A NOVEL LOCATION FOR TWO ENZYMES OF DE NOVO PYRIMIDINE BIOSYNTHESIS IN TRYPANOSOMES AND *LEISHMANIA*

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1. Introduction

African trypanosomes such as *Trypanosoma brucei* are the causative agents for sleeping sickness in man and nagana in cattle, *Trypanosoma cruzi* for Chagas' disease, and species of *Leishmania* for oriental sore and Kala-azar. These important protozoan parasites of man are members of the order Kinetoplastida. This order is characterised by the presence of an atypical organelle rich in DNA, the kinetoplast. Another atypical organelle, the microbody-like glycosome, which contains glycolytic enzymes, has been found in *T. brucei* [1–3] and is probably present in all other members of the Kinetoplastida [4,5]. A common feature now emerging [6–8] is that some of the enzymes of de novo pyrimidine biosynthesis show a subcellular distribution distinct from that observed in other species (review [9]). The first 3 enzymes of this pathway (carbamoylphosphate synthetase, EC 6.3.4.16; aspartate carbamoyltransferase, EC 2.1.3.2; dihydroorotase, EC 3.5.2.3) in the Kinetoplastida, as in other cells, are all soluble. In contrast, the fourth enzyme dihydroorotate dehydrogenase (EC 1.3.3.1) is usually particulate, mitochondrial and intimately connected to the respiratory chain; in the Kinetoplastida it is soluble and is possibly a hydroxylase [10]. The last 2 enzymes of this pathway, orotate phosphoribosyltransferase (EC 2.4.2.10, OPRTase) and orotidine-5′-phosphate decarboxylase (EC 4.1.1.23, ODCase), which are soluble in other cells, are particulate in the Kinetoplastida. Here, we show that OPRTase and ODCase co-purify in isopycnic sucrose gradients with glycosomal enzymes, suggesting they are associated with microbody-like organelles, probably the glycosomes themselves.

2. Materials and methods

Growth, isolation and purification of the bloodstream trypomastigote forms of *T. brucei*, stock 427, was done as in [11]. Epimastigote forms of the Sonya strain of *T. cruzi* were cultured as in [8]. Promastigote forms of *Leishmania mexicana amazonensis* (LV 78) were grown in a modified LIT medium, to be reported subsequently. Organisms were disrupted by grinding with silicon carbide according to [12], except that a homogenization buffer containing 0.25 M sucrose, 3 mM imidazole–HCl and 1 mM EGTA at pH 7.0 was used. Enzymes activities were determined by the methods in [1,8,13] and the results were calculated and presented by methods reviewed in [14].

3. Results

Table 1 shows that the highest relative specific activities for OPRTase of *T. brucei* after differential centrifugation are in the large and small granule fractions. Very little activity is found in the supernatant. This demonstrates the particulate nature of this enzyme from *T. brucei*. Post large-granule extracts were subsequently fractionated by isopycnic centrifugation in sucrose; fig.1 shows that the distribution frequencies of OPRTase and the glycosomal enzyme, hexokinase, after centrifugation in isopycnic sucrose gradients are very similar. The highest activities for both are found at 1.25 g/cm^3. All other enzymes assayed to identify other organelles [13] give different profiles (fig.1). Using similar techniques, OPRTase

from the epimastigote form of *T. cruzi* and the promastigote form of *L. m. amazonensis* show also highest activities at ~1.25 g/cm^3. In these studies, ODCase always co-purified with OPRTase. This is in agreement with the generally accepted idea that these 2 enzymes are catalysed by a bifunctional protein, or are both part of a stable, multi-enzyme complex [9].

