Stanley, E. R., Robinson, W. A., & Ada, G. L. (1968) *Aust. J. Exp. Biol. Med. Sci. 46,* 715.
Stanley, E. R., Hansen, G., Woodcock, J., & Metcalf, D. (1975) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 34,* 2272.
Warren, L. (1959) *J. Biol. Chem. 234,* 1971.
Winter, A., Perlmutter, H., & Davies, H. (1975) *LKB Appl. Note,* 198.
Wrigley, C. W. (1971) *Methods Enzymol. 22,* 559.

Biochemistry of Terminal Deoxynucleotidyltransferase: Mechanism of Inhibition by Adenosine 5′-Triphosphate†

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ABSTRACT: The polymerization of deoxyribunucleoside triphosphate catalyzed by terminal deoxyribonucleotidyltransferase (TdT, EC 2.7.7.31) is severely inhibited by the addition of ribonucleoside triphosphates, ATP being the most potent inhibitor. Examination of the inhibitory effect of ATP using oligo(dA)$_{12-18}$ as well as activated DNA as primers revealed that (a) ATP inhibition is not due to its addition onto a 3′-OH primer terminus as judged by the lack of incorporation of labeled ATP, although under similar conditions incorporation of GTP can be demonstrated, (b) a consistent degree of inhibition was noted independent of primer or enzyme concentration; (c) addition of ATP to an ongoing reaction promptly reduces the rate of polymerization; (d) kinetic studies indicate a competitive (with respect to substrate deoxy triphosphate) pattern of inhibition; (e) addition of excess deoxyribotriphosphate promptly relieves the inhibition. Unlike ATP, other ribotriphosphates yield a mixed pattern of inhibition partly mediated by competitive mechanisms. GTP and CTP and to a minor extent UTP are incorporated into DNA in the presence or absence of deoxy triphosphate. Furthermore, addition of ATP also inhibits incorporation of GTP and CTP.

The confinement of terminal deoxynucleotidyltransferase (TdT,[1] EC 2.7.7.31) to the thymus has been well established (Chang, 1971; Bollum, 1974; Kung et al., 1976). However, it was not until the demonstration of its presence in certain types of leukemic lymphocytes that the interest in the biology and function of this enzyme was renewed vigorously (McCaffrey et al., 1973; Sarin and Gallo, 1974; Gallo, 1975; Coleman et al., 1976; Sarin et al., 1976). The enzyme activity present in leukemic leukocytes clearly suggested a linkage of these cells to thymus and thereby has provided a diagnostic marker for the classification of leukemias as well as marker for T cell differentiation. However, biological function of this enzyme has remained unknown. Baltimore (1974), based on its exclusive presence in immunogenic organ, e.g., thymus and products thereof, has proposed that TdT may be a somatic mutagen and therefore may be responsible for the generation of immunoglobulin diversity. In the absence of any corroborating experimental evidence, this interesting theory remains unproven.

A detailed study of the in vitro properties exhibited by a purified enzyme may be used to infer at least some suggestive in vivo role for that enzyme. So far, demonstration of any specific homo- or heteropolymeric DNA sequence in the cells containing TdT has been lacking. It is therefore difficult to correlate DNA-synthesizing activity of TdT with plausible biological utility. During our studies on the active site of TdT (used as a model system for DNA polymerases) using pyridoxal phosphate as an active-site-specific reagent (Modak, 1976a), it was observed that ribotriphosphates exhibited a strong inhibitory action, ATP being the most powerful inhibitor of the four triphosphates (Modak, manuscript in preparation). Indeed, Bollum and colleagues have described the inhibition of this enzyme by ATP over a decade ago (Kato et al., 1967). Roychoudhury (1972) demonstrated that one or two ribonucleotides could be added to DNA primers and continued his efforts to optimize and characterize the conditions with respect to substrate and primer concentrations and divalent cation requirement for these reactions (Roychoudhury et al., 1976). Admittedly, such ribonucleotide additions provide a very useful tool for the primer extension (Roychoudhury and Kossel, 1971; Roychoudhury, 1972; Padmanabhan et al., 1974; Sekiya et al., 1974), 3′-end labeling (Kossel and Roychoudhury, 1971; Bertazoni et al., 1974), and DNA sequence analysis (Kossel et al., 1974; Wu et al., 1973; Jay et al., 1974). However, these studies do not provide any clue to the in vivo functionality of TdT. The selective inhibitory effect of ATP on TdT in contrast to other replicative DNA polymerases (Bhalla et al., 1977) and the fact that ATP is one of the important cellular micromolecules appears to suggest some (regulatory?) connection between TdT and ATP. We have, therefore, reinvestigated the mechanism of ATP inhibition of this enzyme, and results of this investigation are presented.

