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10. M. A. Pfannenstiel, E. J. Ross, V. C. Kramer, K. W. Nickerson, *Fed. Eur. Microbiol. Soc. Microbiol. Lett.* **21**, 39 (1984) found that this procedure solubilized 80 percent of the crystal proteins, minimized proteolytic degradation (SDS-PAGE analysis gave gel profiles identical to those of intact crystals), and maximized the general cytolytic activity (*2*) exhibited by solubilized but not intact Bti crystals toward human erythrocytes. Considering the multicomponent nature of the Bti crystal, there is no guarantee that the mosquito larval toxin and the general cytolytic factor are identical. Also, since the crystal is incompletely solubilized and the pellet is still highly toxic to mosquito larvae, centrifugation at 100,000*g* is necessary to define the solubilized toxin. Insufficient centrifugation probably explains the erroneous conclusion (*1*) that solubilized Bti crystals have LC_{50} values similar to those of whole crystals.
11. The LC_{50} values for solubilized proteins adsorbed to latex beads were identical in the presence and absence of the polyvinylpyrrolidone coating. The toxin-coated beads do not need to be purified before routine bioassays because the unattached solubilized proteins contribute negligibly to overall toxicity.
12. We have found that, when individual batches of purified Bti crystals are bioassayed for 4 hours against *A. aegypti* larvae, the LC_{50} ranges from 5 to 10 ng/ml. Spontaneous loss of toxicity in Bti is common [H. Kamdar and K. Jayaraman, *Biochem. Biophys. Res. Commun.* **110**, 477 (1983)].
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* Present address: Department of Biochemistry and Biophysics, University of California, Davis 95616.

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The Enzymatic Synthesis of 5-Amino-4-Imidazolecarboxamide Riboside Triphosphate (ZTP)

Abstract. *5-Amino-4-imidazolecarboxamide riboside triphosphate (ZTP) is thought to play a regulatory role in cellular metabolism. Unlike other nucleoside triphosphates, ZTP is synthesized in a one-step reaction in which the pyrophosphate group of 5-phosphoribosyl-1-pyrophosphate is transferred to the riboside monophosphate (ZMP) in a reaction catalyzed by 5-phosphoribosyl-1-pyrophosphate synthetase; reversal of this reaction leads to dephosphorylation of ZTP to ZMP. This unusual route of synthesis (and catabolism) of ZTP may be important in defining its metabolic effects in the cell.*

The discovery of 5-amino-4-imidazolecarboxamide riboside triphosphate (ZTP) in folate-deficient bacteria led to the hypothesis that ZTP is an "alarmone," providing a signal that governs the metabolic economy of the cell during folate deficiency (*1*). This ribotide has also been observed in eukaryotic cells, such as germinating purine auxotrophic fungi (*2*), cardiac and skeletal muscle of animals infused with 5-amino-4-imidazolecarboxamide riboside (Z-riboside) (*3*), and mammalian fibroblasts (*4*) and human erythrocytes (*5*) incubated with Z-riboside. Although no specific biological roles have been ascribed to ZTP in eukaryotic cells, situations under which this metabolite accumulates have been associated with the arrest of growth in mammalian fibroblasts (*6*). Administration of Z-riboside may be useful clinically in replenishing nucleotide pools (*7*) and improving function (*8*) after myocardial ischemia.

Nucleoside triphosphates are usually synthesized from nucleoside monophosphates in a two-step sequence involving a monophosphate kinase that is specific for the nucleobase and a nonspecific diphosphate kinase (*9*). The failure to detect Z-riboside diphosphate (ZDP) in extracts of prokaryotic cells containing the monophosphate and triphosphate of this ribotide has led to speculation that the synthesis of ZTP involves a pyrophosphate transfer to the riboside monophosphate (ZMP) (*1*). Such a one-step synthesis would not be without precedent in nucleotide metabolism as a pyrophosphate transfer has been reported in the synthesis of guanosine 5′-diphosphate 3′-diphosphate (ppGpp) (*10*), the pleiotropic mediator of the stringent response in bacteria [for a review, see (*11*)].

