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TRYPANOSOMA CRUZI ADENINE NUCLEOSIDE PHOSPHORYLASE

PURIFICATION AND SUBSTRATE SPECIFICITY

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Abstract—An adenine nucleoside phosphorylase has been partially purified from extracts of epimastigotes of the Peru strain of *Trypanosoma cruzi*, the causative agent of Chagas' disease. The purification procedure separated this enzyme from the three other nucleoside-cleaving enzymes found in extracts. The adenine nucleoside phosphorylase, which efficiently cleaved 5′-deoxy-5′-methylthioadenosine (MTA), had a particle weight of 68,000 and exhibited a broad pH optimum between pH 6 and 8. In addition to MTA, the purified enzyme cleaved and synthesized adenosine and 2′-deoxyadenosine with high efficiency. This contrasts to the enzyme from S-180 cells which has been reported to cleave adenosine poorly and not to cleave 2′-deoxyadenosine. Several observations suggested that the three substrates, MTA, adenosine and 2′-deoxyadenosine, use a common catalytic site: (a) all served as alternate-substrate inhibitors exhibiting mutually competitive inhibition with K_i values equivalent to their respective K_m values, (b) 5′-chloroformycin A exhibited a competitive K_i value of 4 μM with each nucleoside substrate, and (c) the K_m value of phosphate derived from initial velocity studies (180 ± 20 μM) was independent of the nucleoside substrate. Substrate specificity studies in both the synthesis and cleavage direction indicated that the enzyme had a broad specificity for bases and nucleosides. For the synthesis of nucleosides, the enzyme demonstrated a preference for an amino group in the position equivalent to the 6 position of purine. Compounds containing a hydroxyl group in this position were not substrates. Although a hydrogen or methyl group could substitute for a 6-amino group, a marked decrease in substrate efficiency was observed with these compounds. Alterations in the purine ring led to decreases in the maximal velocity values as evidenced by the substrate or non-substrate properties of 1-, 3-, and 7-deazaadenine and 4-aminopyrazolo[3,4-d]pyrimidine. The K_m values for 5-methylthioribose 1-phosphate, ribose 1-phosphate and 2′-deoxyribose 1-phosphate with adenine serving as acceptor were 21, 150 and 370 μM. For nucleoside cleavage, the *T. cruzi* enzyme catalyzed the phosphorolysis of a variety of 5′-substituted adenine-containing nucleosides including those possessing 5′-hydrogen-, hydroxyl-, halogeno-, alkylthio-, amino- and azido-moieties. Inclusion of an ionized group in the 5′-position, such as 5′-carboxy-5′-deoxyadenosine or AMP, precluded substrate activity. 3′-Deoxyadenosine, arabinosyladenine and α-adenosine did not serve as substrates. These findings indicate that the adenine nucleoside phosphorylase from *T. cruzi* differs from its mammalian counterpart and that this enzyme should be considered as a potential target for selective chemotherapeutic attack against this pathogenic protozoan.

Previous reports [1, 2] noted an adenosine phosphorolytic activity in extracts of *Trypanosoma cruzi* epimastigotes. This type of activity has also been reported in other parasitic protozoa [3–5], bacteria [6, 7] and helminths [8]. In contrast, a specific adenosine phosphorylase has not been found in mammalian systems. The small amount of adenosine cleavage observed in mammals has been attributed to the inefficient substrate activity of adenosine with either purine nucleoside phosphorylase [9] or 5′-deoxy-5′-methylthioadenosine (MTA‡) phosphorylase [10]. Preliminary studies with dialyzed extracts of *T. cruzi* indicated that adenosine was cleaved as efficiently as was MTA. This finding suggested that the parasite possessed either separate adenosine and MTA phosphorylases or a single phosphorylase with a wide substrate specificity. This paper describes studies, indicating that the latter alternative applies.

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‡ Abbreviations: MTA, 5′-deoxy-5′-methylthioadenosine; Pipes, 1,4-piperazinediethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; and Mes, 2-(*N*-morpholino)ethanesulfonic acid.