Materials and Methods

Enzymes. Terminal deoxynucleotidyltransferase was prepared from calf thymus following the procedure of Chang and Bollum (1971). The final preparation consisted of two subunits with molecular weights of ca. 26 000 and 8000, as judged by polyacrylamide gel electrophoresis. The enzyme is at least 98% pure on the basis of intensity of staining of the bands in the gel. The enzyme preparation is completely free of DNA poly-

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[1] Abbreviations used: rNTP, ribonucleoside triphosphate; rNMP, ribonucleoside monophosphate; dNTP, deoxyribonucleoside triphosphate; TdT, terminal deoxyribonucleotidyltransferase; DEAE, diethylaminoethyl; Tris-HCl, 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride.

0006-2960/78/0417-3116$01.00/0

TABLE I: Effect of Various ATP Concentrations on the Oligo(dA) and Activated DNA Primed dGTP Incorporation Catalyzed by TdT.[a]

ATP concn in react. mix. (μM)	oligo(dA)$_{12-18}$ primer		activated DNA primer	
	pmol	% inhibit.	pmol	% inhibit.
none	222		108	
2	143	35.6	66	38.9
5	90	59.5	50	53.8
10	64	71.2	27	75.0
20	33	85.2	23	79.0
40	21	91.6	9	92.7

[a] Assays were performed as described under Materials and Methods. The concentration of oligo(dA) or DNA was 0.5 μg per assay and that of dGTP was 20 μM. Incubation was for 15 min.

merases α, β, and γ. A pH treatment, during the purification procedure, completely destroys the replicative DNA polymerase activity (Chang and Bollum, 1971). This was further confirmed by the poor response of enzyme to any of the known template primers (although some primer-dependent synthesis is always observed), sulfhydril sensitivity, and extreme sensitivity to ATP inhibition. The highly purified DNA polymerase 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 by-product of TdT isolation and was purified using sequential chromatography on phosphocellulose, DEAE-Sephadex, Sephadex G-100, and poly(rC)-agarose and poly(rA)-agarose columns. RLV DNA polymerase was purified using affinity chromatography on a poly(rC)-agarose matrix (Modak and Marcus, 1977).

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 and 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 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 of d[^{3}H]GTP (specific activity 1000 cpm/pmol). The reactions were initiated by the addition of enzyme fraction, incubated at 37 °C for the desired time, and were terminated by addition of 5% Cl_3AcOH containing 0.01 M sodium pyrophosphate. 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 scintillator (Modak, 1976b).

Thin-Layer Chromatography. Thin-layer chromatographic analyses of the product of TdT reaction, for experiments involving ribonucleotide additions, were carried out essentially as described by Roychoudhury (1972). However, no clear-cut separation of oligo(dA)$_{12-18}$ ± 1 NMP residue was achieved. Therefore, activity present within 2 cm from the origin was taken to mean the incorporation of ribonucleotides into product. Unreacted triphosphates moved much further in the system.

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., while ^{32}P-labeled ribotriphosphates were the products of New England Nuclear Corp. Activated DNA was prepared according to the procedure of Aposhian and Kornberg (1962). The possibility of deoxyribonucleoside triphosphates contaminating ribotriphosphates was eliminated by demonstrating the inability of ribotriphosphates to serve as a substrate in Rauscher leukemia virus (RLV) DNA polymerase catalyzed DNA synthesis with appropriate template primers, e.g., poly(dC·dG)-directed synthesis of DNA using dGTP but none with GTP.