The following preliminary studies suggested to us that 5-phosphoribosyl-1-pyrophosphate (PRPP) might participate in the synthesis of ZTP in mammalian cells. (i) In extracts of Chinese hamster cells, ZTP was synthesized from ZMP in a PRPP-dependent reaction (*12*). (ii) Incubation of Chinese hamster fibroblasts with Z-riboside resulted in ZMP and ZTP accumulation, and the PRPP content of the cells was reduced (*4, 6*). (iii) Attempts to phosphorylate ZMP with adenylate and guanylate kinase were unsuccessful when adenosine triphosphate (ATP) was used as the phosphate donor under conditions in which the diphosphates, ADP and GDP, were synthesized from AMP and GMP, respectively (*13*). These observations indicated that ZTP may be synthesized from ZMP in a PRPP-dependent reaction. We therefore undertook studies to determine whether PRPP synthetase (E.C. 2.7.6.1) catalyzed this reaction.

We utilized a preparation of human erythrocyte PRPP synthetase purified to homogeneity (*14*). The usual reaction catalyzed by this enzyme, namely,

$$\text{ATP} + \text{rib-5-PO}_4 \rightleftarrows \text{AMP} + \text{PRPP}$$

where rib-5-PO_4 is ribose 5-phosphate, is reversible in vitro, and the difference in maximal velocities for the "forward" reaction (PRPP synthesis) and "reverse" reaction (PRPP consumption) is approximately one order of magnitude (*15*). The proposed route of ZTP synthesis,

$$\text{ZMP} + \text{PRPP} \rightarrow \text{ZTP} + \text{rib-5-PO}_4$$

would utilize the reverse PRPP synthetase reaction, since PRPP is consumed.

A homogeneous preparation of human PRPP synthetase incubated with PRPP (300 μM) and ZMP (30 μM and 300 μM) catalyzes the synthesis of ZTP in a reaction that is proportional to the time of incubation and amount of PRPP synthetase added (*16*). Omission of PRPP or PRPP synthetase from the reaction mixture results in no detectable formation of ZTP. Table 1 lists the kinetic properties of the PRPP synthetase reaction with adenosine ribotides and Z-ribotides. The ratio of the forward to the reverse reaction with ATP and AMP, respectively, as substrates is 7:1. This is in agreement with the previously reported ratios for the forward to the reverse reaction (*15*). When ATP is replaced by ZTP the maximal velocity (V_{max}) of the forward reaction falls by 77 percent and when ZMP replaces AMP the V_{max} of the reverse reaction falls by 42 percent. With Z-ribotides, the ratio of V_{max} for the forward reaction to V_{max} for the reverse reaction is approximately 3:1. The value of the Michaelis constant (K_m) for PRPP in the ZMP reaction is 32 μM, which is similar to its reported intracellular concentration in many mammalian cells (*17*). The K_m for ZMP is 3.2 mM. These kinetic data are consistent with the following observations made in intact cells (*1–5*). No ZTP is formed in cells at the

very low concentrations of ZMP found under normal conditions. Accumulation of ZTP occurs in cells having enzymatic defects that lead to ZMP accumulation; ZTP also accumulates in cells after administration of Z-riboside, which also leads to ZMP accumulation. In these situations, ZMP concentrations reach millimolar levels (*1, 3–5*), and during the time when ZTP concentrations are increasing, ZMP concentrations greatly exceed those of ZTP (*1, 3, 4*). When Z-riboside administration is discontinued, first ZMP concentrations and then ZTP concentrations decrease (*3*). The requirement of a high concentration of ZMP for ZTP synthesis is consistent with the high K_m for this substrate and the equilibrium of this reaction; the relatively low V_{max} for this reaction may account for the observed delay in ZTP synthesis during periods of ZMP accumulation [illustrations of the accumulation of ZMP and ZTP in vivo can be found in (*1*) and (*3*)]. The lower K_m for ZTP and the equilibrium of the PRPP synthetase reaction may lead to ZTP catabolism by this enzyme when ZMP concentrations are decreasing.

