PURINE METABOLISM IN *TRICHOMONAS VAGINALIS*

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Received 15 March 1982

1. Introduction

The purine metabolism of several genera of parasitic protozoa, is of interest partly due to their sensitivity to purine analogues, and to differences in the enzymes of purine metabolism compared to mammalian systems [1–6]. *Trichomonas vaginalis*, the causative agent of trichomonal vaginitis, a mild, but very common, sexually-transmitted disease, has not been studied in this respect; there is a general lack of knowledge on the biosynthetic ability of the organism. We report here on the ability of washed-cell suspensions of *T. vaginalis* to salvage the purine bases, adenine and guanine, and their nucleosides, and on the absence from the organism of the ability to synthesise purines de novo and to interconvert purine nucleotides. Examination of possible purine salvage enzymes suggests that nucleoside phosphorylase and nucleoside kinase activities are responsible for the conversion of purine bases and nucleosides to nucleoside monophosphates; purine phosphoribosyltransferases are either absent or present at only very low levels.

2. Materials and methods

2.1. *Growth and harvesting of organisms*

Trichomonas vaginalis (Bushby strain), from the Wellcome Research Labs. (Beckenham) was grown in a modified cysteine/peptone/liver infusion/maltose medium [7] at 37°C. Cells were harvested by centrifugation in the late logarithmic phase of growth ($\sim 1 \times 10^6$ cells/ml) and washed twice in phosphate-buffered saline (pH 6.4).

2.2. *Incorporation experiments*

Cells were incubated aseptically at 37°C, initially at $\sim 10^6$ cells/ml in 10 ml of a defined medium, based on that in [8], containing either preformed radiolabelled purines at 80 μM final conc. and 5 μCi/ml, or potential precursors of the purine ring. The precursors used were [U-^{14}C]glycine (200 μM, 5 μCi/ml), Na[^{14}C]bicarbonate (180 μM, 10 μCi/ml), Na[^{14}C]-formate (80 μM, 5 μCi/ml) and [3-^{14}C]serine (200 μM, 0.5 μCi/ml). In each case uracil was added as a pyrimidine source at 80 μM. One millilitre samples were removed at intervals, rapidly mixed with cold 5% (w/v) trichloroacetic acid and treated as in [8]. The radioactivity in the acid-insoluble material (protein, nucleic acid and glycogen) was measured in a toluene-based liquid scintillation system.

2.3. *Cell fractionation*

After incubation, the remaining organisms were washed twice with phosphate-buffered saline and chemically fractionated as in [8]. The nucleic acid fraction was hydrolysed by treatment with 12 N perchloric acid at 100°C for 60 min and the perchlorate removed as the potassium salt at 4°C. The resulting bases were separated by thin-layer chromatography (TLC) on plastic-backed cellulose plates using an isopropanol/HCl (11.6 N)/H_2O (162:42:46, by vol.) solvent system and purine and pyrimidine bases as internal standards. Bases were visualised under ultraviolet light, and radioactivity profiles obtained by cutting the chromatographic strips into 0.5 cm sections and estimating the radioactivity in each section by scintillation counting.

2.4. *Preparation of cell-free extracts*

Washed cells harvested in the late logarithmic phase of growth were routinely resuspended in either 50 mM Tris–HCl (pH 7.6) or 50 mM Hepes (pH 7.4) to $\sim 5 \times 10^7$ cells/ml, broken by two 15 s periods of

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

sonication with a 30 s cooling period and centrifuged at 4°C and 105 000 × *g* for 1 h. The supernatant was used in the following assays after 24 h dialysis against ~500 vol. breakage buffer.

2.5. *Enzyme assays*

The reaction mixtures, in 50 μl total vol., were as follows:

Nucleoside kinases, based on [9]: 50 mM Tris–HCl (pH 7.4); 0.5 mM $MgSO_4$; 0.5 mM ATP; 20 mM KCl; 1.25 mM phosphoenolpyruvate; 10 μg pyruvate kinase; extract; and 0.1 mM radiolabelled nucleosides. Specific activities were 5 mCi/mmol for [8-^{14}C]adenosine and [U-^{14}C]inosine, and 40 mCi/mmol for [8-^{3}H]guanosine.