EXPERIMENTAL PROCEDURES

Materials

The following materials were purchased: [8-^{14}C]adenine (40 Ci/mole), [8-^{14}C]adenosine (34 Ci/mole), and [8-^{14}C]2′-deoxyadenosine (34 Ci/mole) from New England Nuclear, Boston, MA; 5′-deoxy-5′-methylthioadenosine, ribose 1-phosphate, 2-chloroadenine, 8-azaadenine, 2-methyladenine, 2-aminoadenine, 2-hydroxyadenine, purine, 6-methylpurine, N^6-methyladenine and 4-aminopyrazolo[3,4-d]pyrimidine from the Sigma Chemical Co., St.

Louis, MO; 5′-deoxy-5′-iodoadenosine from the Aldrich Chemical Co., Inc., Milwaukee, WI; agarose-adenosine (Type 3, C^8-linkage, 6.4 μmoles adenosine/ml of gel), agarose-AMP (Type 2, N^6-linkage, 5.0 μmoles/ml of gel), AMP, uracil, uridine, 2′-deoxyuridine, thymine, 2′-deoxythymidine, orotic acid and orotidine from PL Biochemicals, Milwaukee, WI; DEAE-Sephadex A-25 from Pharmacia, Piscataway, NJ; and a μBondapak C-18 reverse-phase liquid chromatography column from Waters Associates, Milford, MA. The following compounds were synthesized at Burroughs Wellcome by published procedures and were shown to be >99% pure by high-performance liquid chromatography: 2-fluoradenine, 1-deazaadenine, 3-deazaadenine, 7-deazaadenine, 5′-deoxy-5′-ethylthioadenosine, 2′,5′-dideoxy-5′-azidoadenosine, 2,6-diaminopurine-2′,5′-dideoxyriboside, 2′,5′-dideoxy-5′-phenylthioadenosine, 5′-deoxy-5′-hydrazinoadenosine, 2′,3′-dideoxy-3′-aminoadenosine, and 5′-deoxy-5′-chloroformycin A. Sources of all other materials were as previously reported [2, 11].

Growth of organisms and preparation of cell extracts

Trypanosoma cruzi epimastigotes, Peru strain, were grown and cell extracts were prepared in the laboratory of Dr. J. J. Marr, University of Colorado Health Center, Denver, CO, as previously described [2].

Enzyme purification

All operations were conducted at 4° unless otherwise noted.

Agarose–Adenosine gel chromatography. A 3-ml aliquot of the cell extract was thawed and centrifuged at 40,000 *g* for 60 min. The supernatant fraction was dialyzed against 2 liters of 10 mM sodium Pipes (pH 6.8) (Buffer A) containing 10 g of charcoal for 16 hr [12]. One milliliter of the dialysate (3.4 ml total volume) was passed through (3 ml/hr) a 0.8 × 3.5 cm column of agarose–adenosine (C^8) gel which was equilibrated with Buffer A. The enzyme was also eluted from this column with Buffer A.

Agarose–AMP gel chromatography. The fractions containing the enzyme were pooled (6.1 ml) and applied at 70 ml/hr onto a 1.4 × 3 cm column of agarose–AMP (N^6) gel equilibrated with Buffer A. After washing the column with 42 ml of Buffer A, the enzyme was eluted at 70 ml/hr with Buffer A containing 1 M KCl. The fraction containing the enzyme (5.0 ml) was dialyzed for 16 hr against 1 liter of Buffer A and then stored at −70°.

Enzymatic assays

Spectrophotometric assays. Spectrophotometric assays for the synthesis and cleavage of the ribo- and deoxyribonucleosides were performed as previously described [2]. Additional extinction coefficient changes used in the present study are presented in Table 1. The values were determined at the maximal difference between the ultraviolet spectra of the base and the corresponding nucleoside between 230 and 300 nm.

