is not affected by lowering the dCTP concentration to 0.3 μM, while the activatory effect at low substrate concentration decreases. The inhibitory effect of dCTP at high substrate concentration is observed also when using dCMP as substrate but at dCTP concentrations higher than those studied in Figure 5.

The regulation of the enzyme activity by dCTP and dTTP is reversible. The data of Figure 6 demonstrate the removal of the dTTP inhibition by dCTP. The inhibition by dTTP can be always removed by dCTP; the higher the concentration of dTTP the higher is the concentration of dCTP needed to reverse the inhibition and the same final velocity is observed.

The equation proposed by Hill (1910) to describe the kinetics of hemoglobin oxygenation was used to determine the apparent order of the reaction with respect to the substrate, activator, and inhibitor.

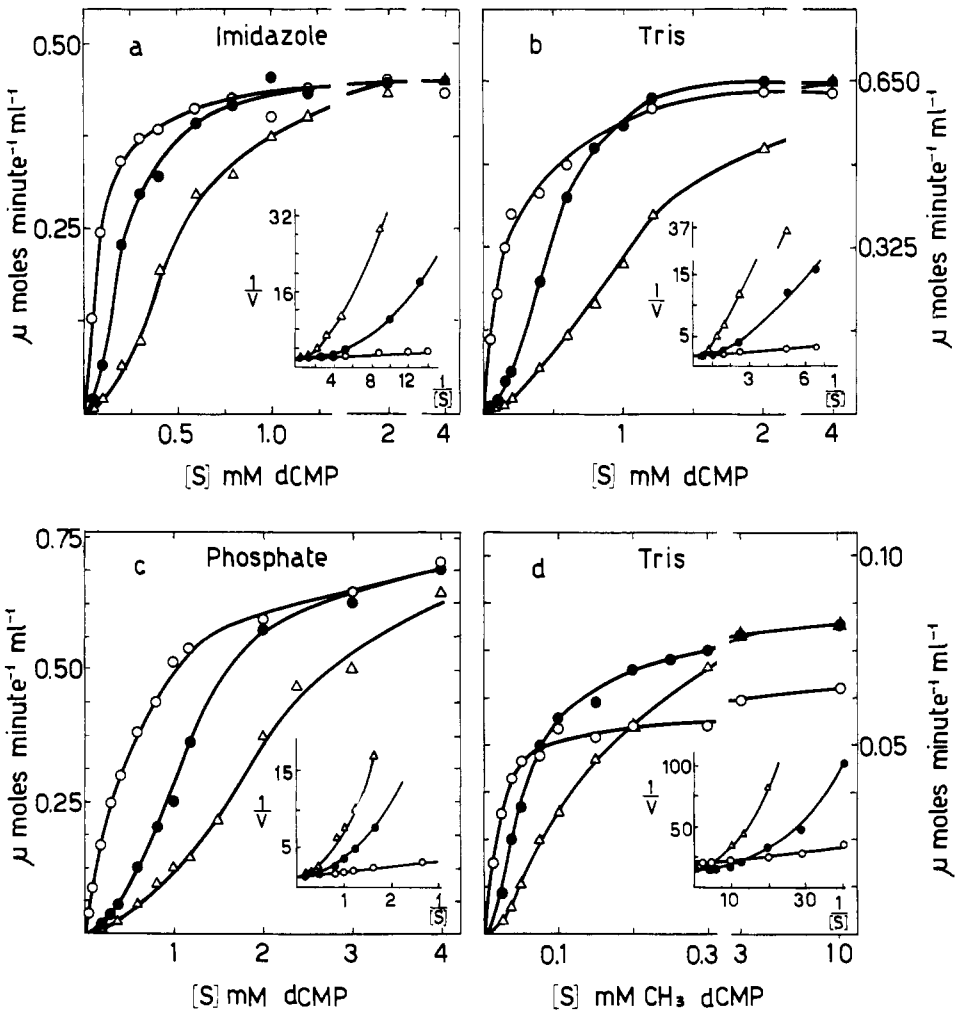

FIGURE 5: Effect of dCMP concentration on the initial reaction rate (a–c) and effect of 5-CH_3-dCMP concentration on the initial reaction rate (d). (a–c) Enzyme (0.7 unit)/ml of incubation mixture in the presence of 1 mM $MgCl_2$. (●—●), no addition; (o—o), 0.3 μM dCTP; (Δ—Δ), 0.6 μM dTTP. In the bottom right corners the double-reciprocal plots of the data are reported. (d) Enzyme (0.22 unit)/ml of incubation mixture in the presence of 1 mM $MgCl_2$. (●—●), no addition; (o—o), 2.5 μM dCTP; (Δ—Δ), 2.5 μM dTTP. The concentration of the buffers was 0.05 M.

