Adenosine Metabolism in the Parasitic Helminth Dirofilaria Immitis

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Summary
Extracts of Dirofilaria immitis surveyed for the enzymes involved in adenosine metabolism. Adenosine deaminase (EC 3. 5. 4. 4) adenine deaminase (EC 3. 5. 4. 2), catabolic adenosine phosphorylase (EC 2. 4. 2. 1) and adenine phosphoribosyl transferase (EC 2. 4. 2. 7) were all detected at relatively high activities, whereas, anabolic adenosine phosphorylase (EC 2. 4. 2. 1) and adenosine kinase (EC 2. 7. 1. 20) were not detected. The result suggest that in D. immitis the main route of adenosine nucleotide synthesis is through the action of phosphorylase and phosphoribosyl transferase.

Introduction
All living organisms require an abundant supply of purine and pyrimidine nucleotides for the synthesis of RNA and DNA needed for cellular proliferation. The important role of adenosine as physiological regulator is well recognized and the name retaliatory metabolite was proposed to describe its action (Newby, 1984).
Adenosine inhibits or stimulates hormone secretion, has an effect on the metabolism of several tissue and modifies a variety of physiological processes, causes cell toxicity and immunosuppressant and acts as neuromodulator (Fox and Kelley, 1978; Braun et.al., 1980).
In man, adenosine cycle have been well delineated for purine metabolism. It was shown that the deficiency of adenosine deaminase is associated with combined immunodeficiency syndrome, characterized by severely impaired humoral and cell mediated immunity and decreased number of peripheral blood lymphocytes (Giblett et.al., 1972; Dissing and Knudsen, 1972). In contrast, adenosine metabolism in parasitic helminthes have been only partially elucidated (Gamble and Pappas, 1981; Emeh et.al., 1986; Jawzaly, 1992). Therefore we have examined the enzymes potentially involved in the salyage of adenosine in Dirofilaria immitis. Such important enzymes or metabolic pathways could be target for chemotherapeutic attack of this parasite by adenosine analogues.

Materials and Methods
Organism: Adults of D. immitis were removed from the pulmonary vessels and heart of a dog immediately after killing, washed in isotonic saline and homogenised with a glass homogeniser in 10 Vol. in 50 mM Tris – HCL. pH 7.2 containing 0.1% Triton X – 100. The crude homogenate produced was then centrifuged at 100.000 g for 60 min at 4 ^{o}C and the resulting supernatant was used as source of the enzymes.
Enzyme assays: The composition of the assays mixture and the extinction coefficients used to calculate the enzyme activities are summarised in Table 1. All enzyme activities were assayed spectrophotometrically at 37 ^{o}C as described previously by Hassan and Coombs (1988) and Hassan and Alchalabi (1993). The routs of change in absorbance resulting from the conversion of substrates to products was monitored at the appropriate wavelength using a Pye unicam SP800 ultraviolet spectrophotometer at a full scale setting of 0 – 0.2 absorbance units. Final assay mixtures had a total volume of 1.0 ml, and 1 cm path length quartz curettes were used. Reaction mixtures were preincubated to 37 ^{o}C, and the reaction was initiated by the addition of enzyme (100 μg protein).
All assays were carried out in triplicate in each experiment. One unit of enzyme activity was defined as the amount of enzyme that will catalyses the conversion of 1 nmol of substrate to product per minute at 37 ^{o}C. All specific activities were calculated with reference to sample protein and measured at the enzymes PH optimum. Protein concentration were estimated by the method of Lowry et.al., (1951) with bovine serum albumin as standard.

Table 1: Spectrophotometer assay conditions of adenosine cycle enzymes.

Enzyme	Substrate	Other constituent of reaction mixture	Product	nm (mM cm)
Adenine deaminase	Adenine	0.02 U XOD	U rate	293 (10.6)
Adenosine deaminase	Adenosine	0.005 H PNP, 0.02 U XOD	U rate	293 (10.6)
Phosphorylase (catabolic)	Adenosine	10 mM KH2P04	Adenine	255 (-2.0)
Phosphorylase (anabolic)	Adenine	1 mM R-1P, 0.4 U ADA	Inosine	261 (-6.4)
Adenosine kinase	Adenosine	10 Mm MgC12, 1mM ATP, 1 mM PEP, 1mM NADH, 0.1 U PK, 0.1 U LDH	AMP	340 (-18.66)
Adenine PRTase	Adenine	5 mM MgSO4, 1mM PRPP	AMP	255 (3.3)
HGPRTase	Hypoxanthine	5 mM MgSO4, 1mM PRPP	IMP	243 (2.2)
	Or Guanine	5 mM MgSO4, 1mM PRPP	GMP	255 (4.2)

a) : The final concentration of the substrate used was 0.1 mM.
b) : The buffer used for the assay of enzymes was 50 mMTris – HCl.
c) : Wavelength monitored (extinction coefficient) .