Radiochemical assays. Reaction mixtures (100 μl) for the initial velocity analysis of the synthesis of nucleosides contained 50 mM sodium Pipes (pH 7), [8-^{14}C] adenine (40 Ci/mole) and either ribose 1-phosphate, 2-deoxyribose 1-phosphate or 5-deoxy-5-methylthioribose 1-phosphate at the concentration indicated.

Reaction mixtures (100 μl) for the initial velocity analysis of the cleavage of adenosine and 2′-deoxyadenosine contained 20 mM sodium Pipes (pH 7),

Table 1. Extinction coefficient changes at pH 7 for nucleoside synthesis

Substrate	Wavelength (nm)	Extinction coefficient change (mM^{-1} cm^{-1})
6-Amino-substituted purines and analogs		
N^6-Methyladenine	285	1.5
N^6-Hydroxyadenine	284	−1.7
2-Fluoroadenine	250	2.3
2-Chloroadenine	260	3.1
2-Hydroxyadenine	252	4.2
2-Aminoadenine	258	4.0
2-Methyladenine	264	3.4
8-Azaadenine	280	3.8
1-Deazaadenine	280	−5.8
3-Deazaadenine	262	2.5
7-Deazaadenine	272	−0.6
4-Aminopyrazolo[3,4]pyrimidine	280	2.8
Miscellaneous purines		
Purine	276	−1.3
2-Aminopurine	250	2.2
6-Methylthiopurine	296	5.9
6-Methylpurine	268	−1.7

* Values represent the extinction coefficient change that occurred upon pentosylation of the analog at the position that corresponds to the 9-position of purine.

[8-^{14}C]adenosine (34 Ci/mole) or [8-^{14}C]2′-deoxyadenosine (34 Ci/mole) and potassium phosphate (pH 7) at the concentration indicated below.

Reaction mixtures (100 μl) for the determination of the inhibition constants contained 20 mM sodium Pipes (pH 7), 40 mM potassium phosphate (pH 7), [8-^{14}C]adenosine (18.3 Ci/mole) or [8-^{14}C]2′-deoxyadenosine (14.3 Ci/mole) and inhibitors at 2- to 4-fold their respective K_i concentrations.

The reaction mixtures were preincubated at 30° for 3 min prior to the reactions being initiated with enzyme. Reactions were terminated by one of two methods: (1) the addition of 10 μl of a 20 mM solution of adenine and the appropriate nucleoside in 50% ethanol and immediate boiling for 3 min; aliquots (10 μl) of the reaction mixture were spotted onto a plastic-backed cellulose thin-layer chromatography plate, or (2) spotting an aliquot (10 μl) directly onto a cellulose thin-layer chromatography plate which had been prespotted with 5 nmoles of adenine and the appropriate nucleoside. The chromatographic plates were developed in water, the radioactive spots containing the adenine (R_f 0.4) and nucleoside (R_f 0.6) were cut out, and the radioactivity was quantitated by liquid scintillation [2]. Results indicate that these procedures for terminating the reactions agreed within ±2%.

The results of the velocity measurements with spectrophotometric assays and the radiochemical assays for the synthesis of MTA, adenosine and 2′-deoxyadenosine and for the cleavage of adenosine and 2′-deoxyadenosine agreed within ±7%.

Preparation of 5-deoxy-5-methylthioribose 1-phosphate

The reaction mixture (4.5 ml) contained 50 mM potassium phosphate (pH 7.4), 7 mM 5′-deoxy-5′-methylthioadenosine, 0.3 mg of *T. cruzi* MTA phosphorylase (sp. ac. 22.4 nmoles/min/mg) and 2.8 I.U. of xanthine oxidase which had been desalted on a Sephadex G-25 column to remove ammonium sulfate. This reaction mixture was incubated for 24 hr at 30° and then centrifuged at 10,000 *g* for 10 min. The supernatant fraction was diluted to 94 ml with water and loaded onto a DEAE-Sephadex A-25 column (1.5 × 9 cm) that had been pre-equilibrated with 5 mM NH_4HCO_3 at room temperature. The column was eluted with a 400-ml gradient of 5–100 mM NH_4HCO_3, and 8.0-ml fractions were collected. A peak of phosphate and 5-deoxy-5-methylthioribose 1-phosphate* appeared to elute at about 50 mM NH_4HCO_3. The pooled peak fractions were repeatedly evaporated to dryness *in vacuo* to remove the majority of the NH_4HCO_3. The resulting fraction was resuspended in 1.6 ml of water. The pH of the concentrated fraction was adjusted to 6 with 6 N HCl. The phosphate and 5-deoxy-5-methylthioribose 1-phosphate were separated by reverse-phase high performance liquid chromatography on a μBondapak C-18 column eluted with water. The concentration of the 5-deoxy-5-methylthioribose 1-phosphate was determined by the phosphate determination procedure [13] with AMP as a standard.

