Published in final edited form as:
Mol Biochem Parasitol. 2014 ; 194(0): 44–47. doi:10.1016/j.molbiopara.2014.04.008.

ISOLATION AND SUBSTRATE SPECIFICITY OF AN ADENINE NUCLEOSIDE PHOSPHORYLASE FROM ADULT *SCHISTOSOMA MANSONI*

Todd M. Savarese[a,b] and **Mahmoud H. el Kouni**[a,c,*]

[a]Division of Biology and Medicine, Brown University, RI 02912

[c]Department of Pharmacology, and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35294

Abstract

An adenine nucleoside phosphorylase (ANP, EC none) activity was identified and partially purified from extracts of *Schistosoma mansoni* by chromatofocussing column chromatography and molecular sieving. The enzyme is distinct from purine nucleoside phosphorylase (PNP, EC 2.4.2.1). ANP is specific for adenine nucleosides which includes adenosine analogues modified in the aglycone, pentose or both moieties. (e.g. 2′-deoxyadenosine, 5′-deoxy-5′-methylthioadenosine, 5′-deoxy-5′-iodo-2-fluoroadenosine, etc.). The enzyme is also distinct from the mammalian 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAP, EC 2.4.2.28) in that it is able of the phosphorolysis of 2′-deoxyadenosine while mammalian MTAP cannot. Because of ANP unique substrate specificity, the enzyme could play a role as a target for chemotherapy of these parasites. Cytotoxic analogs may be designed as subversive substrates that are selectively activated only by the schistosomal ANP.

Keywords

Schistosoma mansoni; Adenosine metabolism; Adenine nucleoside phosphorylase; Purine nucleoside phosphorylase (EC 2.4.2.1) 5′-Deoxy-5′-methylthioadenosine phosphorylase (EC 2.4.2.28)

Living organisms require purine nucleotides for nucleic acid synthesis and energy requiring reactions. Purines nucleotides can be synthesized from simple precursors by the *de novo* pathway and/or from endogenous or exogenous preformed purines by the salvage pathways. In contrast to their mammalian hosts, schistosomes lack the *de novo* purine biosynthetic pathway and are wholly dependent upon salvage pathways for their purine requirements [1].

[*]corresponding author: Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A. Tel.: 205-934-1132, fax: 205-934-8240 *melkouni@uab.edu* (M. H. el Kouni).
[b]Deceased and to whom this paper is dedicated

The reliance of schistosomes on the purine salvage pathways renders the parasites susceptible to chemotherapy by analogues of purine nucleobases and nucleosides. Indeed, we and others have demonstrated that adenosine analogues can be quite effective in the treatment of schistosomiasis [2-7]. Such promising results encourage the further investigation of purine salvage pathways in schistosomes. Detailed studies on the salvage pathways of purines in schistosomes, not only will contribute to the general knowledge of purine metabolism in schistosomes, but may also reveal potential targets for the treatment of schistosomiasis with one or more of the available chemotherapeutic purine analogues.

Previous studies on purine salvage in schistosomes demonstrated that the synthesis of adenine nucleotide form adenosine proceeds mainly by pathways that does not involve adenosine kinase (EC 2.7.1.20) [8-11]. Adenosine is deaminated first to inosine by adenosine deaminase (EC 3.5.4.4). Inosine is then converted to IMP by the sequential of purine nucleoside phosphorylase (PNP, EC 2.4.2.1) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC. 2.4.2.8). Adenine and guanine nucleotides are then formed from IMP [8-9]. However, adenine formation from adenosine was also observed [10-12]. The formation of adenine from adenosine was attributed to the fact that *Schistosoma mansoni*, unlike their mammalian host, contain an adenosine phosphorylase (EC none) activity distinct from PNP [12]. Subsequently, we showed that schistosomes are able to synthesize 2-fluoroadenosine 5′-triphosphate from 5′-deoxy-5′-iodo-2-fluoroadenosine [13]. 5′-Deoxy-5′-iodo-2-fluoroadenosine is not a substrate for adenosine kinase, adenosine deaminase or PNP [14-17]. To be metabolized to 2-fluoroadenosine nucleotides, 5′-deoxy-5′-iodo-2-fluoroadenosine must first be cleaved to yield 2-fluoroadenine. 2-Fluoroadenine is not deaminated in schistosomes [9-12] but the parasite can rapidly synthesize 2-fluoroadenosine 5′-triphosphate from 2-fluoroadenine via the action of adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) [17]. Since 5′-deoxy-5′-iodo-2-fluoroadenosine is not a substrate for PNP, the cleavage of this nucleoside analogue in the schistosomes must proceed by a yet an unidentified 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAP, EC 2.4.2.28) or by the distinctive adenosine phosphorylase identified in this parasite [12]. The aim of the present study is to determine the enzyme(s) responsible for the phosphorolysis of adenine nucleosides in schistosomes.