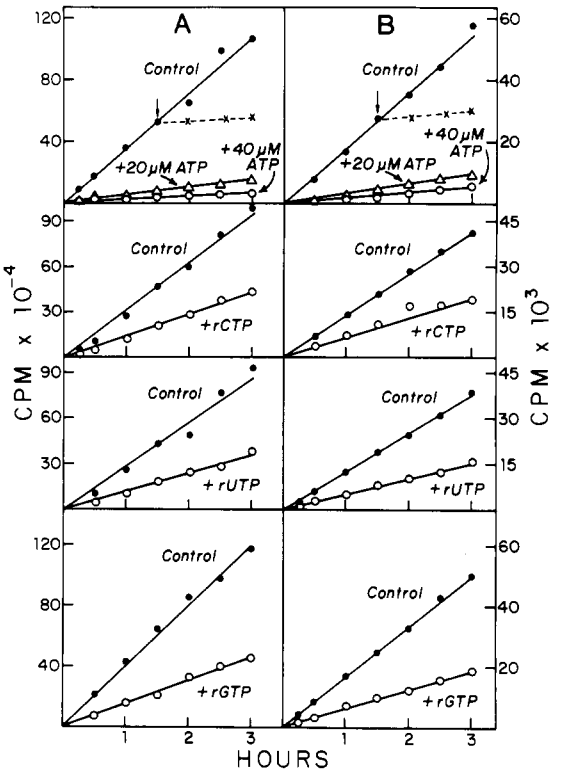

FIGURE 1: Effect of ribonucleoside triphosphates on the relative rates of dGTP polymerization of oligo(dA) (panel A) and activated DNA (panel B) primed reactions catalyzed by TdT. Each frame is self explanatory with regard to triphosphate addition. Concentration of rNTPs was 40 μM, except for ATP where effect of 20 μM is also shown. In top frames, effect of addition of 40 μM ATP to the reaction mixture at 90 min after the initiation of synthesis is shown.

Results

A typical dose-response pattern of ATP concentration of the TdT activity primed by oligo(dA) as well as DNA is presented in Table I. At equimolar concentrations of substrate dGTP and inhibitor ATP approximately 80% inhibition persists irrespective of synthetic or natural primer used. Other ribonucleoside triphosphates also inhibit TdT reaction albeit less severely. The response of TdT to individual ribonucleoside triphosphate with both oligomeric primer [oligo(dA)] and natural DNA primer is depicted kinetically in Figure 1. In all the cases, the incorporation of dGTP in the presence or absence of rNTP is linear for at least 3 h. Furthermore, reaction primed with synthetic or natural as well as oligomeric or polymeric DNA gave almost identical results, suggesting that the observed inhibition is not restricted to homopolymer-primed synthesis. Since ATP is a four to six times stronger inhibitor compared to other rNTPs and is also one of the important intracellular micromolecules, we have examined the effect of ATP in greater detail.

Properties of Inhibition. The top frames in Figure 1 illustrate the kinetics of dGTP incorporation in the presence and absence of ATP. In this experiment, ATP was added to a standard reaction mixture at various times during the course of polymerization, and subsequent rates of synthesis were determined. The data clearly indicate that ATP addition inhibits

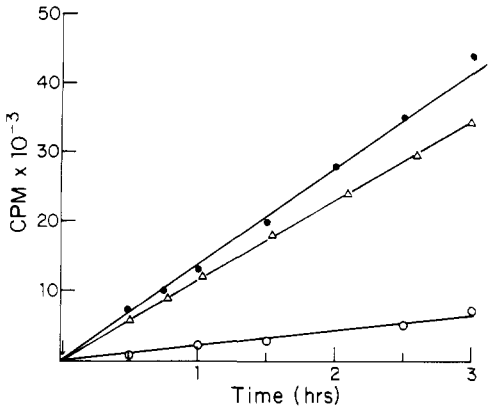

FIGURE 2: Effect of addition of excess dGTP to a TdT-catalyzed polymerization reaction. In this experiment, one set of control and two sets of test (+20 μM ATP) reaction were preincubated in the presence of nonradioactive substrate for the period of 30 min under standard conditions. 200 μM dGTP together with 3 μCi of tritiated dGTP was then added to one set each of control (●-●) and test (△- - -△) sets, while one set containing ATP received only radioactive dGTP (○-○). Incubation was then continued and aliquots were removed at the desired time. The preincubation in this manner (with cold substrates) avoided the necessity for correction of new rates with control when an increase in substrate concentration was effected.

