Biochemistry of Terminal Deoxynucleotidyltransferase. Mechanism of Manganese-Dependent Inhibition by Deoxyadenosine 5′-Triphosphate and Biological Implications†

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ABSTRACT: We have shown that terminal deoxynucleotidyltransferase (TdT) catalyzed DNA synthesis is strongly inhibited in the presence of all the four ribonucleoside triphosphates, ATP being the most potent [Modak, M. J. (1978) *Biochemistry 17*, 3116]. In an activated DNA primed reaction, the effective order of triphosphate inhibition is A > G > C > U. Furthermore, inhibition appears to be more severe in the presence of Mn^{2+} when compared to that observed in the presence of Mg^{2+}. Extension of these findings to deoxynucleoside triphosphates revealed that only dATP, in the presence of Mn^{2+}, strongly inhibits TdT-catalyzed incorporation of the remaining three triphosphates. In contrast to the inhibitory effect of rNTPs and Mn-dATP on TdT, replicative DNA polymerases are resistant to the addition of rNTPs and are either unaffected or stimulated by the addition of dNTPs. An investigation into the mechanism of Mn^{2+}-dependent dATP inhibition of TdT revealed that the observed inhibition is a result of multiple effects involving the three important components of the catalysis, namely, enzyme, primer, and substrate. The rates of incorporation of Mn-dATP are ~50-fold lower than those observed with Mn-dGTP, although the affinity for binding Mn-dATP to enzyme is approximately 1–2 orders of magnitude higher than that of Mn-dGTP. This probably results in the blockage of the substrate binding site on TdT by Mn-dATP. Increasing the primer concentration affords partial protection from dATP inhibition; kinetics studies performed with changing primer concentration and inhibitor dATP have yielded noncompetitive plots. A direct involvement of Mn-dATP as a competitor for the substrate dGTP for binding to the enzyme is revealed by the partial competitive modes of inhibition observed with kinetics studies performed by using these two substrates. Furthermore, the addition of Mn^{2+} to a reaction proceeding in the presence of Mg^{2+} may be instantly inhibited. Biological implications of adenine nucleotide mediated inhibition of TdT have been discussed in light of the present results and the possible involvement of TdT in the differentiation of lymphocytes and immunodeficiency.

Terminal deoxynucleotidyltransferase (TdT)[1] is a DNA polymerizing enzyme, with no known biological function (Bollum, 1974). Due to its organ-specific localization, it is thought to be involved in ontogeny of lymphocytes and/or immunocompetence (Chang, 1971; Baltimore, 1974; Bollum, 1975). Occasionally, TdT has been found at the sites other than thymus and bone marrow, particularly under pathological stress (e.g., chronically infected human tonsil and breast cyst fluid), but it appears to be due to infiltrating immunoblasts responding to local antigenic challenge (Modak et al., 1978). During our studies on the active site of TdT using pyridoxal 5′-phosphate as an active site specific reagent (Modak, 1976a,b; Modak, unpublished experiments), it was observed that ribonucleoside triphosphates exerted a strong inhibitory action. The inhibition caused by each of the four triphosphates is not identical, and the type of divalent cation present in the reaction mixture seems to play a significant role in the process of inhibition. Mechanistic details of the ATP-mediated TdT inhibition as well as its application to the detection of TdT in human leukemia have been published (Modak, 1978; Bhalla et al., 1977). Examination of dNTPs, in a similar manner, revealed an unusually strong inhibitory action with dATP which is comparable to that obtained in the presence of ATP. This inhibitory effect of dATP has not been known and may perhaps indicate a regulatory mechanism for TdT expression in vivo. The results of this investigation together with the mechanistic aspects of the inhibitory process and the possible biological significance of these findings are the subject of this communication.

Materials and Methods

Materials. Ribo- and deoxyribonucleoside triphosphates and oligo$(dA)_{12-18}$ were purchased from P-L Biochemicals Inc. Tritiated deoxyribonucleoside triphosphates were obtained from Amersham/Searle Co. Activated DNA was prepared according to the procedure of Aposhian & Kornberg (1962). The possibility of deoxyribonucleoside triphosphates contaminating ribonucleoside triphosphates was eliminated by demonstrating the inability of ribonucleoside triphosphates to serve as substrates in Rauscher leukemia virus (RLV) DNA polymerase catalyzed DNA synthesis with the appropriate template primers, e.g., poly(dC·dG) directed synthesis of DNA with dGTP but none with GTP.