The parameters of the Hill equation as applied to dCMP-aminohydrolase are defined as follows

$$\log \frac{v_1}{V_1 - v_1} = \log K_1 + n_1 \log [\text{dCMP}] \qquad (1)$$

$$\log \frac{V_2 - v_2}{v_2} = \log K_2 + n_2 \log [\text{dTTP}] \qquad (2)$$

$$\log \frac{v_3}{V_3 - v_3} = \log K_3 + n_3 \log [\text{dCTP}] \qquad (3)$$

where in eq 1 $V_1 = v_1$ maximum; in eq 2 $V_2 = v_2$ in the absence of dTTP; in eq 3 $V_3 = v_3$ at saturating concentrations of dCTP.

The order of the reaction for the substrate, n_1, in the absence of allosteric effectors, is equal to 2 for dCMP and 1.6 for 5-CH_3-dCMP (Figure 7). The effect of dTTP upon n_1 for dCMP is demonstrated in Figure 8. By increasing the concentration of dTTP up to 2.2 μM, n_1 increases to a value of 4. A further increase in the concentration of dTTP up to a value of 20 μM does not produce a further increase in n_1.

Data reported in Figures 6–8 were obtained with a 50% pure enzyme; similar data have been obtained with the homogeneous enzyme in all the instances that have been checked. In the presence of saturating concentrations of dCTP, n_1 for both dCMP and 5-CH_3-dCMP is equal to 1. The apparent order of the reaction for dCTP activation, n_3, and that for dTTP inhibition, n_2, are higher than 1 and do not depend significantly upon the concentration of the substrate (Figure 9).

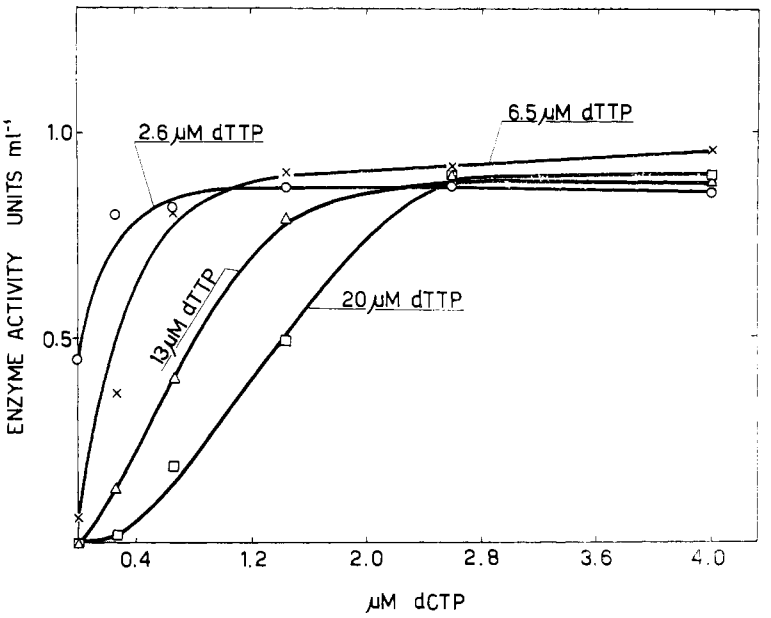

FIGURE 6: Reversal of deoxythymidine triphosphate inhibition by deoxycytidine triphosphate. Incubation mixture contained 0.1 M Tris–2 mM dCMP–1 mM $MgCl_2$, pH 7.5, and 1 unit of enzyme/ml. The enzyme was 50% homogeneous.