Nucleoside phosphorylases, synthetic direction: 50 mM Tris–HCl (pH 7.5); 0.5 mM ribose-1-phosphate; extract; and 0.1 mM radiolabelled purine base (10 mCi/mmol, [8-^{14}C]adenine; 100 mCi/mmol, [8-^{3}H]guanine sulphate and [G-^{3}H]hypoxanthine).

Nucleoside phosphorylases, catabolic direction: 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.0); enzyme extract; and 0.1 mM radiolabelled nucleosides (spec. act., as for kinases).

Deaminases: 50 mM Tris–HCl (pH 7.5); enzyme extract; and 0.2 mM [8-^{14}C]adenine or [8-^{14}C]-adenosine (both at 5 mCi/mmol).

Phosphoribosyltransferases, based on [10]: 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.4) or 50 mM Tris–HCl (pH 7.5); 5 mM $MgCl_2$; 1 mM phosphyribosyl pyrophosphate (PRPP); 25 μg bovine serum albumin; extract; and 0.1 mM [^{3}H]purine base (all at 100 mCi/mmol).

Incubations were at 37°C for 5–30 min. Reactions were stopped by heating at 95°C for 4 min and the precipitated protein was removed by centrifugation. Radiolabelled substrates and products in the supernatants were separated by TLC on PEI-cellulose plates containing a fluorescent indicator, with either distilled water (phosphoribosyltransferases only) or *n*-butanol/methanol/water/ammonia (60:20:20:1, by vol.) [9] as solvents. Appropriate purine bases, nucleosides and nucleotides were included as internal standards at ~5 nmol/spot. After development and visualisation under ultraviolet light, the spots were cut out and the radioactivity in each estimated by scintillation counting.

3. Results

The rates of incorporation of preformed purines into the cold trichloracetic acid-insoluble fraction of *T. vaginalis*, at saturating concentrations, are presented in table 1. Incorporation was linear with time for ~6 h. Adenine, guanine and guanosine were utilised at similar rates whilst the rates for adenosine and deoxyadenosine were considerably lower. The fact that the rate of uptake for AMP was not decreased in the presence of NaF, added to inhibit extracellular phosphatase activity, suggests that the nucleotide is taken up intact albeit relatively slowly by the cell. Of particular interest in table 1 is the virtual lack of hypoxanthine salvage by *T. vaginalis*. The very low level was accounted by an impurity in the [^{3}H]hypoxanthine, thought to be [^{3}H]adenine on the basis of its co-chromatography with standard adenine. The purine base of [U-^{14}C]inosine was also not incorporated into the nucleic acids of *T. vaginalis*.

In competition experiments the addition of 80 μM non-labelled guanine to the incubation medium did not affect the uptake of [^{3}H]adenine. Similarly, the presence of 80 μM adenine had no effect on [^{3}H]guanine incorporation. These results suggested that *T. vaginalis* was not capable of interconverting adenine and guanine nucleotides. This was confirmed by chromatography of the hydrolysed nucleic acid of cells incubated with either radiolabelled adenine or guanine, as shown by the results of a typical experiment presented in fig.1. In each case only large single peaks of radioactivity were obtained corresponding to the original labelled substrated. (Similar experiments with single labelled pyrimidine bases or nucleosides resulted

Table 1
Incorporation of purines into the nucleic acid of *T. vaginalis*

Purine	(nmol . 10^6 cells^{-1} . 4 h^{-1})[a]	
[8-^{3}H]Adenine	1.091 ± 0.279	(12)
[8-^{3}H]Guanine	1.047 ± 0.089	(4)
[G-^{3}H]Hypoxanthine	0.017 ± 0.004	(5)
[2-^{3}H]Adenosine	0.346 ± 0.06	(4)
Deoxy [G-^{3}H]adenosine	0.562	(2)
[8-^{3}H]Guanosine	0.947 ± 0.075	(3)
[2-^{3}H]AMP	0.362	(2)
[2-^{3}H]AMP	(0.419)[b]	(2)