Purity of dATP. A possibility that dATP may be contaminated with inorganic phosphate which is known to be a TdT inhibitor was considered and ruled out. We have earlier shown that phosphate is inhibitory to a variety of DNA polymerases (Modak & Marcus, 1977) and have found TdT, in the presence of Mn^{2+}, to be equally susceptible to P_i (Modak, unpublished experiments). For this reason, dATP was purified by DEAE-Sephadex chromatography as well as by the charcoal adsorption method. dATP purified by these procedures was identical in its efficacy with that of authentic dATP in its inhibitory action on TdT.

Enzymes. Terminal deoxynucleotidyltransferase was prepared from calf thymus by following the procedure of Chang & Bollum (1971). The final preparation consisted of two subunits with molecular weights of approximately 26 000

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[1] Abbreviations used: rNTP, ribonucleoside triphosphate; rNMP, ribonucleoside monophosphate; dNTP, deoxyribonucleoside triphosphate; TdT, terminal deoxyribonucleotidyltransferase; Cl_3AcOH, trichloroacetic acid; ADA, adenosine deaminase; RLV, Rauscher leukemia virus; AMV, avian myeloblastosis virus.

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and 8000, as judged by polyacrylamide gel electrophoresis. The enzyme is at least 98% pure on the basis of the intensity of the staining of the bands in gel. The enzyme preparation is completely free of DNA polymerases α, β, and γ. A pH treatment, during the purification procedure, completely destroys the replicative DNA polymerase activity (Chang & Bollum, 1971). This was further confirmed by the poor response of the enzyme to any of the known template primers (although some primer-dependent synthesis is always observed), sulfhydryl sensitivity, and extreme sensitivity to ATP inhibition. The specific activity of the final preparation of TdT was 9600 units/mg under the assay conditions described below. One unit of enzyme activity is equal to 1 nmol of dGTP incorporation in 1 h. The highly purified DNA polymerases α and γ as well as the homogeneous preparation of DNA polymerase β are totally resistant to ATP inhibition. Homogeneous DNA polymerase β was a byproduct of TdT isolation and was purified by using sequential chromatography on phosphocellulose, DEAE-Sephadex, Sephadex G-100, poly(rC)–agarose and poly(rA)–agarose columns. AMV and RLV DNA polymerases were purified by using affinity chromatography on a poly(rC)–agarose matrix (Modak & Marcus, 1977; Marcus & Modak, 1976).

TdT Assays. TdT can utilize several single-stranded oligonucleotides as a primer. However, the optimum synthesis seems to occur in the presence of oligo$(dA)_{12-18}$ as a primer and dGTP as a substrate (Sarin & Gallo, 1974; Bollum, 1974; McCaffrey et al., 1975; Marcus et al., 1976). Activated DNA under these conditions is also an excellent primer. The assay mixture in the final volume of 100 μL contained the following: 50 mM Tris-HCl, pH 7.8; 1 mM dithiothreitol; 0.01% albumin; 0.5 mM $MnCl_2$; 0.5 μg of oligo$(dA)_{12-18}$ or of activated DNA; and 20 μM [^{3}H]dGTP (sp act. 1000 cpm/pmol). The reactions were initiated by addition of 50–100 ng of enzyme, incubated at 37 °C for the desired time, and terminated by addition of 5% Cl_3AcOH containing 0.01 M sodium pyrophosphate. The rates of reaction in all cases were determined to be linear for 90 min. The acid-insoluble material was collected on GF/B filters, washed extensively with Cl_3AcOH containing sodium pyrophosphate, water, and ethanol, dried, and counted in toluene-based scintillators (Modak, 1976c).

DNA Polymerase Assays. Activated DNA together with all of the four triphosphates (100 μM each except the labeled precursor which was present at 20 μM) in the presence of either 1 mM $MnCl_2$ or 10 mM $MgCl_2$ in a Tris buffer system (similar to that ussed for TdT) constituted the DNA polymerase assays. The products of 1-h reaction at 37 °C were determined by acid precipitation as described above.