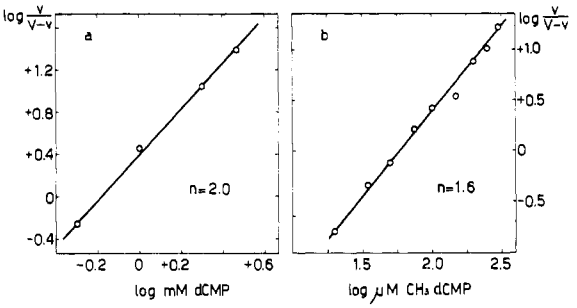

FIGURE 7: Hill plot of the dependence of the enzyme activity upon substrate concentration. (a) Titrimetric assay at 38°. The incubation mixtures contained dCMP–1 mM $MgCl_2$ at pH 7.5 and 0.6 unit of enzyme/ml. The enzyme was 50% homogeneous. (b) Spectrophotometric assay at 38°. The incubation mixture contained 5-CH_3-dCMP–0.05 M Tris–1 mM $MgCl_2$, pH 7.5, and 0.22 unit of enzyme/ml.

TABLE II: Criteria to Distinguish between Isosteric and Allosteric Inhibitors for dCMP-aminohydrolase.

	Isosteric	Allosteric
(1)	Kinetics of first order with respect to the inhibitor	Kinetics of order >1 with respect to the inhibitor
(2)	Effective at millimolar concentration, namely, at concentration of the same order of magnitude than the substrate	Effective at micromolar concentration
(3)	Do not require a divalent cation	Require a divalent cation
(4)	Inhibition not reverted by deoxycytidine triphosphate	Inhibition reverted by deoxycytidine triphosphate
(5)	Inhibition not dependent upon pH	Inhibition dependent upon pH
(6)	Some are activators at low substrate concentration	Do not function as activators at low substrate concentration

In Figure 10 the inhibition by dTMP and by dUMP, which behave as classic competitive inhibitors, is compared with the inhibition by dTTP and by dUTP, which behave as allosteric inhibitors. The two types of inhibitors can be distinguished not only by their different kinetics, but also by the other criteria listed in Table II.

Activation and inhibition of dCMP-aminohydrolase similar to those obtained with dCTP and dTTP can be obtained in the absence of the regulatory nucleotides by other means. Low pH and glycerol bring about an effect similar to the dCTP activatory effect; high pH values and salts cause an inhibition similar to that caused by dTTP (Figures 11–13). No divalent cation is required for the activatory effect of glycerol. Magnesium ions are present in the experiment described in Figure 11 to permit the study of the action of dCTP and/or of dTTP on the enzyme in the presence of glycerol. It is interesting to notice that dCTP releases the inhibition by pH and salts and that dTTP inhibits

