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Review

Aromatic amino acid catabolism in trypanosomatids ☆

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Abstract

Trypanosomatids cause important human diseases, like sleeping sickness, Chagas disease, and the leishmaniases. Unlike in the mammalian host, the metabolism of aromatic amino acids is a very simple pathway in these parasites. *Trypanosoma brucei* and *Trypanosoma cruzi* transaminate the three aromatic amino acids, the resulting 2-oxo acids being reduced to the corresponding lactate derivatives and excreted. In *T. cruzi*, two enzymes are involved in this process: a tyrosine aminotransferase (TAT), which despite a high sequence similarity with the mammalian enzyme, has a different substrate specificity; and an aromatic L-2-hydroxyacid dehydrogenase (AHADH), which belongs to the subfamily of the cytosolic malate dehydrogenases (MDHs), yet has no MDH activity. In *T. cruzi* AHADH the substitution of Ala102 for Arg enables AHADH to reduce oxaloacetate. In the members of the 2-hydroxyacid dehydrogenases family, the residue at this position is known to be responsible for substrate specificity. *T. cruzi* does not possess a cytosolic MDH but contains a mitochondrial and a glycosomal MDH; by contrast *T. brucei* and *Leishmania* spp. possess a cytosolic MDH in addition to glycosomal and mitochondrial isozymes. Although *Leishmania mexicana* also transaminates aromatic amino acids through a broad specificity aminotransferase, the latter presents low sequence similarity with TATs, and this parasite does not seem to have an enzyme equivalent to *T. cruzi* AHADH. Therefore, these closely related primitive eukaryotes have developed aromatic amino acid catabolism systems using different enzymes and probably for different metabolic purposes.

Keywords: Aromatic amino acid metabolism; *Trypanosoma cruzi*; *Trypanosoma brucei*; *Leishmania* spp.; Malate dehydrogenases; Glutamate dehydrogenases; Aromatic aminotransferases; Aromatic lactate derivatives; Aromatic L-2-hydroxyacid dehydrogenase

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1. Introduction

Parasitic protozoa infect hundreds of millions of people every year throughout the World, and are the causative agents of some of the most neglected human diseases. The order Kinetoplastida includes the genera *Trypanosoma* and *Leishmania*. Trypanosomes infect most vertebrate genera. Among them, two species are the agents of important human diseases: *Trypanosoma cruzi* and *Trypanosoma brucei*. The former is the agent of the American Trypanosomiasis, Chagas disease. The latter includes sub-species, such as *Trypanosoma brucei gambiense*, which causes a chronic form of sleeping sickness in west and central Africa; *Trypanosoma brucei rhodesiense*, causing an acute form of the disease in east and southern Africa, and *Trypanosoma brucei brucei*, the agent of the cattle disease nagana (Barrett et al., 2003). *Leishmania* include a total of about 21 species, causing different clinical syndromes, all of them denominated leishmaniasis. These comprise visceral leishmaniasis (kala-azar), which can be fatal; cutaneous leishmaniasis, causing chronic skin ulcers; and mucocutaneous leishmaniasis (espundia) which is grossly disfigurating (Herwaldt,1999; Awasthi et al., 2004).

All these pathogenic protozoa have complex biological cycles, involving in all cases a haematophagous insect vector (triatomine bugs, such as *Triatoma infestans*, for *T. cruzi*; tsetse flies of the genus *Glossina*, for *T. brucei*, and sandflies, of the genera *Lutzomyia* and *Phlebotomus*, for *Leishmania* spp.) in addition to a mammalian host. In the case of *T. cruzi* the cycle involves two replicative forms, the epimastigote in the insect gut and the intracellular amastigote in the mammal, and two non-replicative forms, the metacyclic trypomastigote (the natural infective form) in the insect and the bloodstream trypomastigote in the infected mammal (Barrett et al., 2003). The sub-species of *T. brucei* have also complex cycles, involving only different trypomastigote forms and epimastigotes, but they do not present an intracellular stage (Barrett et al., 2003). *Leishmania* spp. have two major developmental stages, the promastigote in the vector and the intracellular amastigote, which replicates inside macrophages. In addition, the natural infective form is the metacyclic promastigote (Awasthi et al., 2004).