Abbreviations used were as follow; XOD, xanthine oxidase; PNP, purine nucleoside phosphorylase; ADA, adenosine deaminase; PK, pyruvate kinase; LDH, lactate dehydrogenises; PRTase, phosphoribosyl transferase; R-1-P, ribose -1-phosphate; PRPP, phosphoribosyl pyrophosphate; PEP, phosphoenol pyruvate; NADH, nicotinamide adenine dinucleotide; AMP, adenosine monophosphate; IMP, inosine monophosphate; GMP, guanosine monophosphate.

Results and Discussion

Table 2 presents the enzyme activities of the adenosine metabolism which were detected in the soluble fraction of D. immitis. The results suggest that D. immitis has the enzymatic capacity of salvaging adenosine and adenine. This suggestion is supported by the previous observation that D. immitis incorporate exogenous adenine and adenosine in to their nucleic acids (Jaffe and Doremus, 1970). The presence of adenosine deaminase, adenosine phosphorylase and adenine phosphoribosy transferase (APRTase) suggest that exogenous adenosine which is incorporated in large amounts can undergo three possible metabolic fates in D. immitis. It can be (1) converted to inosine via adenosine deaminase; (2) undergo cleavage to adenine by the action of adenosine phosphorylase; (3) converted to adenosine – 5 – monophosphate (AMP) via adenosine phosphorylase and APRTase. In animal cells, the phosphorylase shows little or no detectable action with adenosine (Zannis et.al, 1978). Also the major metabolic pathway for AMP synthesis in animal cells is by way in adenosine kinase. Animal cells have low levels of APRTase (Murray, 1971), by contrast, D. immitis have no detectable adenosine kinase activity but relatively high APRTase and adenosine phosphorylase. The apparent absence of anabolic phosphorylase (the synthesis of adenosine from adenine and ribose – 1 – phosphate) and adenosine kinase activities suggest that the only pathway available for nucleotide synthesis in D. immitis is by APRTase. It would appear that the inhibition of APRTase must affect the intracellular concentration of adenine nucleotide and therefore would inhibit the growth of the parasite. Thus this enzyme may be a potential chemotherapeutic target. The presence of high activity of adenine deaminase is perhaps indicative of a strong salvage system in D. immitis. In each a system adenine deaminase will play a significant role in the conversion of adenine base absorbed from the host in to adenosine monophosphate and guanosine monophosphate (GMP) via hypoxanthine and inosine monophosphate routes (Fig 1).

Knowledge of the adenosine metabolism as well being useful in planning rational chemotherapy with adenosine analogue drugs, could also help to define the potential mode of action of these drugs. The adenosine analogues, tubercidin (7 – deaza – adenosine) and formycin A exhibits potent antischistosomal activity (Dovey et.al., 1985) and it has been observed that Schistosoma could cleave these analogues to their pure bases by phosphorylase before conversion to the nucleotides with APRTase (EL-Kouni and Cha, 1987). The unusual substrate specifity of this phosphorylase which recognizes tubercidin and formycin – A makes it an important target for comparative host parasite studies aimed at developing rational chemotherapy. Further investigations are being undertaken to assess the activities of purine nucleoside enzymes on adenosine analogue compounds.

Table 2: Summary of activities and kinetic data for the D. immitis adenosine cycle enzymes.