The substrate specificity of the reverse reaction catalyzed by PRPP synthetase was examined with a number of purine and pyrimidine nucleotides in addition to ZMP and AMP (Fig. 1). Because of the rotatable nature of the bond between C-5 and C-6 in ZMP (numbers refer to nomenclature of the completed purine ring), this nucleotide can assume two configurations, labeled A and G on the basis of similarities to AMP and GMP, respectively (Fig. 1). Of those nucleotides tested, AMP, 7-deaza-AMP (tubercidin monophosphate), and ZMP were the only substrates that showed activity in the reverse reaction catalyzed by PRPP synthetase. These results suggest that the A conformation of ZMP binds to the active site of PRPP synthetase since AMP is a substrate for this reaction, whereas GMP is not. Additional insight into the structural requirements for nucleoside monophosphate participation in this reaction is obtained by comparing the structures of compounds that are substrates with those that are not substrates. The C-6 position appears to be critical, since any group other than a primary amine at this position eliminates reactivity (AMP versus GMP, IMP, or N^6-*methyl*-AMP). The C-8 position may also be important since addition of an azido group at this position eliminates reactivity. These results may be important to pharmacologists working with nucleotide analogs, especially in cases where synthesis of the triphosphate derivative of the drug is necessary for its action.

Table 1. Kinetics of the PRPP synthetase reaction with adenine and Z-ribotides as substrates.

Substrate	V_{max} (μmole/min-mg)	K_m (μ*M*)
Forward reaction		
ATP and ribose 5-phosphate	3.17	
ZTP and ribose 5-phosphate	0.72	150 (ZTP)
Reverse reaction		
AMP and PRPP	0.43	370 (AMP)
ZMP and PRPP	0.25	32 (PRPP)
		3200 (ZMP)

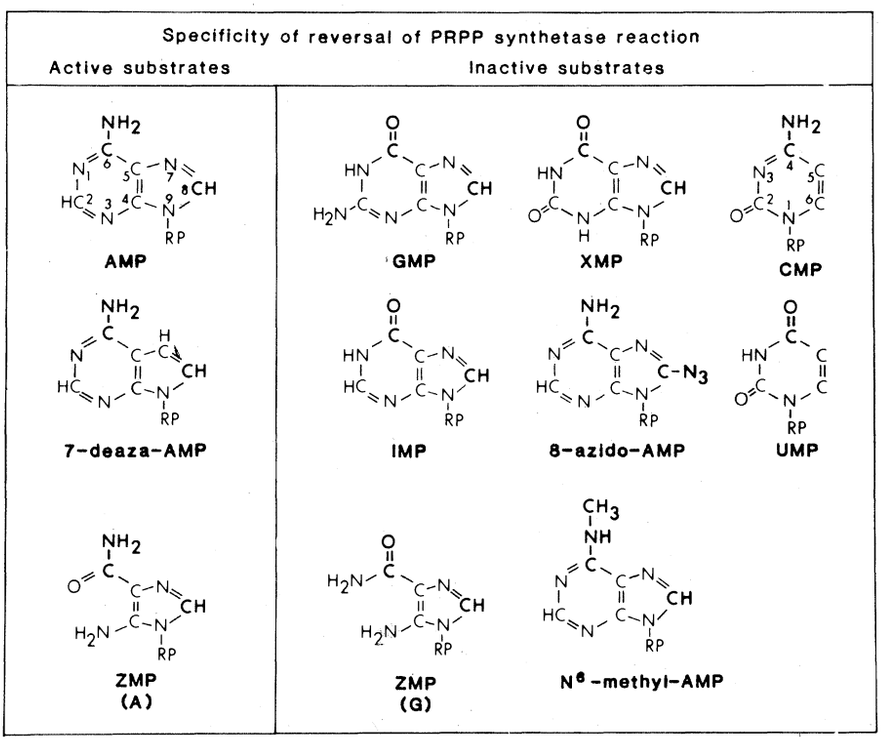

Fig. 1. Substrate specificity of the reverse reaction catalyzed by PRPP synthetase. Abbreviations used are AMP, adenosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; XMP, xanthosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; IMP, inosine 5′-monophosphate; UMP, uridine 5′-monophosphate; and ZMP, 5-amino-4-imidazolecarboxamide ribotide. Numbers refer to nomenclature of the purine and pyrimidine rings.