Results

Effect of Individual Ribo- and Deoxyribonucleotides on TdT by Using Mg^{2+} or Mn^{2+} as a Divalent Cation. The susceptibility of TdT catalysis to the presence of various rNTPs and dNTPs is presented in Table I. The results are expressed as the concentrations of various NTPs required to inhibit 50% of the TdT activity in identical reaction mixtures, with either Mg^{2+} or Mn^{2+} as an effective divalent cation. It is clear that relatively lower concentrations of rNTPs are required to inhibit TdT in the presence of Mn^{2+} when compared to that required with Mg^{2+}. It may also be noted that ATP, particularly in the presence of Mn^{2+}, is the strongest inhibitor among the four rNTPs. Furthermore, the pattern of inhibition appears to remain consistent, irrespective of the substrate used for TdT catalysis (Table I). Unlike, rNTPs, dNTPs are known to be natural substrates for TdT and are, therefore, not expected to inhibit TdT reaction. Indeed, in the presence of Mg^{2+}, all of the four dNTPs are utilized with comparable efficiency and can substitute for each other (data not shown). However, in the presence of Mn^{2+}, only dATP of the four triphosphates exerts a strong inhibitory effect on the polymerization of other dNTPs (Table I). The inhibitory effect of dATP on the incorporation of dGTP in the presence of Mg^{2+} and Mn^{2+} is depicted in Figure 1. Similar patterns of inhibition are noted when oligo(dA) was used as a primer in place of DNA. This type of selective cation–substrate dependent inhibition of DNA polymerase has not been described before.

Table I: Concentration of Various Ribonucleoside Triphosphates and Deoxyriboadenosine 5′-Triphosphates Required to Inhibit Activated DNA Primed DNA Synthesis Catalyzed by TdT[a]

nucleotide	substrate	concn required to inhibit 50% of the act. with Mg^{2+} or Mn^{2+} (μM)	
		Mg^{2+}	Mn^{2+}
rATP	dGTP	40	10
	dTTP	60	12
rGTP	dGTP	50	28
	dTTP	50	20
rCTP	dGTP	80	36
	dTTP	100	44
rUTP	dGTP	50	44
	dTTP	88	48
dATP[b]	dGTP	none	8
	dTTP	none	16
	dCTP	none	20

[a] The assay conditions are as described under Materials and Methods except that substrates were present at 200 μM concentrations. [b] Only dATP which inhibits TdT reaction only in the presence of Mn^{2+} is included.

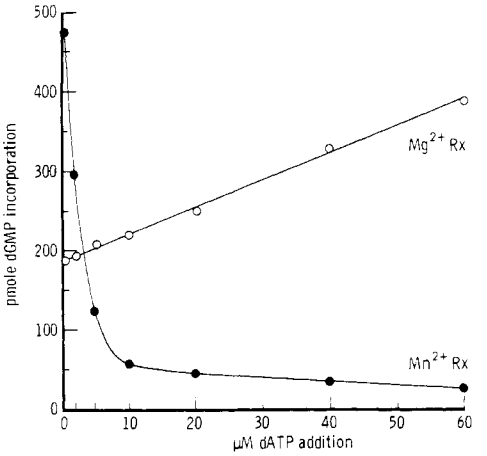

FIGURE 1: Effect of dATP addition on the incorporation of dGTP catalyzed by TdT with DNA primers and either Mg^{2+} or Mn^{2+} as an effective divalent cation. To the reaction mixtures containing Mg^{2+} or Mn^{2+}, the desired concentrations of dATP were added prior to the initiation of DNA synthesis. For computation of the picomoles of incorporation, dATP was considered as the utilizable substrate.