Protein determination

Protein concentrations were determined using the Coomassie blue method [14]. Bovine serum albumin served as the standard.

Determination of kinetic constants

All velocities were calculated from initial linear rates. Reaction velocities of radiochemical assays were determined from time course experiments from the slope of a plot of product formed versus time. Kinetic constants were determined by directly fitting the data to a hyperbola according to the method of Wilkinson [15] by the use of the computer programs of Cleland [16]. Enzyme inhibition was analyzed according to the method of Spector and Hajian [17]. For the graphical presentation (Fig. 1), each line on the double-reciprocal plot was derived from the best fit of the data to the hyperbolic function of rate versus substrate concentration. All of the constants were determined using a minimum of five velocity measurements over a 9-fold range of substrate con-

* 5-Deoxy-5-methylthioribose 1-phosphate was detected by its condensation with [8-^{14}C]adenine to form MTA as catalyzed by the purified *T. cruzi* adenine nucleoside phosphorylase.

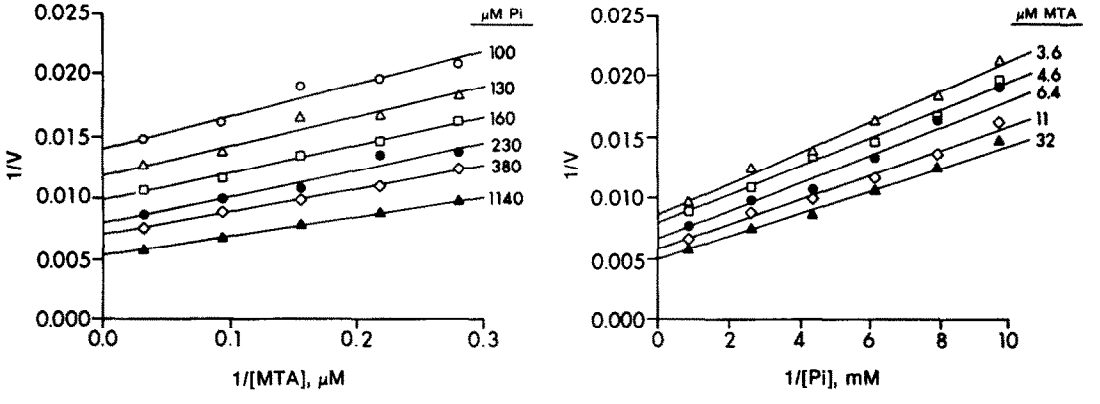

Fig. 1. Double-recripocal plots of initial velocities of MTA phosphorolysis. Velocities are expressed in nmoles/min/mg protein. Lines are drawn to represent the best fit of each individual data set and not to any specific kinetic model.

Table 2. Purification of adenosine phosphorolytic activity of adenine nucleoside phosphorylase from *T. cruzi* extracts

Fraction	Total units (nmoles/min)	Specific activity* (units/mg)
Extract	130	24
Agarose–Adenosine	100	40
Agarose–AMP	75	510

* Reaction velocities were determined in the presence of 100 mM phosphate and 0.1 mM adenosine by the spectrophotometric assay described in Experimental Procedures.

centrations that spanned the K_m value. Inhibition constants were determined using a minimum of two inhibitor concentrations.