[a] Mean ± SD (no. determinations)
[b] Obtained in the presence of 10 mM NaF

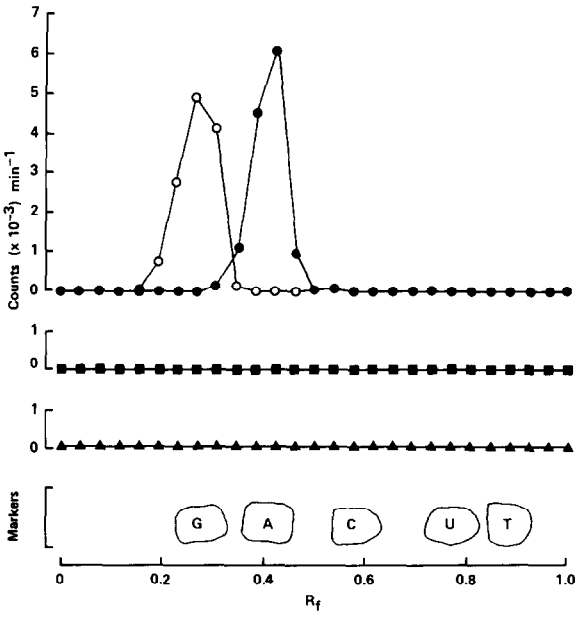

Fig.1. Radioactivity profiles of chromatographed nucleic acid hydrolysates after incubation of *T. vaginalis* with radiolabelled guanine (○), adenine (●), bicarbonate (■) and glycine (▲). Markers: G, guanine; A, adenine; C, cytosine; U, uracil; T, thymine.

in peaks corresponding to both uracil and cytosine.) Fig.1 also shows the total lack of incorporation of two potential purine ring precursors, glycine and bicarbonate, into the nucleic acid bases of the cell. In addition neither [^{14}C]formate nor [^{14}C]serine, both potential, donors of one-carbon units for purine synthesis, were incorporated into purine bases. All 4 precursors were, however, taken up by the cell and used for macromolecular synthesis, as revealed by extensive incorporation of glycine, serine and bicarbonate, and to a lesser extent formate, into the protein fraction, and of serine and bicarbonate into the lipid fraction (not shown).

Analysis of high-speed centrifugal supernatants of *T. vaginalis* revealed an interesting pattern of purine salvage enzymes (table 2). No adenine or hypoxanthine phosphoribosyltransferase activity was detected in either 105 000 × *g* supernatants or pellets despite the use of freshly prepared extracts and the addition of 5 mM Mg^{2+}, 1 mM PRPP and 1 mM dithiothreitol to the extraction buffer. The addition of $MgSO_4$ and PRPP stabilised the purine phosphoribosyltransferase activities of *Leishmania donovani* promastigotes in [11]. We were also unable to detect adenine and adenosine deaminase activities in extracts of *T. vaginalis*.

Table 2
Specific activities of potential purine salvage enzymes in high-speed supernatants of *T. vaginalis*

Enzyme type	Substrate	Activity[a]
Phosphoribosyltransferase	Adenine	n.d.[b]
	Hypoxanthine	n.d.
	Guanine	0.04 ± 0.02 (3)
Kinase	Adenosine	0.52 ± 0.17 (4)
	Inosine	0.13 ± 0.04 (3)
	Guanosine	0.47 ± 0.14 (3)
Phosphorylase (catabolic)	Adenosine	12.1 ± 1.3 (3)
	Inosine	14.3 ± 1.8 (3)
	Guanosine	10.7 ± 2.0 (3)
Phosphorylase (anabolic)	Adenine	26.2 ± 4.3 (4)
	Hypoxanthine	20.7 ± 2.9 (3)
	Guanine	17.6 ± 2.1 (3)
Deaminase	Adenine	n.d.
	Adenosine	n.d.

[a] nmol . min^{-1} . mg protein^{-1} ± SD (no determinations)
[b] n.d., not detected

The limits of detection for the phosphoribosyltransferase and deaminase assays were both ~0.02 nmol . min^{-1} . mg protein^{-1}. Very low levels of guanine phosphoribosyltransferase were detected.

The absence of adenine phosphoribosyltransferase and the low level of the guanine enzyme, implies that the main pathways by which the bases are converted to their respective mononucleotides are via the nucleosides. Purine nucleoside phosphorylase activities for adenosine, guanosine and inosine, capable of acting in both the catabolic and anabolic directions, were detected at relatively high specific activities (table 2). The presence of nucleoside hydrolase activity cannot be excluded, but when the conversion of adenosine to adenine was assayed in the absence of added phosphate, using dialysed extracts, the activity decreased to 0.9 nmol . min^{-1} . mg protein^{-1} (~7% of the control value), suggesting that the majority of the activity is phosphate-dependent. The presence of nucleoside phosphorylase activity was confirmed by the ribose-1-phosphate-dependent formation of nucleosides from the purine bases. Purine nucleoside kinase enzymes, capable of converting adenosine, guanosine and inosine to their corresponding mononucleotides were also detected. These activities, although low, are high enough to account for the rates of purine salvage pre-