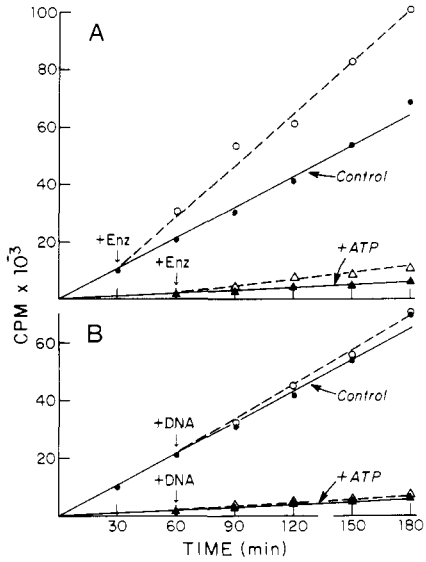

FIGURE 3: Effect of addition of excess enzyme (panel A) or primer (panel B) to the ongoing reaction in the presence and absence of ATP. The arrow indicates the time of addition of enzyme or primer in the volume of 5 μL.

TABLE II: Effect of Increasing Enzyme or Primer Concentration on the Inhibition by ATP.[a]

amount of enz (μL)	act. (pmol/15 min)	act. in the presence of 20 μM ATP (pmol/15 min)	% inhibit.
enzyme concn			
5 μL	55	13	74
10 μL	118	28	73
25 μL	305	83	71
40 μL	600	144	76
primer concn			
0.5 μg	303	85	72
1.0 μg	340	95	72
2.0 μg	282	103	68
4.0 μg	322	106	67

[a] Assay conditions are as described under Materials and Methods.

TABLE III: Incorporation of [^{32}P]Ribotriphosphates in the Presence and Absence of dGTP in an Oligo(dA)$_{12-18}$ and DNA Primed Reaction Catalyzed by TdT.

rNTP	pmol of ribotriphosphate incorp/2 h with oligo(dA)$_{12-18}$ primer (Mn^{2+})	activated DNA primer Mn^{2+}	activated DNA primer Mg^{2+} [c]
rATP	<0.1	<0.1	<0.1
rATP + dGTP	<0.1	<0.1	0.1
rUTP	0.3	0.2	0.15
rUTP + dGTP	0.35	0.22	0.15
rCTP	9.0	1.0	2.0
rCTP + dGTP	5.6	1.5	2.2
rCTP + rATP[b]	1.8	0.2	0.2
rGTP	14.0	2.5	3.5
rGTP + dGTP	15.6	4.1	3.3
rGTP + rATP[b]	2.8	0.4	0.35

[a] The desired ribotriphosphate was added to a standard TdT reaction at the final concentration of 20 μM either individually or together with nonradioactive dGTP at equimolar concentration. Aliquots were removed at various times, and acid-insoluble activity was determined as described (Modak, 1976). In those cases, where incorporation of ^{32}P-labeled ribotriphosphate occurs, it is linear to time of incubation for at least 3 h. The values obtained from the 2-h incubation point are shown here. Incorporation of dGTP under the above conditions was 400 pmol with oligo(dA) primer and 380 pmol with DNA primer. [b] ATP was present at 20 μM. [c] 5 mM $MgCl_2$ was used in these assays in place of $MnCl_2$.

DNA synthesis [in this case, it is poly(dG) formation], regardless of the time of its addition (for clarity, only one time point, i.e., 90 min after the initiation of reaction, is shown). Addition of excess dGTP, on the other hand, to a reaction mixture proceeding in the presence of ATP, promptly restores the rate of dGTP polymerization (Figure 2). The effect of ATP on the activity of TdT as a function of enzyme or primer concentration is detailed in Table II. It may be seen from the table that with over eightfold variation in the enzyme concentration, degree of inhibition remains constant. Similarly, increasing the primer concentration does not appear to relieve ATP inhibition. A kinetic study of both enzyme and primer addition to an ongoing reaction in the presence and absence of ATP (Figure 3) further confirms that the ATP effect is enzyme specific and probably does not involve primer modification.

Incorporation of Ribonucleoside Triphosphates. To confirm that the ATP effect is indeed enzyme specific and not due to primer modification as a result of the incorporation of ATP into the primer terminus, a detailed analysis of incorporation of all four rNTPs into DNA was carried out. The results of the incorporation of rNTPs in the presence and absence of dGTP revealed some interesting patterns (Table III). ATP was not incorporated into DNA in the presence or absence of dGTP when Mn^{2+} was the effective divalent cation. With Mg^{2+} (5 mM final concentration) substituting for Mn^{2+}, there was a small amount (ca. 0.1 pmol) of ATP incorporation into DNA. rGTP, on the other hand, is maximally utilized followed by rCTP and rUTP in the presence of Mn^{2+} or Mg^{2+} (see Table III). The presence of deoxyribonucleoside triphosphate did not alter the above preference pattern, although there was a slight stimulation of incorporation of all rNTPs except ATP. A