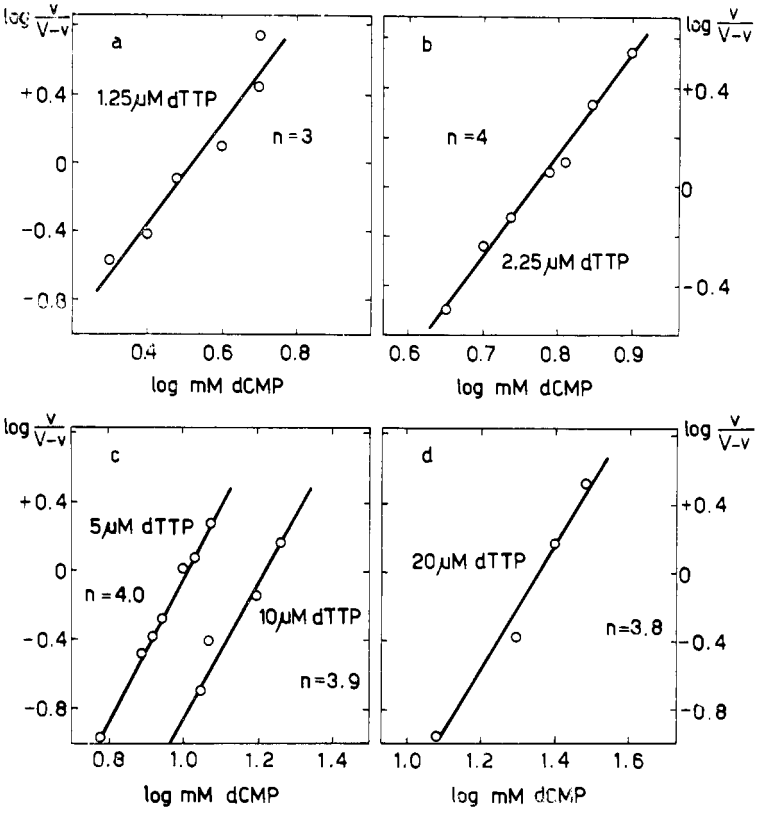

FIGURE 8: Effect of deoxythymidine triphosphate upon the coefficient n_1 of the Hill equation. Titrimetric assay at 38°. The enzyme was 50% homogeneous (0.6 unit/ml of incubation mixture).

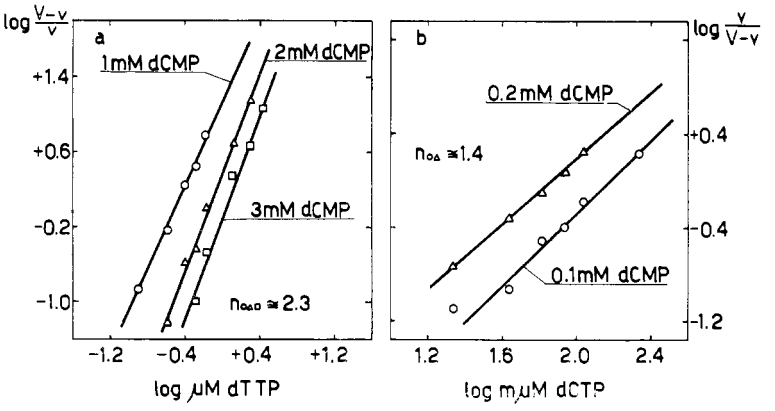

FIGURE 9: Coefficient "n" of the Hill equation for the binding of dTTP (a) and dCTP (b) at different substrate concentrations. (a) The incubation mixture contained 0.05 M Tris–1 mM $MgCl_2$, pH 7.5, and 0.6 unit of enzyme/ml. $V = v$ in the absence of the inhibitor. (b) The incubation mixture contained 0.05 M Tris–1 mM $MgCl_2$, pH 7.5, and 0.45 unit of enzyme/ml. $V = v$ at dCTP saturating.

the enzyme activated by glycerol and low pH. While the presence of concentrations of dCTP, which give rise to the maximum activatory effect at low substrate concentrations, does not decrease the maximum velocity of the enzyme reaction, glycerol concentrations, which activate the enzyme at low substrate concentrations, decrease the maximum velocity.

In the presence of glycerol and in the presence of glycerophosphate the enzyme kinetics can be described with sufficient accuracy in terms of the Michaelis–Menten theory. It seems that glycerol has the same effect on the enzyme as dCTP; the effect of glycerol is reversible. At glycerol concentrations, where the maximum activatory effect occurs, dCTP-Mg does not produce further activation.

