Disruption of Sulphur Amino Acid Pathway Affects Metabolism in *Trypanosoma cruzi*

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Keywords: *Trypanosoma cruzi*, metabolomics, sulphur amino acids.

Chagas disease poses a significant public health challenge across the Americas, particularly affecting economically disadvantaged populations. The causative agent, *Trypanosoma cruzi*, utilises amino acids as a major carbon source and for essential biological processes, including differentiation, stress response, and host-cell invasion. While Cys can contribute to energy metabolism through its conversion to pyruvate, its primary role is likely linked to redox metabolism, serving as a main source of thiol (–SH) groups for sulphur-containing molecules such as glutathione, trypanothione, and ovothiol—all essential for resistance to oxidative stress. Methionine (Met) metabolism in *T. cruzi* has been less studied than that of Cys. There is no evidence of an active Met biosynthetic pathway in the parasite; thus, Met is presumed to be taken up from the extracellular environment. In addition to its role in protein synthesis, Met participates in redox balance and methylation processes via the formation of S-adenosylmethionine (SAM), the principal methyl donor in most organisms. Cys biosynthesis in *T. cruzi* can occur through either the trans-sulphuration or the *de novo* pathways. The *de novo* pathway synthesises Cys from serine (Ser) in two steps using H_2S as the sulphur source. The trans-sulphuration pathway involves the formation of cystathionine from Ser and homocysteine (HCys). Once formed, cystathionine is subsequently converted to Cys. HCys, in turn, is derived from Met through a three-step process: i. Met is converted to SAM by **S-adenosylmethionine synthetase** (**AMS**); ii. SAM donates a methyl group in various methylation reactions, yielding S-adenosylhomocysteine (SAH) as a byproduct; iii. SAH is hydrolysed by **S-adenosylhomocysteine hydrolase** (**AHCH**) to produce HCys, which enters the trans-sulphuration pathway. This study aimed to elucidate the metabolic roles of sulphur amino acids by generating *T. cruzi* knockouts of genes involved in Met catabolism using CRISPR/Cas9 technology in a strain constitutively expressing Cas9 (parental lineage). We successfully obtained partial knockouts for both AMS and AHCH. To assess metabolic changes resulting from these gene deletions, we performed exometabolomic profiling using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Epimastigotes were subjected to nutritional stress by overnight incubation in PBS and then exposed to individual carbon sources (5 mM glucose, Cys, Met, Ser, or PBS as a control) for 6 hours at 28°C. Supernatants were subsequently analysed by ^{1}H-NMR. The analysis revealed significant alterations in acetate excretion in both knockout strains compared to the parental lineage. In the PBS control, both knockout strains exhibited significant increases in acetate excretion, compared to the parental lineage (42% for AMS and 79% for AHCH). Incubation with glucose further amplified this difference, nearly doubling acetate excretion in the mutants. Incubation with Met mirrored the nutritional stress condition (PBS), resulting in similar increments (43% and 78%, respectively). Conversely, supplementation with cysteine or serine led to a marked reduction in acetate excretion (70–85%) relative to the parental lineage under the same conditions. Overall, this approach demonstrated that disrupting sulphur amino acid metabolism reshapes the excreted metabolic profile of *T. cruzi*. These findings highlight the tight integration of sulphur metabolism with redox balance and central metabolic pathways, emphasising these processes as promising targets for new therapeutic strategies.

Acknowledgements: This project was founded by FAPESP (grant N° 2021/12938-0 awarded to AMS, fellowship N° 2022/14959-7 awarded to MSB). This project was partially executed at the Laboratório Nacional de Biociências (LNBio) - Centro Nacional de Pesquisa em Energia e Materiais (CNPEM).