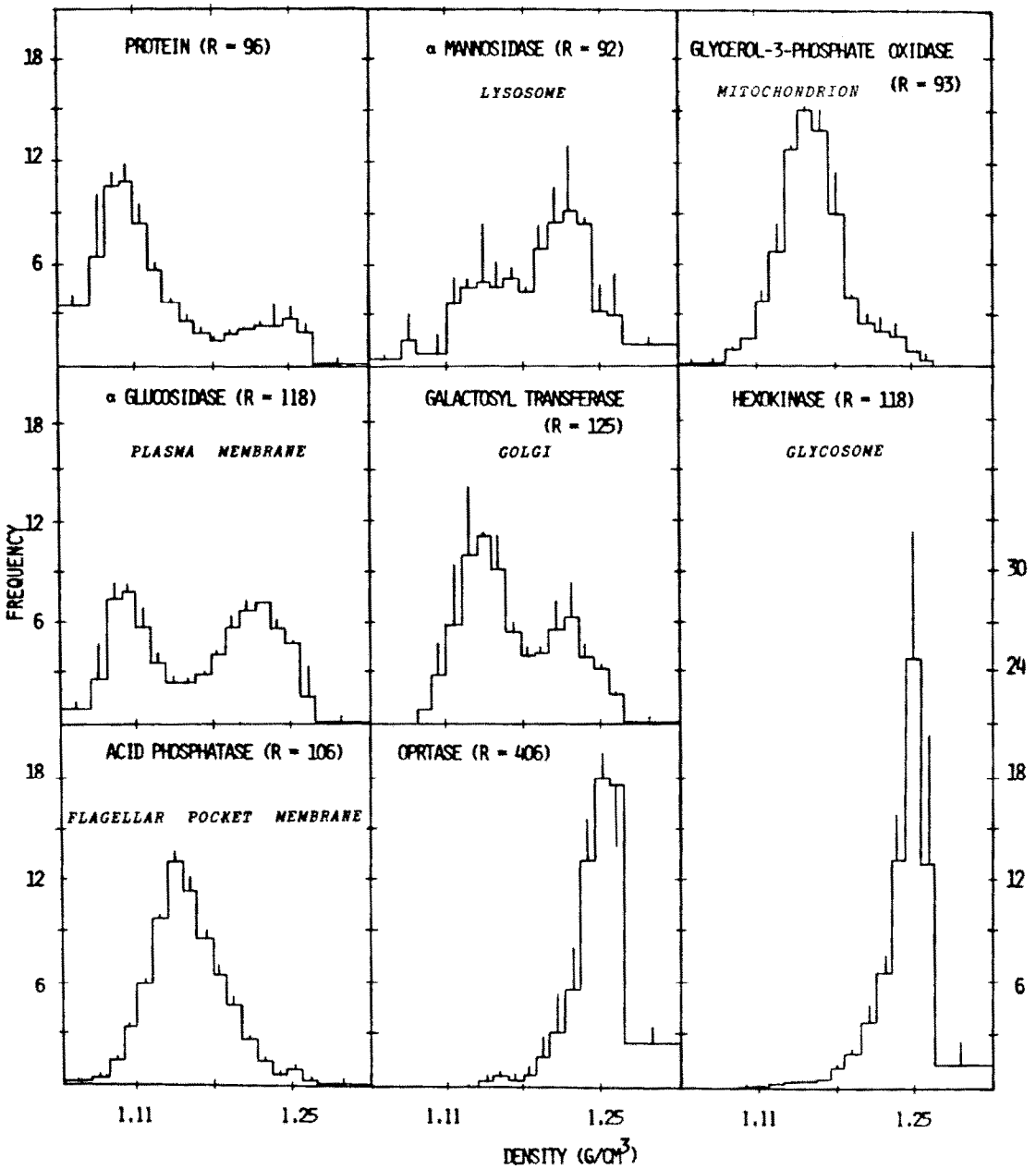

Fig.1. Distribution of *T. brucei* enzymes after isopycnic centrifugation of a post-large granule extract. This was performed in a linear sucrose gradient using the E-40 automatic zonal rotor in [14]. The loading of the rotor, centrifugation and collection of the gradient fractions were done as in [13]. The density of each fraction was calculated from its refractive index. The fractions were diluted 2-fold with 0.15 M NaCl and 0.2% (w/v) Triton X-100 and stored in liquid nitrogen until analysis. The results of 2 experiments were treated and averaged as in [13]. Recoveries of the respective enzymes and protein are given in brackets.

Table 1
Fractionation of *T. brucei* by differential centrifugation

Enzyme		Specific activity[a]	Distribution in fractions					Recovery (%)
			N	LG	SG	M	S	
Protein		117[b]	34	5	6	11	44	91
OPRTase	[c]	0.19	43	16	29	8	4	117
	[d]		(1.3)	(3.2)	(4.8)	(0.7)	(0.09)	
Hexokinase	[c]	628	30	17	35	17	1	108
	[d]		(0.9)	(3.4)	(5.8)	(1.5)	(0.02)	

[a] nmol . min^{-1} . mg protein^{-1}; [b] total protein mg; [c] percentage; [d] (relative specific activity)

Fractionation was done as in [13]. Specific activities are based on the sum of the activities in the nuclear fraction and the cytoplasmic extract. Distributions are expressed as percentages of the sum of the 5 fractions obtained and the relative specific activity as the percentage of total activity/percentage of total protein in each fraction. Recoveries represent ratios of the sum of the activities in the fractions over the total for nuclear fraction + cytoplasmic extract expressed as a percentage. The fractions are nuclear (N, 1000 × *g* pellet), large granule (LG, 5000 × *g* pellet), small granule (SG, 14 500 × *g* pellet), microsomal (M, 139 000 × *g* pellet) fraction and the final supernatant (S)

4. Discussion

These results demonstrate that in isopycnic sucrose gradients, the OPRTase and ODCase activities of 3 representative members of the Kinetoplastida show densities the same as those of early glycolytic enzymes. The latter are known to occur in microbody-like organelles, termed glycosomes. Our observation explains the particulate nature of these terminal pyrimidine biosynthetic enzymes and strongly suggests that these enzymes are also associated with microbodies, probably the glycosomes themselves. Thus it seems likely that the function of this organelle is not limited to glycolysis. Further studies may reveal an even more diverse role for it than has been envisaged.

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