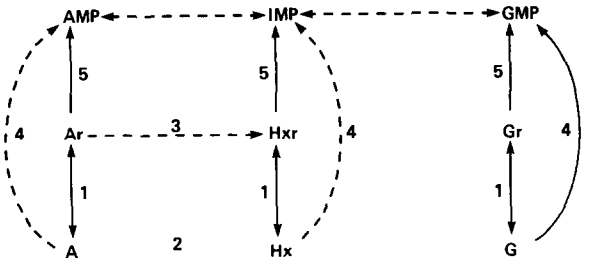

Fig.2. Purine salvage pathways of *T. vaginalis*: (——→) pathways present; (- - -→) pathways apparently absent. Enzymes are: (1) purine nucleoside phosphorylase; (2) adenine deaminase; (3) adenosine deaminase; (4) purine phosphoribosyltransferase; (5) purine nucleoside kinase. A, adenine; Ar, adenosine; Hx, hypoxanthine; Hxr, inosine; G, guanine; Gr, guanosine.

sented in table 1. The pathways of purine salvage present in *T. vaginalis* are shown in fig.2.

4. Discussion

The inability of *T. vaginalis* to incorporate 4 potential purine precursors into nucleic acid bases in the absence of added purines is evidence that this organism, in common with the majority of parasitic protozoa [1], is unable to synthesise the purine ring. Analysis of other chemical fractions clearly indicated that the 4 precursors were being metabolised by the cell in significant amounts, and therefore that the normal biosynthetic processes of the cell were continuing during incubation in the defined medium. Thus the requirement by *T. vaginalis* for preformed purines, as indicated with defined growth medium [12], has now been substantiated by metabolic studies.

In the absence of purine synthesis, the ability to salvage preformed purines is clearly essential. The data in table 1 indicate that *T. vaginalis* can efficiently salvage adenine and guanine bases and their nucleosides. AMP was also taken up by the cell, possibly prior to cleavage by phosphatases. In many cells, including the parasitic protozoa, either unable, or with only a limited capacity to synthesise the purine ring, the phosphoribosyltransferase enzymes appear to play a leading role in purine salvage [3,10,11,13]. The absence of adenine and hypoxanthine phosphoribosyltransferases and the very low level of the guanine enzyme in *Trichomonas* is thus unusual and would appear to place a dependence on nucleoside phosphorylase, acting in the anabolic direction, and nucleoside kinase, both of which were detected in *T. vaginalis* extracts.

Purine nucleoside kinases have been reported in several other genera of parasitic protozoa including *Trypanosoma cruzi* [3], *Plasmodium chabavdi* [9] and *L. donovani* [14]. Adenosine, but not inosine phosphorylase has been detected in extracts of *T. cruzi* [3] and also in *L. donovani* [14], whilst in *T. cruzi* [3], *Crithidia fasciculata* [15], *Trypanosoma gambiense* [16] and *L. donovani* [17] purine nucleoside hydrolases of varying specificities have been reported.

The lack of interconversion between purine mononucleotides in *T. vaginalis* (fig.1) is also an unusual and important finding. Most cells, including the parasitic protozoa, are able to interconvert AMP and GMP via IMP. However, *Tetrahymena pyriformis*, a free-living ciliate, can form AMP from GMP but not vice versa, and thus has an absolute requirement for preformed guanine or guanosine [18]. The apparent absence of purine interconverting enzymes from *T. vaginalis* implies that the organism has an absolute requirement for both adenine and guanine or their nucleosides, and also explains the non-incorporation of hypoxanthine and the purine ring of inosine, into guanine and adenine nucleotides. The presence of inosine kinase and phosphorylase activities may indicate that inosine is an alternative substrate of the kinase and phosphorylase enzymes involved in adenosine and guanosine metabolism, rather than that there are specific enzymes for inosine. The lack of hypoxanthine and inosine utilisation contrasts markedly with the situation in *Plasmodium* [10] and *Babesia* [19] for example, for which hypoxanthine is apparently the major salvage purine.

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