Live *S. mansoni* were obtained as previously described [2,13] and homogenized (1:3, v/v) in 25 mM imidazole-Cl, pH 7.5, 1 mM DTT using a Teflon pestle. The homogenate was centrifuged at 105,000 × g for 1 hr at 4°C in a Beckman L8-M ultracentrifuge and the supernatant fluid (cytosol) was collected. The cytosol contained all of the purine nucleosides cleaving activity. No significant activity could be detected in the particulate fraction (105,000 × g pellet) of live worms. Table I shows the rate of cleavage of various nucleosides by the cytosol extracts of live *S. mansoni* worms. Adenosine, 5′-deoxy-5′-methylthioadenosine (MeSAdo), inosine and guanosine were all cleaved in the presence of phosphate to their respective nucleobases. These activities were abolished in the absence of phosphate (Table I). This indicates that the cleavage of these nucleosides was due to phosphorolytic rather than hydrolytic activity.

Table 1 also shows the effect of 5′-deoxy-5′-chloroformycin A (5′-dClFormycin, synthesized and generously provided by Dr. Shih Hsi Chu, Brown University, Providence,

RI) on the phosphorolysis of these various nucleosides. 5′-dClFormycin is a specific inhibitor of MTAPase [18]. 5′-dClFormycin inhibited the activity towards MeSAdo by 100% and adenosine by 96%. The activities towards inosine or guanosine was not affected by 5′-dClFormycin. These results indicate that in *S. mansoni*, MeSAdo and most of adenosine are cleaved by the same phosphorylase. It also shows that this enzyme is distinct from PNP which is responsible for the phosphorolytic cleavage of other purine nucleosides (e.g. guanosine and inosine) in this parasite. The observation that adenosine phosphorolysis was not completely inhibited by 5′-dClFormycin, suggests that adenosine is cleaved in schistosomes by more than one enzyme. Since an adenosine phosphorylase activity has been previously reported in schistosomes [12] and the fact that all activity towards MeSAdo and the bulk of the activity towards adenosine was inhibited by 5′-dClFormycin, it was imperative to determine whether or not the activity of adenosine/MeSAdo cleavage is due to the distinctive adenosine phosphorylase identified in this parasite [12] or by a yet unidentified enzyme.

To investigate further how many enzymes are involved in phosphorolytic activity towards purine nucleosides in *S. mansoni*, the cytosol from *S. mansoni* was chromatographed on chromatofocussing column employing a pH gradient of 7.4 to 4.0. Two peaks of nucleoside cleaving activity appeared. The First peak, eluted at pH 5.7. The second peak of nucleoside cleaving activity eluted at pH 5.2. Table 2 shows that the first peak has activities that cleave guanosine (100%), inosine (70%), 2′-deoxyadenosine (44%), adenosine (10%), but not MeSAdo. The ratio of inosine/guanosine, adenosine/guanosine, and deoxyadenosine/ guanosine were similar in all fractions containing the four activities. The cleavage of guanosine, inosine, adenosine, and 2′-deoxyadenosine by elutes from this first peak was not inhibited by 100 μM 5′-dClFormycin. The second peak has high adenosine (20 nmol/min/mg protein = 100%), lower MeSAdo (40%) and 2′-deoxyadenosine (37%), but no guanosine and inosine phosphorolytic activities (Table 2). The ratio of MeSAdo/adenosine and deoxyadenosine/adenosine were similar in all fractions containing the three activities. Furthermore, the cleavage of adenosine, 2′-deoxyadenosine and MeSAdo in this peak were all completely (100%) inhibited by 100 μM 5′-dClFormycin.

The chromatofocussing fractions of the first peak were pooled and concentrated (4-6 mg/ml) and stored at -70°C until further use. Under these conditions the enzymes were stable with no loss of activity for over two months. This procedure resulted in retention of the activities towards guanosine, inosine, adenosine, and deoxyadenosine in the same ratio. These results indicate that this activity is a PNP since its activity is not specific to adenosine and cleaves all purine nucleoside tested except MeSAdo and is similar to other PNP studied from different sources. Table 3 shows that by these procedures PNP from *S. mansoni* was purified 41- fold with 38% yield.