High salt concentration increases the substrate co-

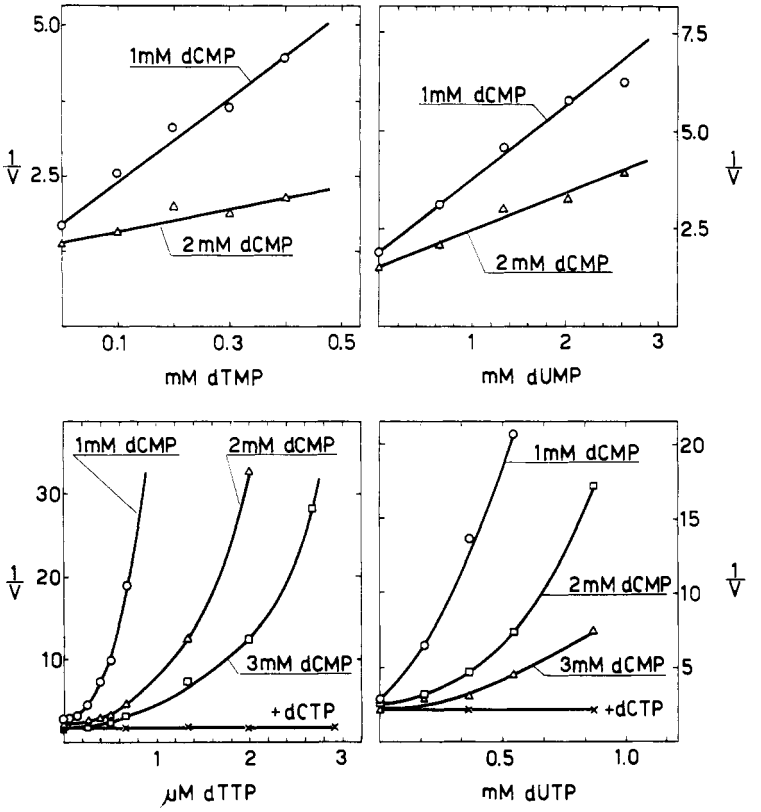

FIGURE 10: Dixon plots of the inhibitory effect of dUMP and dTMP, and of dTTP and dUTP. Incubation mixture contained 0.05 M Tris–1 mM $MgCl_2$, pH 7.5, and 0.6 unit of enzyme/ml.

operative effect; it appears that high concentrations of salts act on the enzyme as dTTP. The salt effect is reversed by dilution and by dCTP. In Figure 12 are shown the results obtained when using ammonium sulfate; similar results are obtained when using KCl.

At different pH values the substrate cooperative interaction changes; at pH values near 6 this interaction is almost absent; at high pH values it increases. However, in glycerophosphate buffer at pH 7–8 the cooperative effect is almost completely eliminated (Figure 13).

Discussion

dCMP-aminohydrolase does not require the presence of a divalent cation for the aminohydrolysis of dCMP and 5-CH_3-dCMP as already reported in previous papers (Scarano *et al.*, 1964; Geraci *et al.*, 1967). The metal is required for the effect of dCTP and dTTP on the enzyme activity. Mg^{2+}, Ca^{2+}, and Mn^{2+} ions have the same qualitative effect although they show quantitative differences. The pH dependence of dCTP activation and of dTTP inhibition (Scarano *et al.*, 1964) indicate that the true allosteric effectors are complexes of the deoxynucleoside triphosphates with a divalent cation. In fact, the apparent binding constant of ATP for Mn^{2+} ions is higher than those for Mg^{2+} and Ca^{2+} (Johnson, 1960; Phillips *et al.*, 1965) and the pH dependence of the apparent binding constants of ATP for divalent cations is similar to that of the dCTP and of the dTTP effect on the enzyme activity.

In the presence of Mg^{2+} ions 5-CH_3-dCTP except for a very small difference in the effective concentrations has the same activatory action as dCTP on the enzyme. On the other hand, dUTP in the presence of Mg^{2+} ions has the same inhibitory effect as dTTP, but at concentrations 100 times higher. Thus, the substitution of an amino group with a hydroxyl group, changes an allosteric activator, 5-CH_3-dCTP-Mg, into an allosteric inhibitor, dTTP-Mg. By contrast, the substitution in position 5 of a hydrogen with a methyl group does not change the type of allosteric effect of the nucleotides.

In the buffers used the substrate saturation curves can be interpreted on the basis of a cooperative interaction of multiple substrate sites. The maximum interaction of the substrate sites is obtained in the presence of saturating concentrations of dTTP and the value of the apparent order of the reaction under these conditions might correspond to the number of the catalytic sites. The absence of cooperative interactions, when the enzyme is assayed at saturating concentration of dCTP, is demonstrated by the kinetics of order one which is observed under these conditions. As reported in Table I, the substrate concentration at half-enzyme saturation depends upon the nature of the buffer used,