Effect of Various Ribo- and Deoxyribonucleotides on the Replicative DNA Polymerases. It was of interest then to examine whether other template-dependent (replicative) DNA polymerases are under similar restrictions, namely, cation-dependent inhibition of substrate incorporation. Table II enumerates the response of DNA polymerase β as a representative enzyme for replicative DNA polymerase to the addition of various rNTPs and dNTPs. It is clear from the table that incorporation of TTP in activated DNA directed reactions catalyzed by the DNA polymerase β is totally unaffected by the addition of rNTPs. Addition of dATP has no effect or actually stimulates the synthesis regardless of the type of divalent cation used in the reaction mixture. Identical

Table II: Effect of Various Ribo- and Deoxyribotriphosphates on the Incorporation of [^{3}H]dTTP in an Activated DNA Directed DNA Synthesis Reaction Catalyzed by DNA Polymerase β by Using Mg^{2+} or Mn^{2+} as an Effective Divalent Cation[a]

addition (200 μM)	pmol of dTTP incorporation per 30 min	
	Mn^{2+}	Mg^{2+}
none	12	16
ATP or UTP	13	15
CTP or GTP	14	17
dATP	10	18
dGTP	14	17
dCTP	13	17
dTTP[b]	60	60

[a] A standard reaction mixture is described under Materials and Methods under DNA polymerase assays. [b] This addition reduces the specific activity of [^{3}H]TTP.

Table III: Response of TdT to Mn-dATP in Various Buffers by Using Oligo(dA) as a Primer[a]

buffer	pmol of dGTP incorporation per 30 min		
	control	+2 μM dATP	+5 μM dATP
Tris-HCl	173	48	32
Hepes	189	52	33
cacodylate (50 mM)	89	39	20
cacodylate (200 mM)	135	31	19
barbital	149	9	9

[a] The reaction mixture contained standard components except for the type of buffer. Barbital buffer had a pH of 8.1 while the rest of the buffers ranged in pH from 7.2 to 7.8. Note that in the presence of Mg^{2+}, dATP has no inhibitory effect on TdT activity in any of these buffers (data not shown).

results are obtained with DNA polymerase α and AMV and RLV reverse transcriptases (data not shown).

Properties of dATP-Mediated Inhibition of TdT. Since the assay of TdT has been known to be influenced by both the buffer and divalent cation used in the reaction mixture, it was desirable to determine if the inhibitory effect of dATP in the presence of Mn^{2+} was restricted to the Tris buffer that we routinely use. The examination of inhibition of TdT catalysis by dATP in the presence of various buffers revealed no such restriction (Table III). For all of the subsequent experiments, therefore, Tris buffer was employed.

The rates of polymerization of dGTP in the presence or absence of dATP are linear with time for at least 90 min, and addition of dATP anytime during the catalysis results in the instantaneous inhibition of dGTP incorporation (Figure 2).

Incorporation of dATP under these conditions was detectable and amounted to only 1–2% of dGTP incorporation (Table IV). Apparently, the newly synthesized, mixed DNA sequence containing dG and dA residues seems to be unfavorable for further chain extension and results in the inhibition of synthesis. If this was the case, addition of excess primer would be expected to overcome, at least partly, the inhibitory effect of the newly extended primer molecules. Indeed, addition of excess primer to an ongoing reaction, in the presence of dATP, results in some increase in the synthesis (Figure 3b), suggesting utilization of at least a part of the freshly added primer. Similarly, increasing the primer concentration seemed to afford some protection from dATP inhibition (Figure 4), indicating that extension of a primer with a mixed sequence of A and G residues might be the rate-limiting step. However, the fact that the incorporation of dATP was not proportional to primer concentration (Table IV) strongly suggests that the

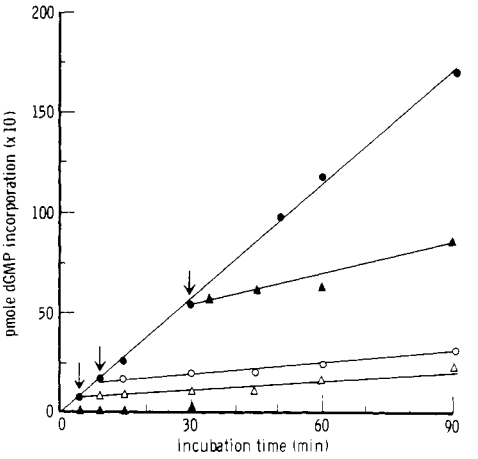

FIGURE 2: Effect of addition of dATP on the TdT-catalyzed rates of dGTP incorporation when added at various times during catalysis. At the desired times indicated by the arrows, dATP was added to the final concentration of 5 μM. Subsequently, aliquots of the reaction mixtures were withdrawn at the desired times, and insoluble radioactivity was determined as described under Materials and Methods.