The combined results of the intact cell and kinetic studies show that Z-ribotides can be metabolized in an unusual way in mammalian cells. Even though adenine nucleotides can also be metabolized in this way, the PRPP synthetase reaction is not the predominant route of synthesis and catabolism of ATP in the cell. In contrast, the PRPP synthetase reaction may be the predominant route for ZTP synthesis and catabolism in the cell, and this route of metabolism of Z-ribotides may be important in defining the metabolic effects of this proposed regulator of intracellular metabolism.

RICHARD L. SABINA*
EDWARD W. HOLMES
Howard Hughes Medical Institute Laboratories, Departments of Medicine and *Biochemistry, Duke University Medical Center, Durham, North Carolina 27710*
MICHAEL A. BECKER
Department of Medicine, University of Chicago Medical Center, Chicago, Illinois 60637

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12. Eight log-phase plates of Chinese hamster ovary fibroblasts (CHO-K1) were extracted in 50 m*M* sodium phosphate buffer, *p*H 7.4. The extract was passed over a G-25 column equilibrated in buffer, and 50 μl of eluent was incubated with 6 m*M* $MgCl_2$, 300 μ*M* PRPP, and 500 μ*M* ZMP. The reaction was performed at 37°C for 60 minutes. Results were analyzed by anion-exchange high-performance liquid chromatography with the nucleotide buffer system described earlier (*3*).
13. Adenylate and guanylate kinase were purchased from Sigma Chemical Co., St. Louis, Mo. One unit of enzyme was incubated in 50 m*M* tris-HCl, *p*H 7.4, with 10 m*M* $MgCl_2$, 100 m*M* KCl, 5 m*M* ATP, and 100 μ*M* of AMP, GMP, or ZMP. Assays were performed at 37°C for 1 hour. Analyses were performed as described in (*12*). For explanation of abbreviations, see legend to Fig. 1.
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16. Purified enzyme was stored at −70°C at a concentration of 0.5 mg/ml in 50 m*M* sodium phosphate with 0.3 m*M* ATP, 6 m*M* $MgCl_2$, and 1 m*M* dithiothreitol (*p*H 7.4). For assay of the reverse reaction, purified enzyme was diluted 1:30 with buffer containing 70 m*M* tris-HCl, *p*H 7.4, plus 700 μ*M* EDTA, 1.7 m*M* β-mercaptoethanol, 7 m*M* $MgCl_2$, 33 m*M* potassium phosphate, and bovine serum albumin (1 mg/ml). All reactants were also dissolved in this buffer. All assays were performed at 37°C, and product formation was linearly related to time of incubation and amount of enzyme added for various times and at a fixed substrate concentration. When varying the ZMP concentration, 0.2 to 2 m*M*, PRPP concentration was fixed at 300 μ*M*. When varying the PRPP concentrations 10 to 300 μ*M*, ZMP concentration was fixed at 10 m*M*. For assay of the forward reaction, purified enzyme (diluted 1:60 with buffer) was incubated in the buffer described above. Ribose 5-phosphate was fixed at 300 μ*M* and the concentration of ATP was fixed at 300 μ*M* or the concentration of ZTP varied from 20 to 500 μ*M*, respectively.
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* Correspondence should be addressed to R.L.S.
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Novel Viral Sequences Related to Human T-Cell Leukemia Virus in T Cells of a Seropositive Baboon

Abstract. *Antibodies reactive with proteins of human T-cell leukemia virus (HTLV) can be found in Old World monkeys. A T-lymphocyte cell line established from a seropositive baboon (*Papio cynocephalus*) was analyzed for the presence of viral DNA sequences. The provirus found in these cells was related to but distinct from HTLV subgroup I. These results add to recent evidence from human studies that HTLV represents a spectrum of infectious T-lymphotropic retroviruses that includes closely and distantly related members.*