Enzyme	Specific activity	pH optimum	K_m	V_{max}
Adenine deaminase	34 + 2	7.2	3.7 + 0.5	176 + 15
Adenosine deaminase	12 + 1	6.8 – 7.4	52 + 5	156 + 6
Adenosine Phosphorylase (anabolic)	nd < 0.3	---	---	---
Adenosine Phosphorylase (catabolic)	25 + 3	7.4	111 + 12	85 + 4
Adenosine kinase	nd < 0.1	---	---	---
A PRTase	17 + 1	7.6	13 + 4	189 + 11
HGPRTase	14 + 2	7.4	9 + 2	751 + 13

The specific activities given are in p mol / min / mg, protein and are the means + SD from three experiments
K_m is given in uM + S. E.
V_{max} is given in p mol / min / mg, protein + S. E.
Nd : not detectable.

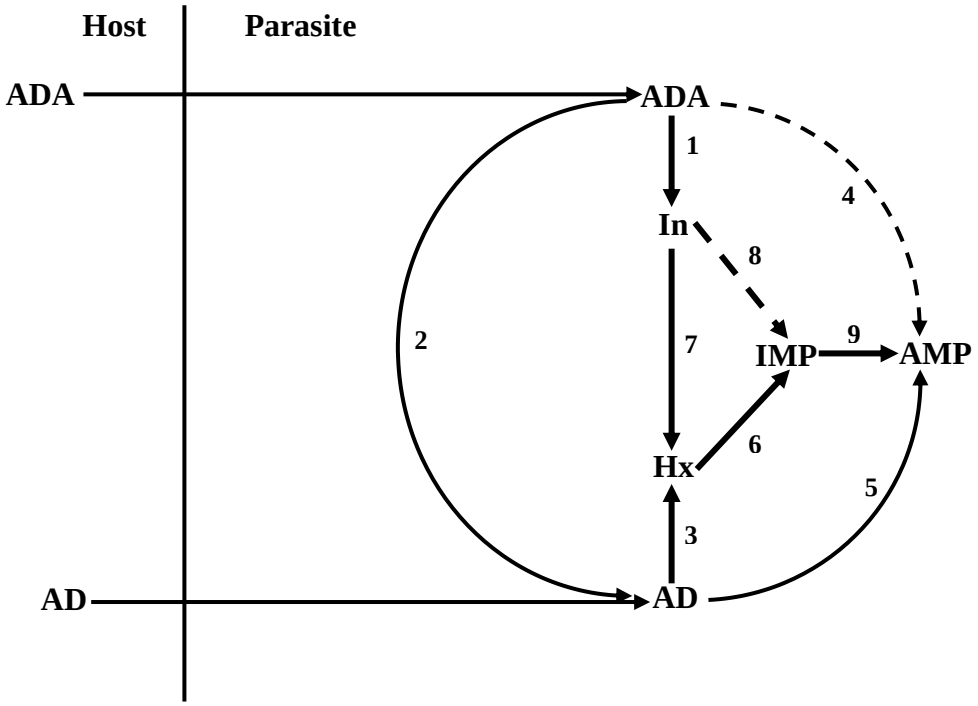

Fig. 1. Adenosine cycle in Dirofilaria immitis

Enzymes: 1, Adenosine deaminase;2, adenosine phosphorylase; ;3, adenine deaminase; 4, adenosine kinase; 5, APRTase; 6,HRRTase; 7, phosphorylase; 8, Inosine kinase; 9, adenylosuccinate synthetase and AMP Lyase.

Abbreviation: AD, adenine; ADA, adenosine; inosine; Hx, hypoxanthine; IMP, inosine monophosphate; AMP, adenosine monophpsphate. Solid lines, represent enzymes that detectable whereas dotted lines indicate enzymes that are not exist.

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ايض الادينوسين في الدودة المتطفلة دايروفيلاريا ايميتس

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الخلاصة

تم دراسة فعالية أنزيمات ايض الادينوسين من مستخلص دودة الدايروفيلاريا ايميتس ووجدت فعالية الأنزيمات التالية: ادينوسين دي امينيز والادينين دي امينيز والادينوسين الفوسفوريليز الهدمية والادينين فوسفور ايبوسيل ترانسفريز بينما افتقرت هذه الديدان إلى أنزيم الادينوسين الفوسفوريليز البنائية وأنزيم الادينوسين كاينيز. توحي النتائج إلى أن المسار الرئيسي لصنع نيوكليوتيدات الادينوسين في الدايروفيلاريا ايميتس هو بفعل أنزيمي الفوسوريليز والفسفور ايبوسيل ترانسفريز .