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

A. M. GERO* and G. H. COOMBS
Department of Zoology, University of Glasgow, Glasgow, G12 8QQ, Scotland

Received 26 June 1980

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:

$$\text{Orotate} + \text{P-Rib-PP} \xrightarrow{Mg^{2+}} \text{OMP} + PP_i \quad (1)$$

$$\text{OMP} \longrightarrow \text{UMP} + CO_2 \quad (2)$$

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 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 $CO_2 . h^{-1} . mg\ protein^{-1}$. 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

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

* Present address: School of Biochemistry, University of New South Wales, Kensington, NSW, Australia

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

	Leishmania m. mexicana		*Crithidia fasciculata*
	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. Differences 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 hylomysci* 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

	Leishmania m. mexicana		*Crithidia fasciculata*	*Plasmodium berghei*	*Babesia hylomysci*	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.

Acknowledgements

We thank M. Y. Ghafoor, J. A. Craft and D. T. Hart for assistance with parts of this work and the Wellcome Trust for financial support.

References

[1] Kelley, W. N. and Smith, L. H. jr (1978) in: The metabolic basis of inherited disease (Stanbury, J. B. et al. eds) 4th edn, pp. 1045–1071, McGraw-Hill, New York.
[2] Jones, M. E. (1971) Adv. Enz. Reg. 9, 19–49.
[3] O'Sullivan, W. J. and Ketley, K. (1980) Ann. Trop. Med. Parasitol. 74, 109–114.
[4] Hart, D. T., Vickerman, K. and Coombs, G. H. (1980) submitted.
[5] Berens, R. L., Brun, R. and Krassner, S. M. (1976) Parasitology 62, 360–365.
[6] Coombs, G. H. and Gutteridge, W. E. (1975) J. Protozool. 22, 555–560.
[7] Hart, D. T., Ghafoor, M. Y., Craft, J. A. and Coombs, G. H. (1980) Parasitology in press.
[8] Fox, R. M., Wood, M. H. and O'Sullivan, W. J. (1971) J. Clin. Invest. 50, 1050–1060.
[9] Smithers, G. W., Gero, A. M. and O'Sullivan, W. J. (1978) Anal. Biochem. 88, 93–103.
[10] Lowry, O. H., Rosebrough, N.-J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Perrin, D. D. and Dempsey, B. (1974) in: Buffers for pH and metal ion control, pp. 137–149, Chapman and Hall Ltd, London.
[12] Kidder, G. W. and Nolan, L. L. (1973) Biochem. Biophys. Res. Commun. 53, 929–936.
[13] Gutteridge, W. E., Dave, D. and Richards, W. H. G. (1979) Biochim. Biophys. Acta 582, 390–401.
[14] Taylor, W. H. and Taylor, M. L. (1964) J. Bacteriol. 88, 105–110.
[15] Chen, J.-J. and Jones, M. E. (1976) Arch. Biochem. Biophys. 176, 82–90.
[16] Gero, A. M. and Coombs, G. H. (1980) Parasitology, in press.
[17] Hill, B., Kilsby, J. and Ginger, C. D. (1979) J. Protozool. 26, 74A.
[18] Gero, A. M. and Coombs, G. H. (1980) J. Protozool. in press.
[19] Gero, A. M. and O'Sullivan, W. J. (1979) unpublished.