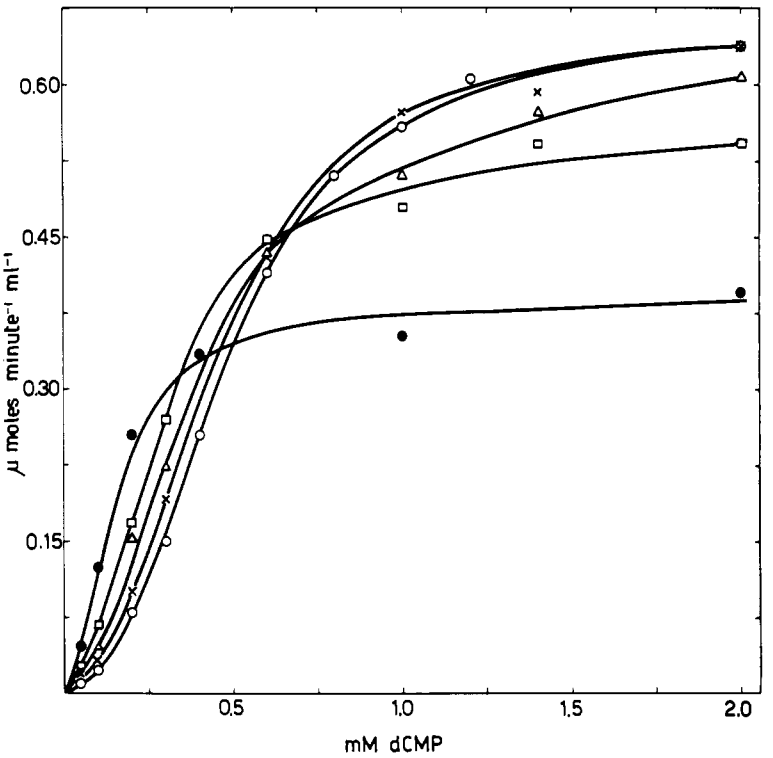

FIGURE 11: Effect of glycerol on initial reaction rates. Incubation mixture contained 0.05 M Tris–1 mM $MgCl_2$, pH 7.5, 0.7 unit of enzyme/ml. Glycerol: (x—x), 5%; (Δ—Δ), 10%; (□—□), 15%; (●—●), 20%; (○—○), no addition.

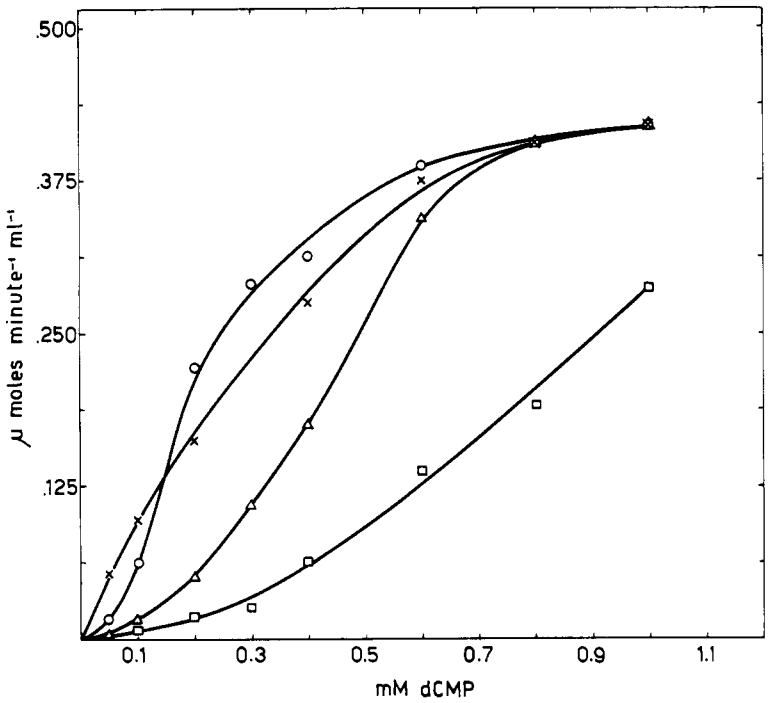

FIGURE 12: Effect of ammonium sulfate on initial reaction rates and its reversal by dCTP. The incubation mixture contained 0.05 M Tris–1 mM $MgCl_2$, pH 7.5, and 0.7 unit of enzyme/ml. Ammonium sulfate: (Δ—Δ), 0.05 M; (□—□), 0.15 M; (x—x), 0.15 M + 2.5 μM dCTP; (○—○), no addition.

while in the presence of dTTP this value is almost independent of the buffer. It is interesting to notice that the maximum regulatory effects are observed at low substrate concentrations which more likely occur *in vivo*.