Table 4 shows the results of the partial purification of Peak 2 by the use of chromatofocussing column chromatography and molecular sizing. This activity was purified 10-fold with 31% yield. The concentration of the pooled chromatofocussing fractions of the second peak resulted in co-concentration of the activities towards adenosine, 2′-deoxyadenosine and MeSAdo. Administration of 5′-dClFormycin A, a known MTAP inhibitor [18], caused 100% inhibition of the activity whether adenosine, 2′-deoxyadenosine

or MeSAdo was used as a substrate, suggesting that the cleaving activities of these nucleosides reside with a single enzyme. This is further supported by the fact that when samples of enzyme preparations from the second peak were incubated with saturating concentrations of both MeSAdo (200 uM) and Ado (500 uM), the resulting rate of adenine formation was less than the sum of the rates that were observed in the presence of saturating concentrations of either substrate alone. If two separate cleaving enzymes were present then the rate of product formation would have been additive under these conditions. Furthermore, experimental evidence indicate that the enzyme in the second peak is a phosphorylase and not a hydrolase. First, the cleavage activity towards adenosine, 2′-deoxyadenosine and MeSAdo in the concentrate of Peak 2 was abolished in the absence of phosphate. Secondly, aliquot from this peak were capable of synthesizing adenosine from adenine and ribose-1-phosphate and MeSAdo when incubated with adenine and methylthioribose-1-phosphate. This demonstrates that the activity is indeed a phosphorylase, not a hydrolase, specific for adenine nucleosides. Therefore, the cleaving activity associated with this peak is designated adenine nucleoside phosphorylase (ANP).

In conclusion, the present results confirm that *S. mansoni*, unlike their host, do not contain a distinct MTAP and that MeSAdo and adenosine as well as 2′-deoxyadenosine are cleaved in this parasite by ANP. The enzyme is specific for various ribo-, 2-′ or 5′-deoxyriboadenine nucleosides as well as their analogues, and can use substrates modified in the aglycone, pentose or both moieties [12,13 and the present results]. ANP differs from the host MTAP in substrate specificity as it can cleave 2′-deoxyadenosine while the mammalian MTAP cannot. ANP has been identified in *Trypanosoma brucei* and *Leishmania donovani*, but is absent from *Giardia lamblia*, *Plasmodium falciparum*, and *Entamoeba invadens* [19 and references therein]. Thus, ANP does not appear to be common among all parasites

It should be mentioned here that ANP could be considered as a target for chemotherapy in schistosomes as well as other parasites which contains ANP. Since *S. mansoni* lacks *de novo* purine biosynthesis, they are totally dependent on purine salvage mechanism such as ANP. Adenosine analogues which are substrates for ANP but not adenosine kinase and adenosine deaminase (e.g. 2-fluoroadenosine, 5′-deoxy-5′-iodo-2-fluoroadenosine) could be used as subversive substrates in the chemotherapy of such parasites. The phosphorolysis of such type of subversive substrates by ANP would result in the selective liberation of the toxic nucleobase, 2-fluoroadenine, as well as potentially cytotoxic analogues of 5-methylthioribose-1-phosphate, solely in the parasites but not in the host cells where 2-fluoroadenine is quite toxic. The cytotoxic action of such subversive substrates would depend primarily on their conversion to their respective 2-fluoroadenine nucleotides by APRT which is indeed the case in Schistosomes [1,12,13.17]. It should be mentioned here that the rate of adenine salvage is quite high in *S. mansoni* [1]. It has to be kept in mind, however, that for this strategy to work and achieve selective toxicity, the designed adenosine analogue should neither be a substrate for the adenosine kinase nor inactivated by adenosine deaminase. In addition, the released adenine analogue should be a good substrate for the parasite APRT and its nucleotide is toxic to the parasite.

It is also known that 5-methylthioribose-1-*P* is recycled to methionine and is an important source for methionine salvage [20 and reference therein]. Therefore, substrate analogues of

5′-deoxy-5′-methylthioadenosine that release toxic methionine analogues (e.g., ethionine) may also be useful as antischistosomal drugs

Acknowledgments

We would like to thank Mr. Steven Harrington and Ms. Norma Messier for technical assistance. This work was supported in part by PHS grant AI-22219 from NIAID, DHHS.

