The importance of glutamine for *T. cruzi* survive throughout the life cycle

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Introduction

Trypanosoma cruzi is the causative agent of American trypanosomiasis, also known as Chagas disease. *T. cruzi* life cycle alternates between the mammalian hosts, among them the humans and the insect vector, a reduviid insect. Inside the both host *T. cruzi* differentiate into five mainly stages: amastigote, intracellular epimastigote and trypomastigote in the mammalian host and epimastigote and metacyclic trypomastigote in the insect vector (**Fig. 1**). Thus, *T. cruzi* needs to colonize different environments, such as, the bloodstream and cells cytoplasm in the mammalian host, and the intestine in the insect vector.
In our work we demonstrate the important role of Glutamine (Gln) in the parasite survival in the different environments during their life cycle.

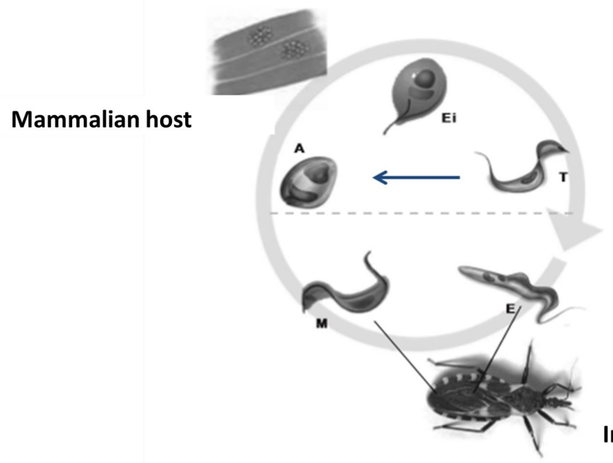


Figure 1- *T. cruzi* life cycle. The life cycle of *T. cruzi* alternates between the mammalian host and the insect vector.
A (amastigote)
iE (intracellular epimastigote),
T (trypomastigote)
E (epimastigote)
M (metacyclic trypomastigote).
(Adapted from: Paes et al. 2011)[1].

Results

Gln uptake and Gln biosynthesis

T. cruzi is able to uptake Gln from the external medium[(2)] and also can biosynthesize it[(3)], both systems act as complementary Gln sources during the life cycle of the parasite.

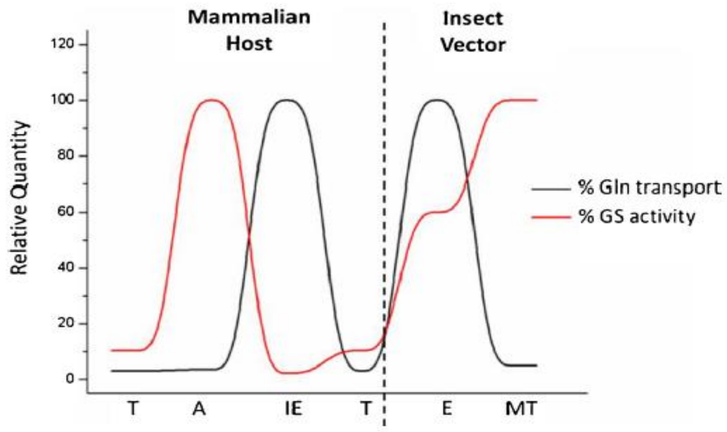


Figure 2- Gln uptake and Gln biosynthesis. Comparasion between Gln uptake and Gln synthetase activity during the life cycle of *T. cruzi*. 100% of transport or GS activity was attributed to the stage that showed the transport or GS maximum activity. The proportion of these activities with respect to the maximum was calculated for the others stages (Crispim et al. 2018; Damasceno et al. 2018).

Gln as energy source

Gln is completely oxidized to CO_2 and feed the electron transport chain, contributing to ATP biosynthesis and maintain the intracellular ATP level in epimastigote forms.

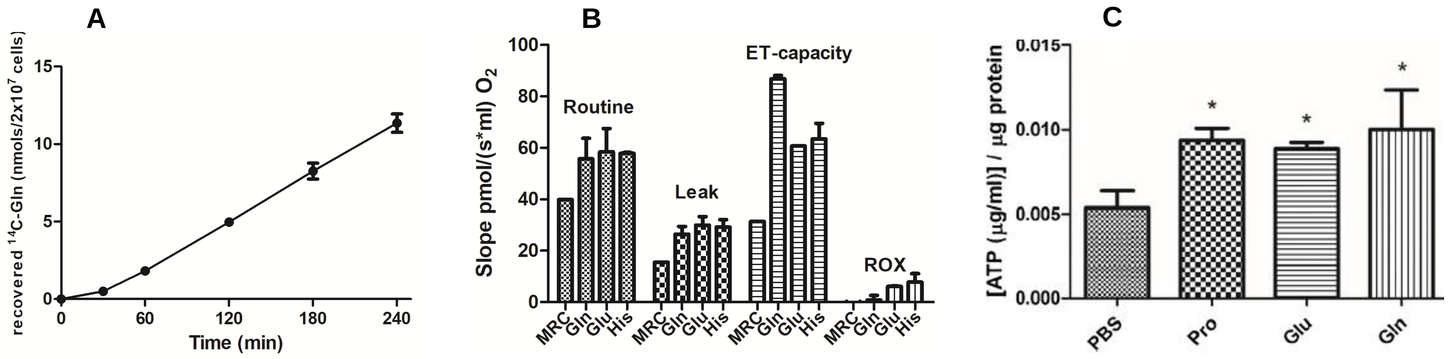


Figure 4- Gln as energy source. A- Gln oxidized to CO_2. *T. cruzi* epimastigote forms were incubated in the presence of L-[^{14}C(U)]-Gln. $^{14}CO_2$ was captured and radioactivity was measured using a scintillation counter. **B- Oxygen consumption in the presence of Gln**. Oxygen consumption was measured using intact cells in a high-resolution oxygraph (OROBOROS). Cells were incubated in the presence of Gln, histidine or glutamate as external carbon source and the buffer MRC was used as control without external carbon source. Routine (basal respiration), Leak (uncoupled of the ATP synthesis after addition of oligomycin A) ET capacity (uncoupled states of respiration after addition of FCCP), ROX (residual respiration after addition of antimicin A). **C- Intracellular ATP levels.** Cells were incubated in the presence of Gln, Glu, Pro or PBS. The ATP levels were quantified using luciferase assay kit (from Sigma-Aldrich).