The dCTP-activatory effect at low substrate con-

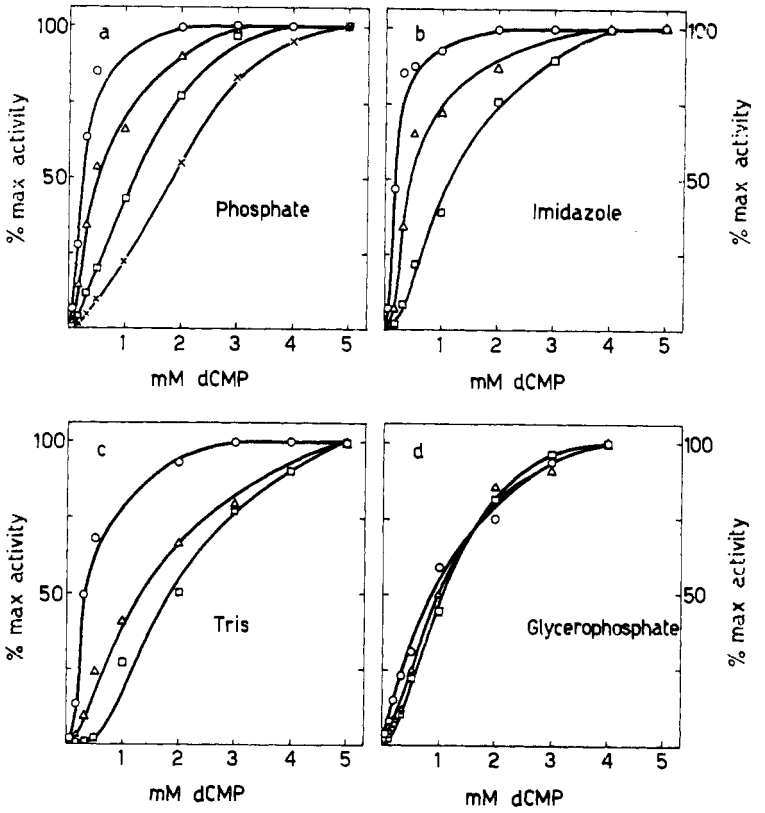

FIGURE 13: Influence of pH on initial reaction rates in different buffers. (a) Sodium–phosphate (0.05 M) (O—O), pH 6.5; (Δ—Δ), pH 7.0; (□—□), pH 7.5; (x—x), pH 8.0. (b) Imidazole–HCl (0.10 M) (O—O), pH 7.0; (Δ—Δ), pH 8.0; (□—□), pH 8.5. (c) Tris–HCl (0.10 M) (O—O), pH 7.0; (Δ—Δ), pH 8.0; (□—□), pH 8.5. (d) Sodium–glycerophosphate (0.05 M) (O—O), pH 7.0; (Δ—Δ), pH 7.5; (□—□), pH 8.0.

centration is observed also using 5-CH_3-dCMP as substrate, but at high substrate concentration there is a marked inhibitory effect by dCTP that cannot be overcome by the substrate. The inhibition is only partially dependent on the dCTP concentration since a limiting value of inhibition is rapidly attained.

As shown in Figure 6, dCTP removes the inhibition by dTTP and the same maximal velocity is reached independently of the dTTP concentration, but higher concentrations of dTTP require higher concentrations of dCTP. It is possible to overcome both the activatory and the inhibitory effect by increasing the dCMP concentration.

The analysis of the kinetic data by means of the Hill equation indicates that the apparent order of the reaction with respect to the substrate has a limiting value close to 4. The apparent order of the reaction with respect to dCTP or dTTP is also higher than one. A model of the enzyme composed of four subunits, each having a catalytic site and at least one regulatory site to bind dCTP and dTTP, is in agreement with the experimental data. It is not possible to distinguish whether dCTP binds to the dTTP sites or to independent sites because the experimental data can be accounted for in both cases.

The multimolecular kinetics might be explained by interaction among sites. The interaction of the enzyme substrate sites would be enhanced by the binding of dTTP to the enzyme molecule and eliminated by the binding of dCTP. The changes of the interaction among substrate sites might be based on changes of interaction among subunits. The plausibility of the model of subunit interaction is strengthened by the experiments described in Figure 5, in which the influence of the nature of the buffer on the substrate concentration at half-enzyme saturation is demonstrated. Moreover, the kinetics as function of the substrate concentration in the presence of different concentrations of salts, different concentrations of glycerol, and at different pH values are also indicative of subunit interactions.