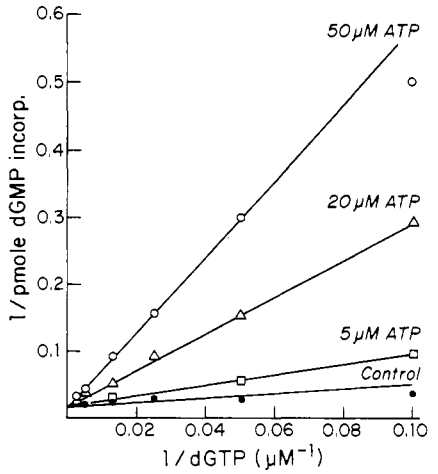

FIGURE 4: Double-reciprocal plots of 1/velocity vs. 1/dGTP concentration with three different concentrations of ATP. Units of velocity are expressed as picomoles of substrate incorporated under standard assay conditions (see Materials and Methods).

similar pattern of response was observed with either primer, although the absolute quantity of rNTP incorporation varied (Table III) with the individual primer.

A possibility for our apparent failure to observe ATP incorporation into the primer site due to the acid-soluble nature of the small-sized product was considered and ruled out. A comparison of Cl_3AcOH-precipitable counts with those obtained by thin-layer chromatographic procedures (Roychoudhury, 1972), for both DNA and oligo(dA)-primed reactions using either ATP or GTP as a substrate, showed a good agreement. Additional evidence, that acid solubility of the primer extended with addition of a single ATP was not the reason for our failure to observe ATP incorporation, was obtained by the demonstration that the same template–primer remained acid insoluble when only a single deoxyribotriphosphate was added using DNA polymerase and any one of the four dNTPs.

Mechanism of Inhibition. Since increasing the enzyme or primer concentration (Table II) did not alter the degree of inhibition caused by ATP, the effect of increasing substrate at three different inhibitor concentrations was examined. Both ATP and GTP were used as inhibitors. Results are presented as a double-reciprocal plot in Figure 4 (data for GTP not shown). The results indicate that ATP inhibition is competitive with respect to dGTP concentration. The inhibition caused by GTP, however, was characterized as a mixed type. The apparent K_i value is calculated to be 5 μM for ATP as against 30 μM for rGTP. A low K_i value for ATP as compared to the K_m for substrate dGTP (20–25 μM) is indicative of stronger affinity of enzyme for ATP than dGTP or rGTP. A Hill plot indicated that there is only a single binding site for either ATP or dGTP. Higher affinity of ATP among the four ribotriphosphates is also shown by the fact that ATP inhibits incorporation of other ribotriphosphates (Table III). However, inhibition of deoxyribotriphosphate incorporation by ATP may be further enhanced by the additions of other ribotriphosphates (Table IV).

TABLE IV: Effect of ATP and GTP Individually and in Combination on the Incorporation of dGTP in the Activated DNA-Primed Reaction Catalyzed by TdT.[a]

GTP concn (μM)	ATP concn (μM)	act. (pmol/30 min)	% inhibit.
none	none	87.0	none
	7.5	32.7	62
10		57.5	34
20		48.8	47
40		32.0	63
10	7.5	21.3	76
20	7.5	20.0	77
40	7.5	15.0	83

[a] A similar pattern of results was obtained when rGTP was substituted with rCTP in the above experiments.