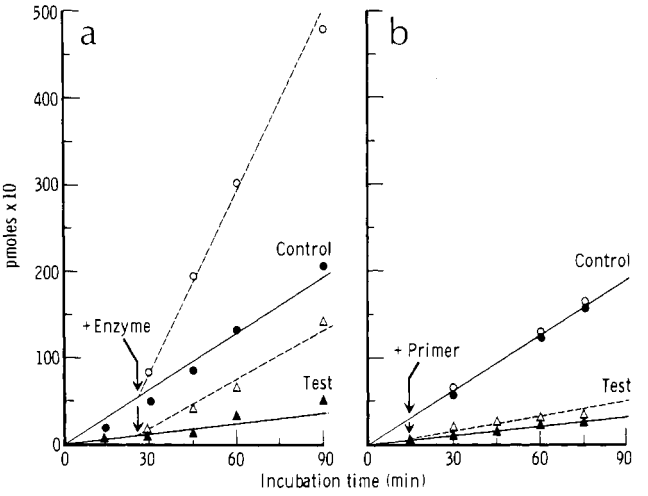

FIGURE 3: Effect of the addition of excess enzyme (a) or primer (b) to the reaction proceeding in the presence or absence of 5 μM dATP. At the times indicated by the arrows, excess enzyme or primer, equivalent to that present in the reaction mixture, was added to both the control and the test reaction, and polymerization was monitored by using acid-insoluble activity.

Table IV: Effect of Changing Primer Concentration on the Incorporation of dATP in the Presence and Absence of dGTP by TdT-Catalyzed Oligo(dA)-Primed Reaction[a]

primer concn (μg/mL)	pmol of dATP incorporation	
	control	+dGTP (20 μM)
1.4	16	14
2.8	27	24
7.0	33	31
14.0	37	36

[a] Reaction mixture is as described under Materials and Methods. dATP is present at 20 μM. Incubation is for 30 min. Similar results were obtained when DNA was used as a primer in place of oligo(dA).

extension of a primer with a mixed sequence of A and G residues was only a minor catalytic reaction. Thus, additional mechanisms appeared to be involved in the inhibitory process.

Increasing the substrate concentration results in a significant decrease in the inhibition (Figure 4). Similarly, addition of dGTP to an inhibited reaction permits a significant increase in the rate of synthesis (Figure 5) on the existing primer. These results indicate that the inhibitory effect is reversible

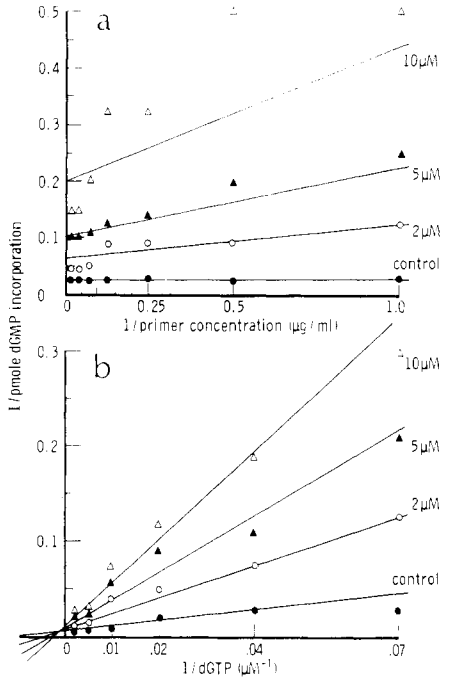

FIGURE 4: Double-reciprocal plot of 1/velocity vs. 1/primer concentration (a) and 1/dGTP (b) with three different concentrations of dATP. Rates of velocity are expressed as picomoles ($\times 10^{-3}$) of substrate incorporated under standard assay conditions (see Materials and Methods).