2. Amino acid metabolism

All these parasites spend part of their life cycle in the gut of a haematophagous insect, where the nutrients available are essentially proteins and amino acids originated from the blood meal, since the small amounts of carbohydrate present are likely to be consumed very soon. This means that all of them must have catabolic pathways connected to an (at least partially) operative Krebs cycle and respiratory chain, allowing the utilization of amino acids as fuel. When these organisms infect the mammal, in the case of *T. brucei* a dramatic change in metabolism occurs, the slender bloodstream trypomastigotes being entirely dependent on glucose to obtain energy (Besteiro et al., 2005; van Hellemond et al., 2005). In the case of both, *T. cruzi* and *Leishmania* spp., since the major form in the mammal is the intracellular amastigote, less dramatic changes in metabolism are expected. Unlike *T. brucei*, *T. cruzi* and *Leishmania* spp. seem to be able to oxidize varied substrates, such as amino acids and fatty acids during their whole life cycle (Hart and Coombs, 1982). Due to the difficulties for the large scale production of homogeneous mammalian stage-specific cell populations of *Leishmania* spp. and *T. cruzi*, most of our knowledge regarding the functionality of metabolic routes in the mammalian stages derives from detailed studies performed in *T. brucei* (Besteiro et al., 2005; van Hellemond et al., 2005). Most of the life cycle stages of these pathogenic Trypanosomatids, with the notorious exception of the slender trypomastigotes of *T. brucei*, are able to catabolize amino acids. Both, *T. cruzi* epimastigotes and *Leishmania* spp. promastigotes, have a considerable intracellular pool of free amino acids, which, in addition to being used for the synthesis of proteins or some derived substances, and for energy production, are involved in osmoregulation (Rohloff et al., 2003; Blum, 1994). Proline, being an abundant amino acid in insects, is prominent among oxidizable substrates, both in *T. cruzi* epimastigotes (Sylvester and Krassner, 1976; Silber et al., 2005) and in *T. brucei* procyclic trypomastigotes; in the latter case, it has been recently demonstrated that its uptake and catabolism are down-regulated in the presence of glucose (Lamour et al., 2005). Despite the probable relevance of amino acid catabolism for Trypanosomatids, little is known about the fate of their carbon chains. Degradation of L-threonine to acetyl-CoA by *T. brucei* (Cross et al., 1975) and production of sterols and fatty acids utilizing L-leucine as carbon source in *Leishmania* spp. (Ginger et al., 2001) are exceptions in our knowledge of the fate of the amino acid carbon chains. The major enzymes involved in the exchange and disposal of amino nitrogen are the aminotransferases, many of which transfer the amino group to 2-oxoglutarate to give L-glutamate. The latter represents the substrate of the glutamate dehydrogenases (GDHs), which liberate the amino group as NH_3. These enzymes have been detected in all three parasites, and some of them have been subjected to rather detailed studies. *T. cruzi* epimastigotes (Juan et al., 1978; Walter and Ebert, 1979; Barderi et al., 1998), as well as *Leishmania mexicana* promastigotes and amastigotes (Mottram and Coombs, 1985) contain two different GDHs, one NADP-linked and one NAD-linked. The latter enzymes very likely correspond to the *L. major* counterparts identified in the genome project with the systematic names: *LmjF28.2910* (accession number: CAJ05749) potentially the NADP-linked enzyme and *LmjF15.1010* (accession number: CAJ03327) which might represent the NAD dependent enzyme, probably localized in the mitochondrion. These parasites, therefore, resemble bacteria, fungi and plants in this respect, and clearly differ from higher animals, which possess only one, coenzyme-unspecific, GDH. However, *T. brucei* appears to be the exception among these pathogenic protozoa since only a single gene encoding a protein with high sequence similarity with *LmjF28.2910* (accession number: CAJ05749) could be identified in the *T. brucei* genome (*Tb09.160.4310*, accession number: EAN76644). The functionality of *T. brucei* GDH has not been proved yet, and the leishmanial enzymes have been