RESULTS

Purification

A summary of the purification of the adenosine-cleaving activity of the adenine nucleoside phosphorylase is presented in Table 2. The ratio of adenosine to MTA phosphorolytic activity remained constant throughout the purification procedure (data not shown). Although the agarose–adenosine gel chromatography column offered little purification of the adenosine-cleaving activity, it retained the inosine/guanosine hydrolase present in the extracts [2]. The final enzyme preparation did not detectably cleave 2′-deoxyinosine, inosine, guanosine or uridine (Table 3), indicating that it was free of the other nucleoside-cleaving activities known to be present in *T. cruzi* extracts; 2′-deoxyinosine hydrolase, inosine/guanosine hydrolase and uridine phosphorylase [2]. Storage of the purified enzyme at −70° resulted in <5% loss in activity over a period of 12 months.

Physical properties

The particle weight of the purified adenosine-cleaving activity of the adenine nucleoside phosphorylase was 68,000 ± 3,000 as determined by G-100 Sephadex column chromatography in 50 mM sodium Pipes, pH 7, containing 100 mM KCl. Blue dextran, ovalbumin, chymotrypsinogen, myoglobin and cytochrome *c* served as molecular weight standards (data not shown). The purified enzyme exhibited a broad pH optimum between pH 6 and 8 as determined using 50 mM buffers of the sodium salts of Mes, Pipes and Hepes in overlapping pH ranges (data not shown).

Catalytic properties

The cleavage of MTA, adenosine and 2′-deoxyadenosine was dependent upon the presence of phosphate (Table 3). In the absence of phosphate, cleavage of these nucleosides was undetectable. In addition, the enzyme catalyzed the synthesis of MTA, adenosine and 2′-deoxyadenosine from adenine and the appropriate pentosyl-1-phosphate donor. These findings indicate that the enzyme is a phosphorylase rather than a hydrolase.

Table 3. Substrate specificity and phosphate dependence of purified adenine nucleoside phosphorylase from *T. cruzi*

Substrates*	Concentration† (mM)	Relative rate of synthesis‡	Relative rate of cleavage§ $+P_i$*	$-P_i$
Ade/Ado	0.1/1.0	920	100	<0.2
Ade/dAdo	0.1/1.0	1410	190	<0.2
Ade/MTA	0.1/1.0	260	66	<0.2
Hyp/Ino	0.1/1.0	<3	<0.3	<0.3
Hyp/dIno	−/1.0	—	<0.9	<0.9
Gua/Guo	0.04/0.04	<0.9	<0.9	<0.9
Gua/dGuo	−/0.04	—	<0.9	<0.9
Xan/Xao	−/1.0	—	<0.4	<0.4
Ura/Urd	0.1/0.4	<2	<2	<2
Ura/dUrd	−/0.4	—	<2	<2
Thy/dThd	−/0.1	—	<4	<4
Oro/Ord	−/0.1	—	<0.7	<0.7

* Abbreviations: P_i, phosphate; Ade, adenine; Ado, adenosine; dAdo, 2′-deoxyadenosine; Hyp, hypoxanthine; Ino, inosine; dIno, 2′-deoxyinosine; Gua, guanine; Guo, guanosine; dGuo, 2′-deoxyguanosine; Xan, xanthine; Xao, xanthosine; Ura, uracil; Urd, uridine; dUrd, 2′-deoxyuridine; Thy, thymine; dThd, 2′-deoxythymidine; Oro, orotic acid; and Ord, orotidine.

† Values represent the concentration of the purine or pyrimidine used for nucleoside synthesis followed by the concentration of the nucleoside used for nucleoside cleavage assays.

‡ Nucleoside synthesis rates were determined in the presence of 1 mM ribose 1-phosphate, 2-deoxyribose 1-phosphate or 5-deoxy-5-methylthioribose 1-phosphate.

§ Rates were normalized by setting the rate of adenosine cleavage at 100. The actual rate was 510 nmoles/min/mg protein. Less than values represent the lower limit of detectability.