Human T-cell leukemia virus (HTLV) is a family of related T-cell tropic retroviruses associated with a specific subtype of mature T-cell malignancy in man (*1*). Two subgroups of HTLV have been identified thus far. Members of the first subgroup, HTLV-I, are highly related to each other, if not identical. These include the initial isolates of the virus in the United States (*2, 3*) as well as additional isolates from patients in this country (*4, 5*), Israel (*4*), South America (*4*), Japan (*4, 6*), and the Caribbean (*4*). Members of the second subgroup, HTLV-II, are only distantly related to HTLV-I as determined by serological cross-reactivities of their proteins (*7*) and molecular hybridization studies with cloned viral genomes (*8, 9*). Only two HTLV-II isolates have been obtained, the first from a patient with a T-cell variant of hairy cell leukemia, and the second from a patient with acquired immunodeficiency syndrome (AIDS) (*10*). Recently, antibodies against HTLV antigens have been found in several Old World monkey species, including Japanese and Chinese macaques, African green monkeys, and baboons (*11–13*). It has been proposed that HTLV can be spread between macaques and humans in Japan (*11*). However, we have evidence that macaques are also seropositive in regions of Japan where HTLV is not endemic in the human population (*13*), suggesting independent entries of the virus into man and other primates. We have analyzed DNA from a T-cell line established from a baboon that was seropositive for HTLV antigens. We found the viral sequences to be related to but distinct from HTLV-I and HTLV-II.

A T-lymphocyte cell line (991-ICC) was established from peripheral leukocytes of a baboon (*Papio cynocephalus*) that is seropositive for HTLV antigens by the enzyme-linked immunosorbent assay (ELISA). The baboon was a recipient of cellular material (bone marrow, lymph node, spleen, and peripheral blood) from a leukemic baboon from Sukumi, U.S.S.R., as part of a U.S.–U.S.S.R. joint virology study during 1973–1975 (*14*). The cell line releases typical type-C particles and extracellular reverse transcriptase, and reacts positively with a monoclonal antibody against the 19,000 dalton core protein (p19) of HTLV as well as a hyperimmune antibody against HTLV p24 (*15*).

High molecular weight DNA from 991-ICC was examined for the presence of HTLV-related sequences by Southern blot hybridization (Fig. 1). DNA from cell lines infected with HTLV-I (lane 1) and HTLV-II (lane 3) were used as positive controls and uninfected human cells were used as a negative control. DNA from many primates, including baboons, lacks sequences closely related to HTLV (data not shown). The restriction endonuclease Sst I cuts in the long terminal repeat (LTR) of many HTLV-I isolates and in the LTR, as well as internally in HTLV-II. When it was used to cut 991-ICC DNA a single band of 8.5 kb was produced that hybridized to the cloned HTLV-I genome. A similar size band was observed in the DNA of HTLV-I infected cells, corresponding to a complete genome with one LTR. This result indicates that like HTLV-I, the provirus in 991-ICC is only cut in the LTR by Sst I. However, Bam HI, which yields a characteristic internal band in all known HTLV-I isolates and two internal bands

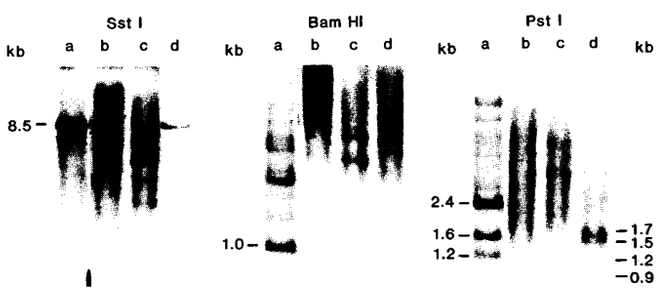

Fig. 1. The distinction between the provirus in 991-ICC and HTLV-I and HTLV-II. DNA from CH, an HTLV-I infected cell line (lane a), normal human thymus (lane b), MO, an HTLV-II infected cell line (lane c), and 991-ICC (lane d) were digested with the enzymes Sst I, Bam HI, and Pst I as shown, subjected to electrophoresis in 0.8 percent agarose gels, and transferred to nitrocellulose filters (*20*). Hybridization was carried out with ^{32}P-labeled DNA representing the complete HTLV-I genome that has been purified away from the vector on agarose gels. After incubation at 37°C for 20 hours in a solution containing triple-strength standard saline citrate, 50 percent formamide, 20 percent dextran sulfate, and 5× Denhardt's solution [0.1 percent bovine serum albumin, Ficoll, polyvinylpyrrolidone] and ^{32}P-labeled HTLV DNA (2×10^6 count/min), the filters were washed with two changes of double-strength SSC and 0.1 percent sodium dodecyl sulfate (SDS) at room temperature for 10 minutes each and at 65°C for another 10 minutes. Autoradiography was for 20 to 48 hours.