Discussion

The major objective of the present study was to examine the mechanism of inhibitory action of ATP on TdT. This study seemed to be warranted because we have demonstrated the usefulness of ATP as a selective inhibitor of TdT in monitoring this enzyme in certain leukemias (Bhalla et al., 1977). The insensitivity of other replicative DNA polymerases, including reverse transcriptase (RT) to ATP has also been used to identify putative reverse transcriptase from human leukemic cells with some certainty (Modak, manuscript in preparation). A mechanistic explanation of this inhibition was, therefore, highly desirable. Bollum and colleagues had demonstrated that ATP is a competitive inhibitor of dATP in TdT-catalyzed polymerase reactions and that ATP could be incorporated into the primer molecule in the presence of substrate. Roychoudhury (1972), on the other hand, established the conditions for the incorporation of all four ribotriphosphates using oligo(dT)$_6$ as a primer molecule. In light of these results, it was then concluded that rNTP inhibition was mediated through "suicidal" incorporation of rNTPs at the primer terminus which was then not elongated (Bollum, 1974). Such a mode of inhibition is not consistent with competitive inhibition (with respect to substrate), since addition or availability of excess 3′-OH ends should permit synthesis to occur. In addition, these studies were carried out only with synthetic oligomeric DNA and may not be applicable to heteropolymeric DNA. Indeed, with ϕX 174 DNA, Roychoudhury and colleagues (1976) have reported the addition of only 25% A residues, which also required prolonged incubation and relatively high enzyme concentrations. A significant incorporation of ATP and other rNTPs was achieved only when Co^{2+} was used as an effective divalent cation. Since the ultimate objective of our studies was to get some insight into the possible in vivo role of this enzyme, it was highly desirable to confirm and establish the validity of these results using natural DNA as a primer and a physiological metal ion such as Mg or Mn. In this study, we have shown that ribonucleoside triphosphates inhibit the TdT reaction to different degrees, ATP being the most potent inhibitor, in the presence of Mg^{2+} or Mn^{2+}. In the presence of Co^{2+}, ATP addition does result in inhibition, although not to the same degree as that observed with Mg^{2+} or Mn^{2+}. However, the TdT reaction itself appears to be highly sluggish (at least a 50-fold decrease in rate of polymerization of deoxynucleotides) in the presence of Co^{2+} ions. In the presence of Mn^{2+}, which is a preferred divalent cation for the in vitro assay of TdT in an oligo(dA) or activated DNA-primed dGTP incorporation (McCaffrey et al., 1975; Bollum, 1974; Sarin and Gallo, 1974; Marcus et al., 1976), ATP strongly inhibits polymerization of dGTP. This inhibition seems to be mediated through blockage of the deoxyribotriphosphate binding site on the enzyme, rather than primer modification via an addition of a ribo residue on the primer terminus for the following reasons: (1) kinetic analysis (Figure 4) clearly indicates a competitive (with respect to substrate dGTP) mode of inhibition; (2) a negligible amount

of ATP is incorporated into the DNA chain; (3) addition of excess primer does not overcome the inhibition; (4) addition of excess dGTP to a reaction proceeding in the presence of ATP promptly restores the rate of dGTP polymerization (Figure 2); (5) other ribonucleotides can be shown to be incorporated in the product under the identical assay condition.

An additive inhibitory effect may be shown to occur in the presence of ATP and any one of the remaining rNTPs, suggesting that other rNTPs may inhibit the reaction by a similar mechanism. However, the kinetics of inhibition with rGTP are indicative of a mixed inhibition, and the fact that a significant amount of rGTP is incorporated into product (Table III) may imply a different mode of inhibition. The susceptibility of even ribonucleotide incorporation to ATP inhibition (Table III) and the high affinity of TdT for ATP as compared to both rGTP and dGTP may be indicative of a possible regulatory role for ATP in the expression of TdT activity in vivo. One possible biological situation appears to exist wherein a regulatory role for ATP may be invoked. Such a biological implication assumes that TdT is in some way involved in conferring immunocompetence to both T and B lymphocytes. Hutton and Bollum (1977) provide some evidence to this assumption by finding the presence of TdT in bone-marrow stem cells of an athymic (nude) mouse. If this is true, then one might expect that either TdT deficiency or an aberrant in vivo condition in which TdT is present, but inhibited, may result in immunologically incompetent lymphocytes. Indirect evidence to such an interpretation exists in the form of the clinically well-known condition called adenosine deaminase deficiency (ADA). ADA deficiency in man as well as animals (e.g., mouse) seems to occur in conjunction with immunodeficiency (Giblett et al., 1972; Brady and O'Donovan, 1965; Green, 1975). In ADA-deficient cells, intracellular levels of adenine nucleotides rise very sharply (Green and Chan, 1973; Green, 1975). This increase in adenine nucleotides, and particularly in ATP, may then be postulated to inhibit or repress TdT action in vivo analogous to an in vitro effect reported in this paper. Does the observed immunodeficiency in an ADA patient thus result from functional inhibition of TdT in vivo? Further exploration of this aspect would shed more light on the possible connection between TdT and development of immunologically competent cells.