Table 4. Kinetic parameters of substrates for adenine nucleoside phosphorylase purified from *T. cruzi*

Substrate	K_m (μM)	V_{max} (μmoles/min/mg protein)
5'-Methylthioadenosine	3 ± 0.3	0.48 ± 0.02
Phosphate	200 ± 20	
Adenosine	18 ± 2	0.52 ± 0.02
Phosphate	171 ± 26	
2'-Deoxyadenosine	12 ± 1	0.60 ± 0.02
Phosphate	160 ± 20	
Adenine	1 ± 0.1	0.73 ± 0.07
5-Methylthioribose 1-phosphate	21 ± 2.1	
Adenine	3 ± 0.3	2.6 ± 0.2
Ribose 1-phosphate	150 ± 10	
Adenine	6 ± 0.8	5.1 ± 0.5
2-Deoxyribose 1-phosphate	370 ± 50	

Nucleoside synthesis values were determined using the radiochemical assay. Nucleoside cleavage values were determined using the spectrophotometric assay.

The data derived from initial velocity studies for the synthesis and cleavage of adenosine, 2'-deoxyadenosine and MTA are presented in Table 4. In each case, an apparently parallel pattern of lines was obtained (Fig. 1). These data cannot distinguish between a ping–pong mechanism or a sequential mechanism in which the dissociation constant for the first substrate was less than its K_m value [18].

The results of a study in which radioactive adenosine, 2'-deoxyadenosine or MTA served as a substrate with the other compounds (non-radioactive) serving as alternate substrate inhibitors are presented in Table 5. In each case, competitive inhibition was observed. The values of the K_i obtained for a given alternate substrate-inhibitor were not significantly different when determined with different varied

Table 5. Inhibition of *T. cruzi* adenine nucleoside phosphorylase

Substrate	K_i Ado (μM)	K_i 2'-dAdo (μM)	K_i MTA (μM)	K_i 5'ClForA (μM)
Adenosine	—	21 ± 4.1	3.3 ± 0.4	4.1 ± 0.4
2'-Deoxyadenosine	20 ± 3	—	3.9 ± 0.9	4.0 ± 0.4
MTA	ND*	ND	—	3.8 ± 0.4

* Not determined.

Table 6. Purine substrates for adenine nucleoside phosphorylase

Substrate*	K'_m (μM)	Relative maximal velocity	Relative substrate efficiency (V_{max}/K'_m)
Adenosine	3 ± 0.3	100	30
2-Fluoroadenine	1 ± 0.1	65	65
2-Chloroadenine	4 ± 0.6	49	12
8-Azaadenine	4 ± 0.5	2.7	0.7
2-Methyladenine	9 ± 1	11	1.2
2-Aminoadenine	16 ± 1.7	47	2.9
N^6-Hydroxyadenine	41 ± 2	26	0.6
2-Hydroxyadenine	55 ± 5	5.4	0.1
1-Deazaadenine	58 ± 6	4.1	0.07
Purine	180 ± 20	34	0.2
6-Methylpurine	220 ± 47	15	0.07
N^6-Methyladenine		<0.09†	
6-Methylthiopurine		<0.02	
3-Deazaadenine		<0.5	
7-Deazaadenine		<1.6	
4-Aminopyrazolo[3,4-d]pyrimidine		<0.02	

* The bases were tested at a 1 mM ribose 1-phosphate concentration.
† The less than values were obtained at a base concentration of 100 μM.