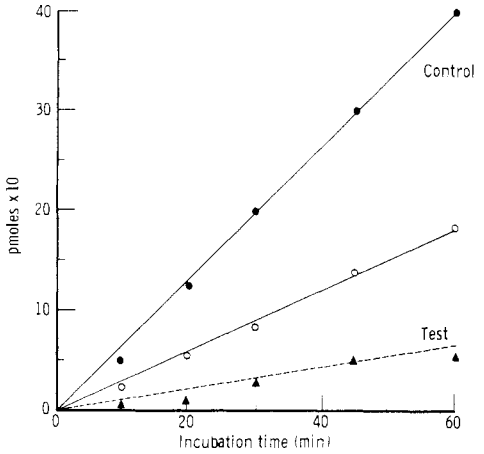

FIGURE 5: Effect of addition of excess dGTP to a TdT-catalyzed polymerization reaction. In this experiment, one set of control and two sets of test (+5 μM dATP) reactions were preincubated in the presence of nonradioactive substrate (20 μM) for the period of 30 min under standard conditions. dGTP (200 μM) together with 5 μCi of tritiated dGTP was then added to one set of control (●) and test (○) sets, while one set containing dATP received only radioactive dGTP (▲). Incubation was then continued and aliquots were removed at the desired times. Preincubation in this manner (with cold substrates) avoided the necessity for correction of the new rates with the control when an increase in the substrate concentration was effected.

and is exerted at the substrate level. Since incorporation of dATP into the primer is not accomplished with efficiency, when compared to other dNTPs, by TdT, binding of dATP to the enzyme with much higher affinity and yet failing to polymerize that residue onto a primer terminus seems to be one way by which inhibition of catalysts is effected. A direct support for this interpretation may be found by determination of kinetics constants for various dNTPs. From the kinetics constants listed in Table V, it is clear that the lowest K_m and V_{max}, among the four dNTPs, are both the exclusive properties of dATP.

Table V: K_m and V_{max} Values of Various dNTP Substrates for TdT Catalysis by Using Oligo(dA) and Activated DNA as Primers in the Mn^{2+} System[a]

	primer			
	oligo(dA)$_{12-18}$		activated DNA	
substrate	K_m (μM)	V_{max} (pmol/h)	K_m (μM)	V_{max} (pmol/h)
dGTP	10	1300	20	460
dTTP	25	600	25	170
dCTP	22	650	45	200
dATP	2	34	10	40

[a] The reaction mixture contained either 7 μg/mL oligo(dA) or 25 μg/mL activated DNA as a primer with the indicated substrate. Approximately 100 ng of enzyme was used in each assay. Results are the average of at least two experiments. Other standard components of the reaction mixture are described under Materials and Methods.

Mechanism of dATP Inhibition. Since the increase in the substrate and to a minor degree in the primer concentration resulted in the reduction of dATP-mediated inhibition of TdT, a detailed kinetics analysis was carried out with changing substrate and primer concentration in response to changing inhibitor concentration. Results of this investigation are presented in the form of double-reciprocal plots in Figure 4. The results indicate that dATP inhibition with respect to the substrate is at least partially competitive. The apparent K_i value for dATP in the presence of Mn^{2+} is calculated to be 2.5 μM. Low K_i and K_m values for dATP when compared to the K_m for the substrate dGTP (10–20 μM) are indicative of a much higher affinity for dATP. However, the fact that dATP is polymerized at the rate which is 1–2 orders of magnitude lower than that observed for dGTP under identical conditions (Table V) strongly suggests that dATP remains bound to the enzyme, resulting in the blockage of substrate binding sites. Alternatively, the Mn-dATP–enzyme complex may bind with stronger affinity for the 3′-OH end of the primer and yet may transfer the substrate nucleotide with poor efficiency. The observations that dATP incorporation in the presence of dGTP remains unaffected and that addition of excess primer reduces the inhibition only to a minor extent support this interpretation.