In the presence of glycerol the enzyme obeys the Michaelis–Menten kinetics and cannot be activated by dCTP; thus, the glycerol effect resembles the activatory effect of dCTP. On the other hand, increase of ionic strength has an inhibitory effect on enzyme activity, which can be overcome by addition of dCTP. Glycerol and high ionic strength protect the enzyme from inactivation (Geraci *et al.*, 1967). It seems that the conformation of the activated enzyme can be

induced by dCTP and by glycerol while the conformation of the inhibited enzyme can be induced by dTTP and by high ionic strength. The demonstration reported in the preceding paper (Geraci *et al.*, 1967) that no polymer–monomer conversion occurs in the presence of dCTP and of dTTP, strengthens the hypothesis that the changes of enzyme activity caused by the regulatory effectors, are based only on conformational changes of the enzyme molecule.

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References

Geraci, G., Rossi, M., and Scarano, E. (1967), *Biochemistry 6*, 183 (this issue; preceding paper).

Grippo, P., Iaccarino, M., Rossi, M., and Scarano, E. (1965), *Biochim. Biophys. Acta 95*, 1.

Hill, A. V. (1910), *J. Physiol. (London) 40*, 4p.

Johnson, M. S. (1960), *Enzymes 3*, 416.

Monod, J., Wyman, J., and Changeux, S. P. (1965), *J. Mol. Biol. 12*, 88.

Phillips, R., Phyllis, S. J., Eisemberg, P. G., and Rutman, R. J. (1965), *J. Biol. Chem. 240*, 4393.

Scarano, E. (1964), *Intern. Congr. Biochem., 6th, New York*, 692.

Scarano, E., Geraci, G., Polzella, A., and Scarano, E. (1963), *J. Biol. Chem. 238*, PC1556.

Scarano, E,, Geraci, G., and Rossi, M. (1964), *Biochem. Biophys. Res. Commun. 16*, 239.

Scarano, E., Geraci, G., and Rossi, M. (1965a), *2nd Meeting Federation Europ. Biochem. Soc., Vienna*, 203 (A299).

Scarano, E., Geraci, G., and Rossi, M. (1965b), *Federation Proc. 24*, 356.

Smith, M., and Khorana, H. G. (1958), *J. Am. Chem. Soc. 80*, 1142.

Effect of Salt Solutions on Glycogen Phosphorylase. A Possible Role of the Phosphoryl Group in Phosphorylase a*

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ABSTRACT: The structure and catalytic activity of phosphorylases a and b have been found to be particularly sensitive to salts. Kinetic studies with respect to adenosine monophosphate (AMP) for phosphorylase b indicate that the saturation curve is hyperbolic in NaF but sigmoidal in its absence with a Hill coefficient of $n = 1.5$. In contrast to NaF, phosphorylase b is less active in $NaClO_4$ and does not follow Michaelis–Menten kinetics for AMP. These salts had little effect on the sedimentation of phosphorylase b in the absence of AMP, but in the presence of 10^{-3} M AMP and NaF, the enzyme sedimented as a tetramer, and in AMP and $NaClO_4$, as a mixture of dimer and tetramer In NaF phosphorylase a is stimulated little by AMP, but in $NaClO_4$ the requirement for AMP for activity is nearly complete. NaF has no effect on the sedimentation of phosphorylase a, but in $NaClO_4$ the enzyme is partially dissociated to a dimeric form. A model, based upon the assumed effect of the covalently bound phosphate groups of phosphorylase a on activity coefficients of certain groups at a specific site, is proposed to explain the differences in structure and activity of phosphorylases a and b. The action of salts is discussed in relation to this model.

It is well recognized that the interconversion of phosphorylases b and a from skeletal muscle arises from specific enzymic phosphorylation and dephosphorylation reactions and that these transformations constitute, in part, an important mechanism by which glycogen metabolism is controlled *in vivo*. The incorporation of phosphate that occurs in the phosphorylase b to a reaction (Krebs and Fischer, 1956) results in an enzyme that differs from its precursor in sensi-

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