Table 7. Nucleoside substrates for adenine nucleoside phosphorylase

Substrate*	K'_m (μM)	Relative maximal velocity	Relative substrate efficiency (V_{max}/K'_m)
Adenosine	18 ± 2	100	10
5'-Deoxy-5'-methylthioadenosine	3 ± 0.3	92	29
5'-Deoxy-5'-iodoadenosine	3 ± 0.06	28	9
5'-Deoxy-5'-ethylthioadenosine	4 ± 0.4	49	20
5'-Azido-2',5'-dideoxyadenosine	4 ± 0.2	107	27
2',5'-Dideoxyadenosine	5 ± 0.7	145	29
5'-Azido-5'-deoxyadenosine	6 ± 0.1	31	5
5'-Chloro-5'-deoxyadenosine	7 ± 1	37	16
5'-Chloro-2',5'-dideoxyadenosine	8 ± 0.4	117	14
5'-Deoxyadenosine	15 ± 0.6	121	8
2-Amino-2',5'-dideoxyadenosine	250 ± 30	67	4
2',5'-Dideoxy-5'-phenylthioadenosine		27†	
α-L-Lyxosyladenine		13	
5'-Deoxy-5'-hydrazinoadenosine		8.3	
5'-Amino-5'-deoxyadenosine		3.9	
5'-Deoxy-5'-tosyladenosine		3.1	
2'-Amino-2'-deoxyadenosine		2.6	
4-Aminopyrazolo[3,4-d]pyrimidine riboside		<0.3	
4-Hydroxypyrazolo[3,4-d]pyrimidine riboside		<0.3	
3'-Amino-2',3'-dideoxyadenosine		<0.1	
5'-Carboxy-5'-deoxyadenosine		<0.04	
3'-Deoxyadenosine		<0.04	
Arabinosyladenine		<0.04	
α-Adenosine		<0.04	
Glucopyranosyladenine		<0.04	
AMP		<0.04	

* The nucleosides were tested at a phosphate concentration of 40 mM.

† Where no K_m value is presented, the velocity values were obtained at a nucleoside concentration of 100 μM.

nucleoside substrates. Further, the K_i values obtained were essentially equivalent to their respective K_m values. In addition, 5'-chloroformycin A (Cl For A), a potent competitive inhibitor of the S-180 enzyme [19], inhibited the *T. cruzi* enzyme. The K_i values obtained with adenosine, 2'-deoxyadenosine and MTA as substrates were identical (Table 5). These findings are consistent with the hypothesis that adenosine, 2'-deoxyadenosine and MTA bind at a common catalytic site.

Substrate specificity

The data in Table 3 indicate that the ability of the enzyme to synthesize and cleave nucleosides was limited. Of the common naturally occurring heterocyclic bases and nucleosides, it only accepted adenine-containing compounds as substrates. However, the purified adenine nucleoside phosphorylase catalyzed the synthesis of ribonucleosides from several adenine analogs (Table 6). All of the 2-substituted adenosines tested served as substrates, indicating that a wide variety of substituents could be tolerated in this position. Although compounds possessing a 6-amino group had the highest substrate efficiencies (V_{max}/K_m), the 6-amino group was not absolutely essential as noted by the substrate activity of purine and 6-methylpurine. The observation that N^6-hydroxyadenine served as a substrate whereas N^6-methyladenine did not suggested that substitution on the 6-amino group could be tolerated but only in some cases. Compounds with 6-hydroxyl groups did not serve as substrates. In some cases, alterations in the purine ring of adenine resulted in non-substrates, the exceptions being 1-deazaadenine and 8-azaadenine.

The effect of the pentosyl moiety on substrate activity was investigated in the nucleoside cleavage reaction. Data in Table 7 indicate that the enzyme required either a β-ribosyl moiety or a 2'-deoxyribosyl moiety for optimal activity. Neither adenine arabinoside, 3'-deoxyadenosine nor α-adenosine served as substrates for the enzyme. Many, but not all, compounds with 5'-modifications of the ribosyl or 2'-deoxyribosyl moieties had substrate activity comparable to their 5'-hydroxyl counterparts. Of the substrates studied (Table 7), 2'-deoxyribonucleosides exhibited higher substrate velocities (1.2- to 3-fold) than the corresponding ribonucleosides.

DISCUSSION

An earlier study demonstrated that extracts of *Trypanosoma cruzi* epimastigotes contain four distinct nucleoside-cleaving activities [2]. Two of these activities were attributable to nucleoside hydrolases: one specific for inosine/guanosine and the other specific for 2'-deoxyinosine. The other two activities were reported to be nucleoside phosphorylases: one specific for uridine and the other specific for adenosine. The present study demonstrates that the previously reported adenosine

phosphorylase also catalyzed the phosphorolysis of MTA and 2′-deoxyadenosine.