Manganese Dependency of the Inhibition. As noted before, the presence of Mn^{2+} was essential for the effective inhibition of TdT catalysis to occur in the presence of dATP. The reactions that were preincubated in the presence of Mg^{2+} become readily susceptible to strong inhibition by the addition of small quantities of Mn^{2+} (Figure 6). For example, addition of 0.1 mM Mn^{2+} to a reaction that has commenced synthesis in the presence of 5 mM $MgCl_2$ and both dGTP and dATP results in approximately 75% inhibition of dGTP incorporation, irrespective of the synthetic or natural primer used. Similarly, addition of a 100-fold excess of Mg^{2+} to an inhibited reaction does not relieve the degree of inhibition (data not shown). These results strongly indicate that the effect of dATP is indeed mediated through Mn-dATP and that magnesium ions are incapable of reversing the inhibitory effect.

Discussion

Ribonucleotide-mediated inhibition of TdT has been known for a long time (Kato et al., 1967; Ratliff, 1972; Roychoudhury, 1972) and is thought to be due to "suicidal" incorporation of one or two riboresidues on a deoxyribopolymer chain (Bollum, 1974). In our detailed study of ATP inhibition (Modak, 1978), we found no evidence for the incorporation of ATP with Mn^{2+} as an effective divalent cation although

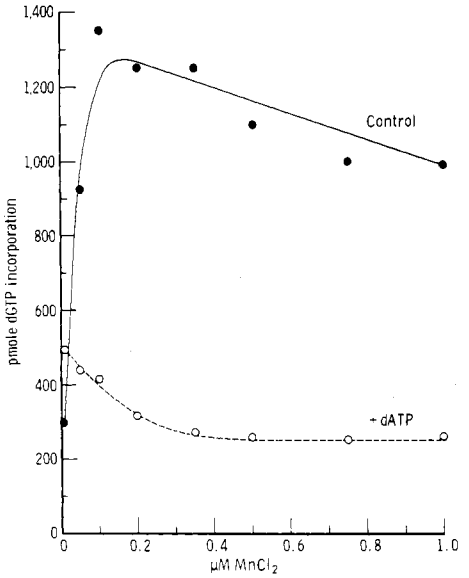

FIGURE 6: Inhibition of Mg^{2+}-utilizing TdT reaction by the addition of Mn^{2+} in the presence of dATP by using activated DNA as a primer. The standard reaction contains 5 mM $MgCl_2$, and a solution of $MnCl_2$ was added in or under 5 μL to give the desired concentration.

other rNTPs could be shown to be incorporated. The ATP inhibition, under the assay conditions, appeared to be mediated by blocking the substrate binding site on TdT for which ATP has 4–6 times greater affinity (Modak, 1978) than dNTPs. From the present studies, it is clear that the individual rNTP causes differential inhibition of TdT and that the divalent cation used in the reaction mixture determines the severity of the inhibition (Table I). Perhaps the most interesting and unique aspect of the present study is the finding that dATP addition to TdT reactions in the presence of Mn^{2+} results in the drastic inhibition of that reaction (Figure 1). Replicative DNA polymerases, e.g., DNA polymerases α and β, and reverse transcriptases are not inhibited by the additions of both rNTPs and dATP (as well as other dNTPs) in the presence of Mn^{2+} or Mg^{2+} (Table II). This differential susceptibility of TdT vs. other DNA polymerases to adenine nucleotides may be indicative of distinct control mechanisms for the two types of enzymes in those cells which contain these enzymes. Investigations were then designed to characterize and determine the mode of dATP-mediated inhibition of TdT catalysis. dATP appears to inhibit polymerization of all the other substrates (Table II), although to varying degrees, irrespective of the synthetic or natural primer used in the assay. The detailed analysis revealed that dATP-mediated inhibition is due to multiple mechanisms, and evidence for each one of them is discussed below.

At least a partial involvement of primer molecules in the inhibitory process may be inferred from the observation that (1) increasing the concentration of the primer affords some protection from dATP inhibition (Table VI) and (2) the addition of excess primer to an inhibited reaction allows additional limited synthesis to occur (Figure 3b). The newly synthesized sequence toward the 3′ end of a primer appears to be the region involved in the process of inhibition since the sequence present at the distal end (5′ end) does not seem to have any influence in the process of catalysis as judged by the identical response obtained with homooligomeric or heteropolymeric DNA primers. However, the "primer extension" reaction as a rate-limiting step does not account for the entire, observed inhibition, for excess primer only partially relieves the inhibition.

