

OROTATE PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE-5'-PHOSPHATE DECARBOXYLASE IN TWO PARASITIC KINETOPLASTID FLAGELLATES

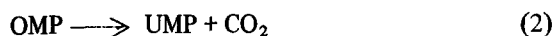
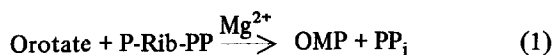
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1. Introduction

The pyrimidine biosynthetic enzymes, orotate phosphoribosyltransferase (OPRTase; EC 2.3.2.10) and orotidine-5'-phosphate decarboxylase (ODCase; EC 4.1.1.23) catalyse the last two steps, reactions (1) and (2), respectively, of the de novo pyrimidine pathway:



These enzymes have been found to be soluble in all organisms studied [1–3]. Here we report that the enzymes present in the human pathogen, *Leishmania mexicana mexicana*, and in a related flagellate protozoan, *Crithidia fasciculata*, are particulate and possibly associated with the mitochondrial membrane. In addition, the activity in the obligate intracellular form of *L. m. mexicana*, the amastigote, which is the stage parasitic to man, is ~40-times lower than that in the promastigote, the form characteristically present in the sandfly vector of the disease. We have found also that the preceding enzyme, dihydroorotate dehydrogenase (DHO-DHase, EC 1.3.3.1), occurs in an unusual form in these parasites.

2. Materials and methods

2.1. Isolation, cultivation and fractionation of cells

Leishmania m. mexicana amastigotes were isolated

Abbreviations: OMP, orotidine-5'-phosphate; UMP, uridine-5'-phosphate; P-Rib-PP, 5-phosphoribosyl-1-pyrophosphate

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from cutaneous lesions in NIH mice by a method, involving Saponin lysis of host cells, anion exchange chromatography and isopycnic centrifugation, which will be detailed in [4]. The purified amastigotes were viable and essentially free from host cell and acellular contamination. Promastigotes of *L. m. mexicana* were grown in vitro in HOMEM medium [5] with 10% (v/v) heat-inactivated foetal calf serum at 26°C. *Crithidia fasciculata* (Anopheles strain, ATCC 11745) was maintained in vitro as in [6]. Crude homogenates of cells in 0.25 M sucrose were obtained by 3 cycles of freezing (–180°C) and thawing (30°C) and fractionated by centrifugation at 100 000 × g for 1 h. To investigate the subcellular localisation of enzymes, *L. m. mexicana* promastigotes were gently lysed by mixing with acid-washed alumina (Sigma type 305) and fractionated by differential centrifugation at 2100 × g for 10 min, 15 800 × g for 10 min and 240 000 × g for 1 h. The 4 fractions produced were characterised as in [7].

2.2. Assay of OPRTase, ODCase and DHO-DHase

The sequential activity of OPRTase and ODCase was assayed by the method in [8] and DHO-DHase as in [9]. Protein concentrations were measured by the Lowry method [10] using bovine serum albumin as standard. Enzyme activities for these enzymes were measured at 30°C and expressed as nmol orotate or L-dihydroorotate converted to CO₂ · h^{–1} · mg protein^{–1}. The effect of pH (range 6.0–10.0) on reaction velocity was determined in 20 mM Tris–maleate–glycine buffer [11].

3. Results

The activities of OPRTase/ODCase in the crude homogenates of *L. m. mexicana* amastigotes and

Table 1
OPRTase/ODCase activity in *Leishmania m. mexicana* and *Crithidia fasciculata*

	<i>Leishmania m. mexicana</i>		<i>Crithidia fasciculata</i>
	Amastigote	Promastigote	
Homogenate	1.9 ± 0.4 ^a	81.8 ± 9.1	35.5 ± 1.5
Soluble fraction	14 ^b	6	<1
Particulate fraction	86 ^b	94	>99
Recovery %	65	92	96
pH optimum	8.5	7.8	nd

^a Standard deviation; ^b activity in fraction given as a percentage of the total activity recovered in both fractions

n.d., not done; The data presented is from ≥2 expt

promastigotes and of *C. fasciculata* and the activities recovered in the various cell fractions are shown in table 1. In each case the enzyme activity was found to be associated with the 100 000 × g pellet, indicating the particulate nature of the enzymes. Differ-

ences between the amastigote and promastigote activities were observed, that in the promastigote homogenate being ~40-fold higher and with a lower pH optimum. The OPRTase/ODCase activity in *L. m. mexicana* promastigotes was shown to be associated with the small organelle fraction (table 2), which contains most of the mitochondrial segments, together with organelles in which many glycolytic enzymes are found [7]. In contrast, DHO-DHase activity was shown to be cytosolic (table 2).

4. Discussion

The subcellular localisation and nature of OPRTase/ODCase in *L. m. mexicana* and *C. fasciculata* are very different from those of the enzymes in mammalian cells and the sporozoan parasites *Babesia hyalomysci* and *Plasmodium berghei* (table 3) in that they appear to be membrane bound and located in the small organelle fraction of the organisms. The much lower enzyme activity in the amastigotes of *L. m. mexicana* suggests that there are differences in the importance

Table 2
Subcellular localisation of enzymes in *L. m. mexicana* promastigotes

Activity in fractions ^a :	OPRTase/ ODCase	DHO- DHase	Phosphofructo- kinase ^b	Pyruvate kinase ^b	Succinic dehydrogenase ^b
P ₁ , nuclear fraction	10	1	12	1	29
P ₂ , small organelle fraction	82	3	65	4	38
P ₃ , microsomal fraction	7	1	17	<1	29
S, cytosolic fraction	<1	95	6	94	<4

^a Percentage of recovered activity; ^b data from [7]; ^c data from ≥2 expt

Table 3
OPRTase/ODCase and DHO-DHase in several parasitic protozoa

	<i>Leishmania m. mexicana</i>		<i>Crithidia fasciculata</i>	<i>Plasmodium berghei</i>	<i>Babesia hyalomysci</i>	Human spleen
	Amastigotes	Promastigotes				
OPRTase/ODCase						
Spec. act.	1.9	82	36	96 ^a	5.3 ^c	1200 ^d
Location	Particulate	Particulate	Particulate	Soluble	Soluble	Soluble
DHO-DHase						
Spec. act.	8.8	8.7	8.3	13 ^b	17 ^c	4 ^d
Location	Cytosolic	Cytosolic	Cytosolic	Particulate	Particulate	Particulate

^{a-d} Data are from [17], [13], [18] and [19], respectively

of the pyrimidine de novo biosynthetic pathway at different stages of the life cycle of the parasite. The location in *L. m. mexicana* of the preceding enzyme, DHO-DHase, is also unusual in that it is cytosolic. A soluble DHO-DHase has been reported in other kinetoplastida [12,13], however in all other eukaryotes and the majority of prokaryotes investigated, this enzyme has been found to be membrane bound [14,15]. A partial characterisation of DHO-DHase from *L. m. mexicana* promastigotes and amastigotes with respect to substrate specificity, coenzyme requirement, mechanism and sensitivity to inhibitors is in [16]. The qualitative differences found between the last 3 enzymes of the pyrimidine de novo biosynthetic pathway in *L. m. mexicana* and mammalian cells may make this area of metabolism a potentially useful target at which to aim in the search for much needed new antileishmanial drugs.

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