The *T. cruzi* adenine nucleoside phosphorylase cleaved MTA, adenosine and 2′-deoxyadenosine (Table 4) with essentially equivalent efficiencies. This specificity is unlike that of the mammalian MTA phosphorylase which does not cleave 2′-deoxyadenosine and cleaves adenosine inefficiently with a K_m value of 1400 μM compared to 4 μM for MTA [10].

Savarese *et al.* [19–21] proposed that MTA phosphorylase may serve as a delivery system for introducing potentially "toxic" adenine analogs into mammalian cells. Recent reports indicate that the cytotoxic effects of 5′-deoxy-5′-iodoadenosine are due, in part, to the formation of 5-deoxy-5-iodoribose 1-phosphate catalyzed by MTA phosphorylase [22]. In addition, cells lacking MTA phosphorylase or adenine phosphoribosyltransferase are insensitive to the effects of the MTA analogs containing potentially "toxic" purine bases [20, 23]. In light of the substrate specificity differences between the *T. cruzi* and mammalian MTA phosphorylases and the presence of adenine phosphoribosyltransferase in extracts of *T. cruzi* epimastigotes [1], it appears that chemotherapy of *T. cruzi* via MTA analogs may be feasible.

Several advantages might arise from the delivery of adenine analogs to the parasite *in situ* via the adenine nucleoside phosphorylase. First, since adenosine is a poor substrate for mammalian purine nucleoside phosphorylase [9], most adenosine analogs would not be expected to serve as substrates for this enzyme. This suggests that these nucleosides would not be cleaved by the host cell, thus allowing them to reach the parasite intact. Second, 5′-substitution of adenosine either eliminates or greatly reduces deamination of these compounds by the mammalian adenosine deaminase [24]. Thus, 5′-substituted nucleosides would be less susceptible to inactivation by deamination in the host prior to reaching the parasite. Third, 5′-substituted nucleosides would not be expected to serve as substrates for adenosine kinase or purine nucleoside phosphotransferase, thus alleviating phosphorylation by the host cell. Fourth, the difference in substrate specificity exhibited by the *T. cruzi* enzyme (Table 7) relative to the mammalian MTA phosphorylase [10] could allow specificity to be built into chemotherapeutic agents such that they would only be cleaved inside the parasite and not by the host cell.

In addition to the above strategy, another approach for the design of anti-trypanosomal drugs is suggested. The presence of adenosine kinase in this organism [25] and the ability of the *T. cruzi* adenine nucleoside phosphorylase to catalyze the synthesis of a variety of adenine nucleosides (Table 6) suggest that the parasite possesses all of the machinery for the synthesis and phosphorylation of potentially "toxic" adenosine analogs from purine bases. This approach would rely on finding an adenine analog that does not serve as a substrate for the host cell adenine phosphoribosyltransferase but would serve as a substrate for the parasite adenine nucleoside phosphorylase. Irrespective of how the nucleoside monophosphate analog forms, the enzymes for further phosphorylation of the monophosphate to its di- and triphosphate forms are present in the parasite [26].

Elevation of *in vivo* MTA conncentrations has been noted to have a variety of *in vivo* and *in vitro* effects [27, 28]. MTA inhibits spermine synthase and spermidine synthase [29], is a suicide inactivator of adenylhomocysteine hydrolase [30, 31], and causes an increase in intracellular cyclic AMP concentrations by inhibiting cyclic AMP phosphodiesterase [32]. Thus, another potential route of attack upon this parasite could involve the inhibition of the *T. cruzi* adenine nucleoside phosphorylase, resulting in elevations of MTA levels.

All of the mentioned potential routes of chemotherapeutic attack upon this organism (delivery of "toxic" adenine or pentose analogs, synthesis of "toxic" adenosine analogs or the inhibition of the parasite adenine nucleoside phosphorylase) must await the availability of the desired nucleoside analogs. It is hoped that the specificity data presented here will provide a basis for their design.

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