Table VI: Effect of Changing Enzyme Concentration on the dATP-Mediated Inhibition of TdT Catalysis[a]

	pmol of dGMP incorporation		
enzyme (ng)	control	+10 μM dATP	% inhibn
40	660	58.0	91.3
100	1316	109.0	91.7
200	3033	256.0	91.6
400	6574	604.0	90.8

[a] Reaction mixture is described under Materials and Methods.

A direct effect of dATP on the enzyme itself is concluded from the fact that inhibition is proportional to enzyme concentration (Table VI), and a newly added enzyme to an ongoing reaction becomes equally susceptible to inhibition (Figure 3a). Kinetics experiments clearly suggest a competitive (with respect to substrate) mode of inhibition which is indicative of the involvement of the substrate binding site on the enzyme. Indeed, the low K_i value, as well as low K_m when compared to other dNTPs (Table V), indicates a much higher affinity of TdT for Mn-dATP. Since Hill's plots indicate that there is only a single binding site for both dATP and dGTP, the triphosphate binding site could remain blocked through the triphosphate that is bound but not transferred to the 3′ OH of the primer. Approximately 50-fold lower rates of Mn-dATP polymerization (Table V) certainly support this contention. The principal inhibitory effect thus appears to be exerted through a paradoxical situation where Mn-dATP is bound to the active site of TdT (or the Mn–dATP–enzyme complex to the primer terminus) with greatest affinity but is transferred (for the polymerization reaction) with lowest efficiency among the four Mn-dNTPs. It may be recalled here that in the presence of Mg^{2+}, all of the four dNTPs are bound and polymerized with comparable efficiency. Further support for the inhibitory role of Mn-dATP may be found in the observation that a reaction initiated in the presence of Mg^{2+}, dATP, and dGTP may be severely inhibited by the addition of small quantities of Mn ions, irrespective of the synthetic or natural primer (Figure 6). Under these conditions, manganese may bind to substrates to form Mn-dNTPs, displacing Mg^{2+}, and can effect its inhibition through Mn-dATP. The formation of Mn-dGTP under these conditions may be shown by the significant stimulation of dGTP incorporation when supplemented with Mn^{2+} ions which are known to be preferred over Mg^{2+} for the polymerization of dGTP (Bollum, 1974; Sarin & Gallo, 1974; McCaffrey et al., 1975; Marcus et al., 1976). The fact that only micromolar quantities of Mn^{2+} are required for the inhibition and that excess addition of Mn^{2+} does not have additional inhibitory effects strongly suggests that the inhibitory component is, indeed, manganese deoxytriphosphate. Whether this unusual property of TdT has any biological relevance remains to be established; nevertheless, some interesting correlations of these metal–nucleotide effects with unique location and phenomenon can be made which are discussed below.

Biological Implications of ATP and dATP Inhibition. The presence of TdT only in the precursor T cells and not in the mature T lymphocytes implies a role for TdT in some stages of lymphocyte differentiation. It may be reasonable to assume then that the functional "repression" or inhibition of TdT in vivo may result in the production of lymphocytes abnormal in some function, perhaps immunological reactivity (immunocompetence). Severe combined immunodeficiency associated in patients with adenosine deaminase (ADA) deficiency may be one biological situation where the observed increase

in adenine nucleotides, particularly in that of ATP (Green, 1975) and dATP (Coleman et al., 1978), may be postulated as a basis for the development of immunodeficient lymphocytes through the aberrant TdT function during differentiation.

Another biological correlation may be drawn between possible involvement of TdT in conferring immunocompetence to T cells and a recent report wherein Mn^{2+}-dependent failure of T cells to respond to antigen has been described. As low as 100 μM concentration of Mn^{2+}, if present within the first 8 h of the growth cycle of T cells established in a culture, results in the inability of these otherwise normal, cultured cells to respond to mitogen (Hart, 1978). The Mn^{2+}-dependent dATP effect on TdT that we have described in this manuscript may offer one explanation for the failure of these cells to develop immunological reactivity mediated through aberrant TdT catalysis. A detailed study of the biochemical environment within the cell under specific conditions together with the known properties of TdT may ultimately provide some clue toward the understanding of the function of this enzyme in the specialized cell.

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