Metacyclogenesis in the presence of Gln

Epimastigotes differentiate to metacyclics in the presence of only Gln as carbon and energy source.

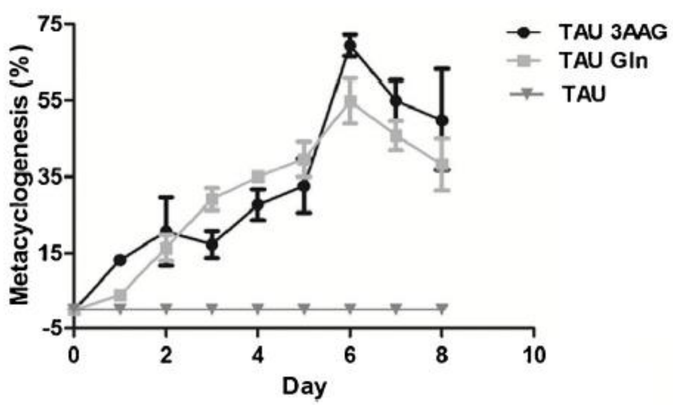
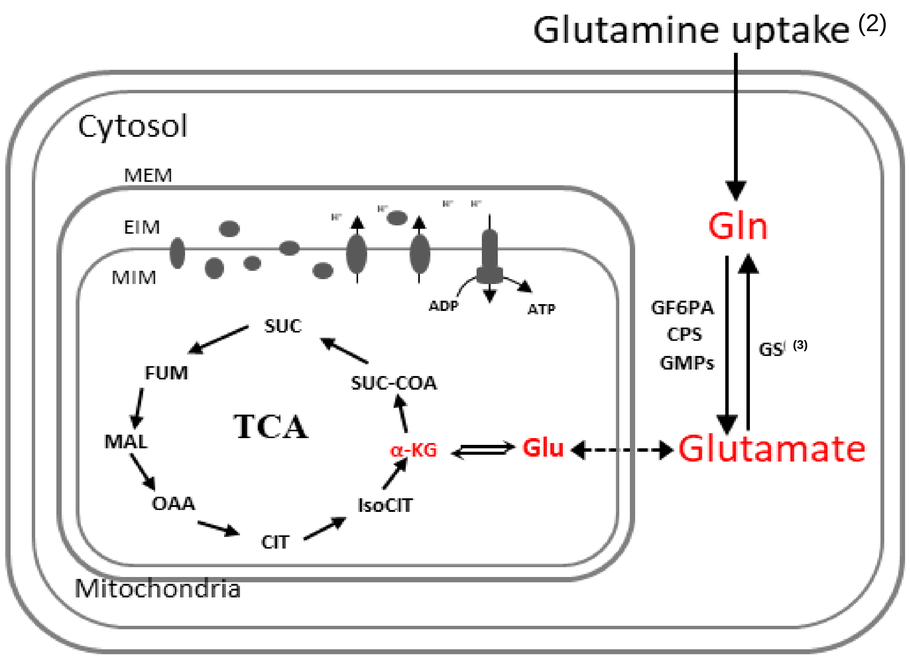


Figure 3 – Metacyclogenesis in the presence of Gln. Differentiation from epimastigote forms to metacyclic trypomastigote in the presence of TAU 3AAG (Salts supplemented with glucose, aspartate, glutamate and proline, standard differentiation medium), TAU supplemented with Gln or TAU without supplementation. The differentiation rate was follow-up for 8 days by counting in Neubauer chamber (Damasceno et al. 2018).

Overview of the Gln metabolism in *T. cruzi*



T. cruzi can obtain Gln by uptake from the external medium and or biosynthesis. Inside the cell, Gln is converted to Glu by the ezymes GF6PA, CPS or GMPs acting as nitrogen donor. The resultant Glu may be transported into the mitochondria and participates in the TCA as α-ketoglutarate. Thus contibuted to ATP biosynthesis.
GS- Glutamine synthetase
GF6PA- Glutamine fructose-6-phosphate aminotransferase
CPS- Carbomoil phosphate synthase
GMPs- Guanosine monophosphate synthase.

Conclusions

- *T. cruzi* can obtain Gln by the uptake from the external medium or can synthesize it using Glu, ATP and NH_4^+ as substrates.
- Gln uptake and biosynthesis act as complementary source of Gln through the life stages of the parasite.
- Gln is a relevant metabolite to differentiation from epimastigotes to metacyclic trypomastigote, a process call metacyclogenesis.
- Gln participates as energy source; it is completely oxidized to CO_2 and can feed the electron transport chain, contributing to ATP biosynthesis.
- These data showed that Gln is an example of adaptation of *T. cruzi* according to nutrient availability.

References

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2- Damasceno, F. S., M. J. Barison, M. Crispim, R. O. O. Souza, L. Marchese and A. M. Silber (2018). "L-Glutamine uptake is developmentally regulated and is involved in metacyclogenesis in Trypanosoma cruzi." Mol Biochem Parasitol 224: 17-25.3-
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