Abbreviations

ANP	adenine nucleoside phosphorylase
MeSAdo	5′-deoxy-5′-methylthioadenosine
MTAP	5′-deoxy-5′-methylthioadenosine phosphorylase (EC 2.4.2.28)
PNP	purine nucleoside Phosphorylase (EC 2.4.2.1)
5′-dClFormycin	5′-deoxy-5′-chloroformycin A

REFERENCES

1. Senft AW, Miech RP, Brown PR, Senft DG. Purine metabolism in Schistosoma mansoni. Int J Parasitol. 1972; 2:249–260. [PubMed: 4652611]
2. el Kouni MH, Diop D, Cha S. Combination therapy of schistosomiasis by tubercidin and nitrobenzylthioinosine 5’-monophosphate. Proc Natl Acad Sci USA. 1983; 80:6667–6670. [PubMed: 6579551]
3. el Kouni MH, Knopf PM, Cha S. Combination therapy of Schistosoma japonicum by tubercidin and nitrobenzylthioinosine 5’-monophosphate. Biochem Pharmacol. 1985; 34:3921–3923. [PubMed: 3933515]
4. el Kouni MH, Messier NJ, Cha S. Treatment of schistosomiasis by purine nucleoside analogues in combination with nucleoside transport inhibitors. Biochem Pharmacol. 1987; 36:3815–3821. [PubMed: 3689423]
5. Baer HP, El-Soofi A, Selim A. Treatment of Schistosoma mansoni- and Schistosoma haematobium-infected mice with a combination of tubercidin and nucleoside transport inhibitor. Med Sci Res. 1988; 16:919.
6. el Kouni MH, Diop D, O'Shea P, arlisle R, Sommadossi J-P. Prevention of tubercidin host toxicity by nitrobenzylthioinosine 5’-monophosphate for the treatment of schistosomiasis. Antimicrob Agents Chemother. 1989; 33:824–827. [PubMed: 2764531]
7. el Kouni MH. Efficacy of combination therapy with tubercidin and nitrobenzylthioinosine 5’-monophosphate against chronic and advanced stages of schistosomiasis. Biochem Pharmacol. 1991; 41:815–820. [PubMed: 1900158]
8. Senft AW, Senft DG, Miech RP. Pathways of nucleotide metabolism in Schistosoma mansoni. II. Disposition of adenosine by whole worms. Biochem Pharmacol. 1973; 22:437–447. [PubMed: 4691873]
9. Senft AW, Crabtree GW, Agarwal KC, Scholar EM, Agarwal RP, Parks RE Jr. Pathways of nucleotide metabolism in Schistosoma mansoni. III. Identification of enzymes in cell-free extracts. Biochem Pharmacol. 1973; 22:449–458. [PubMed: 4691874]
10. Crabtree GW, Senft AW. Pathways of nucleotide metabolism in schistosoma mansoni. V. Adenosine cleavage enzyme and effects of purine analogues on adenosine metabolism in vitro. Biochem Pharmacol. 1974; 23:649–660. [PubMed: 4822748]
11. Senft AW, Crabtree GW. Purine metabolism in the schistosomes: potential targets for chemotherapy. Pharmacol Ther. 1983; 20:341–356. [PubMed: 6412258]

12. Miech RP, Senft AW, Senft DG. Pathways of nucleotide metabolism in Schistosoma mansoni - VI. Adenosine phosphorylase. Biochem Pharmacol. 1975; 24:407–411. [PubMed: 1125049]

13. el Kouni MH, Cha S. Metabolism of adenosine analogues by Schistosoma mansoni and the effect of nucleoside transport inhibitors. Biochem Pharmacol. 1987; 36:1099–1106. [PubMed: 3566804]

14. Savarese TM, Cannistra AJ, Parks RE Jr, Secrist JA III, Shortnacy AT, Montgomery JA. 5'-deoxy-5'-methylthioadenosine phosphorylase--IV. Biological activity of 2-fluoroadenine-substituted 5'-deoxy-5'-methylthioadenosine analogs. Biochem Pharmacol. 1987; 36(12):1881–93. [PubMed: 3109431]

15. Parks, RE., Jr; Stoeckler, JD.; Cambor, C.; Savarese, TM.; Crabtree, GW.; Chu, SH. Purine nucleoside phosphorylase and 5'-methylthioadenosine phosphorylase: chemotherapeutic target enzymes.. In: Sartorelli, AC.; Lazo, JS.; Bertino, JR., editors. Molecular Actions and Targets for Cancer Chemotherapeutic Agents. Academic Press; New York: 1981. p. 229-252.