Acknowledgments

I thank Drs. Stuart Marcus and Steven Smith for several stimulating discussions and Peter J. Gomatos and Robert A. Good for support and encouragement. The excellent technical assistance of Ms. H. Bhatt is well appreciated.

References

Aposhian, H., and Kornberg, A. (1962), *J. Biol. Chem. 237,* 519–526.

Baltimore, D. (1974), *Nature (London) 248,* 409–410.

Bertazzoni, U., Ehrlich, S. D., and Bernardi, G. (1974), *Methods Enzymol. 29E,* 355–359.

Bhalla, R., Schwartz, M., and Modak, M. J. (1977), *Biochem. Biophys. Res. Commun. 76,* 1056–1061.

Bollum, F. J. (1974), *Enzymes, 3rd Ed., 10,* 145–171.

Brady, T. G., and O'Donovan, C. I. (1965), *Comp. Biochem. Physiol. 14,* 101–114.

Chang, L. M. S. (1971), *Biochem. Biophys. Res. Commun. 44,* 124–131.

Chang, L. M. S., and Bollum, F. J. (1971), *J. Biol. Chem. 246,* 906–916.

Coleman, M. S., Greenwood, M. F., Hutton, F. J., Bollum, F. J., Lampkin, B., and Holland, P. (1976), *Cancer Res. 36,* 120–127.

Gallo, R. C. (1975), *New Engl. J. Med. 292,* 804–805.

Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B., and Meuwissen, H. J. (1972), *Lancet* 1067–1069.

Green, H. (1975), in Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency: A Molecular Defect, Meuwissen, H. J., Pollara, B., and Pickering, R. J., Ed., New York, N.Y., Academic Press, pp 141–155.

Green, H., and Chan, T. (1973), *Science 182,* 836–837.

Hutton, J. J., and Bollum, F. J. (1977), *Nucleic Acids Res. 4,* 457–460.

Jay, E., Bambara, R., Padmanabhan, R., and Wu, R. (1974), *Nucleic Acid. Res. 1,* 331–354.

Kato, K., Goncalves, J. M., Houts, G. E., and Bollum, F. J. (1967), *J. Biol. Chem. 242,* 2780–2789.

Kossel, H., and Roychoudhury, R. (1971), *Eur. J. Biochem. 22,* 271–276.

Kossel, H., Roychoudhury, R., Fischer, D., and Otto, A. (1974), *Methods Enzymol. 29E,* 322–341.

Kung, P. C., Gottlieb, P. D., and Baltimore, D. (1976), *J. Biol. Chem. 251,* 2899–2906.

Marcus, S. L., Smith, S. W., Jarowsky, C., and Modak, M. J. (1976), *Biochem. Biophys. Res. Commun. 70,* 37–44.

McCaffrey, R., Smoler, D. F., and Baltimore, D. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 521–525.

McCaffrey, R., Harrison, T. A., Parkman, R., and Baltimore, D. (1975), *New Engl. J. Med. 292,* 775–780.

Modak, M. J. (1976a), *Biochemistry 15,* 3620–3626.

Modak, M. J. (1976b), *Anal. Biochem. 75,* 340–344.

Modak, M. J., and Marcus, S. L. (1977), *J. Biol. Chem. 252,* 11–19.

Padmanabhan, R., Wu, R., and Calendar, R. (1974), *J. Biol. Chem. 249,* 6197–6207.

Roychoudhury, R. (1972), *J. Biol. Chem. 242,* 3910–3917.

Roychoudhury, R., and Kossel, H. (1971), *Eur. J. Biochem. 22,* 310–320.

Roychoudhury, R., Fischer, D., and Kossel, H. (1971), *Biochem. Biophys. Res. Commun. 45,* 430–435.

Roychoudhury, R., Jay, E., and Wu, R. (1976), *Nucleic Acid Res. 3,* 863–877.

Sarin, P. S., Anderson, P. N., and Gallo, R. C. (1976), *Blood 47,* 11–20.

Sarin, P. S., and Gallo, R. C. (1974), *J. Biol. Chem. 249,* 8051–8053.

Sekiya, T., Van Ormondt, H., and Khorana, H. G. (1974), *J. Biol. Chem. 250,* 1087–1098.

Wu, R., Tu, C. D., and Padmanabhan, R. (1973), *Biochem. Biophys. Res. Commun. 55,* 1092–1099.