16. Agarwal RP, Sagar SM, Parks RE Jr. Adenosine deaminase from human erythrocytes: purification and effects of adenosine analogs. Biochem Pharmacol. 1975; 24:693–701. [PubMed: 1125070]

17. Stegman RJ, Senft AW, Brown PR, Parks RE Jr. Pathways of nucleotide metabolism in Schistosoma mansoni. IV. Incorporation of adenosine analogs in vitro. Biochem Pharmacol. 1973; 22:459–468. 17. [PubMed: 4691875]

18. Savarese TM, Crabtree GW, Parks RE Jr. Reaction of 5'-deoxyadenosine and related analogs with the 5'-methylthioadenosine cleaving enzyme of sarcoma 180 cells, a possible chemotherapeutic target enzyme. Biochem Pharmacol. 1979; 28:2227–2230. [PubMed: 315229]

19. el Kouni MH. Potential Chemotherapeutic Targets in the Purine Metabolism of Parasites. Pharmac Therapeut. 2003; 99:283–309.

Highlights

- Adenine nucleoside phosphorylase (ANP, EC none) activity was identified and partially purified from extracts of *Schistosoma mansoni*
- ANP is distinct from the parasite purine nucleoside phosphorylase (EC 2.4.2.1) and mammalian 5′-deoxy-5′-methylthioadenosine phosphorylase (EC 2.4.2.28).
- ANP is specific for adenine nucleosides which includes adenosine analogues modified in the aglycone, pentose or both moieties
- ANP could play a role as a target for chemotherapy of these parasites by the use of subversive substrates that are selectively activated only by this enzyme.

Table 1

Rates of nucleoside cleavage by cytosol extracts of *S. mansoni* and inhibition by 5'-dClFormycin (5'-deoxy-5'-chloroformycin A).

Nucleoside	Control	No Phosphate	5'-dClFormycin [100 μM]
Adenosine	490± 80[a]	0.1 ± 0.1	20.7 ± 6 (96)
MeSAdo	650 ± 60	0	2 ± 2 (100)
Inosine	807± 12	0	802 ± 16 (1)
Guanosine	798 ± 30	0	800 ± 35 (0)

[a]Values are pmol/min/mg protein ± standard deviation from at least three experiments. Percent inhibition by 5'-dClFormycin is shown between parentheses

Table 2

Rates of nucleoside cleavage catalyzed by Partially purified Activities of Polled Fractions of Peak 1 (Purine Nucleoside Phosphorylase, PNP) and Peak 2 (Adenosine nucleoside Phosphorylase, ANP) Resulting from Chromatofocusing chromatography of Cytosol of *S. mansoni*.

Nucleoside	Peak 1 (PNP)	Peak 2 (ANP)
Adenosine	8.3[a] (10)	20 (100)
2′-Deoxyadenosine	37 (45)	7.4 (37)
MeSAdo	0.0	8.0 (40)
Inosine	58 (70)	0.0
Guanosine	83 (100)	0.0

Percentages relative to Peak 1 (PNP) activity with guanosine and Peak 2 (ANP) with adenosine are shown between parenthesis

[a] nmol/min/mg protein assayed as described in Table 1.

Table 3

Isolation and Partial Purification of purine nucleoside phosphorylase (PNP) from *S. mansoni* by the use of chromatofocussing column chromatography and molecular sieving

Fraction	Total volume (ml)	Total protein	Total units (nmol/min)	Specific Activity[a] (nmol/min/mg protein)	Yield %
Cytosol	7.7	88.9	73	0.82	100
Chromatofocussing	20	14.2	118	8.2	162
Molecular sieving	1.5	0.84	28	34.0	38

Table 4

Partial Purification of Adenine Nucleoside Phosphorylase (ANP) from *S. mansoni* by the use of chromatofocussing column chromatography and molecular sizing concentration

Fraction	Total volume (ml)	Total protein (mg)	Total units (nmol/min)	Specific Activity[a] (nmol/min/mg protein)	Yield (%)
Cytosol	7.7	88.9	107	1.09	100
Chromatofocussing	50.0	11.3	130	10.8	10
Molecular sieving	1.5	11.2	39.4	120	10.9

Chromatofocusing and molecular sieving by Centriprep-30 were performed as described in Table 3. ANP activity eluted from the column at pH 5.2.

[a] Measured with MeSAdo as a substrate as described in Table 1.