DEVELOPMENT OF RNA APTAMERS AS INHIBITORS OF THE GLUCOSE PERMEASE IN TRYPANOSOMA CRUZI

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Trypanosoma cruzi, the etiological agent for Chagas' disease, presents a high rate of glucose consumption. The glucose transporter is being considered as a target for the development of anti-trypanosome drugs. In this study we have obtained, by the first time to our knowledge, an aptamer which inhibits glucose permease. Firstly, the number of glucose binding sites for one epimastigote parasite cell was estimated, to be 0.075 fmol. Ten rounds of *in vitro* selection using live epimastigotes as target and 30 mM glucose to elute RNA molecules bound to glucose permease were performed to enrich the combinatorial RNA pool for RNA aptamers binding to these proteins and inhibiting their activity. RNA *in vitro*-transcribed from seventh and tenth cycles of *in vitro* selection were used to evaluate the inhibition of glucose uptake into the parasites. The transport of glucose was determined as the incorporation of [^{14}C]-U-glucose by the parasite for 30 seconds in the presence or absence of selected RNA molecules. Saturation analysis revealed a competitive inhibition profile, with an inhibition constant (K_i) for RNA-glucose transport inhibition of 1.2 -2.0 μM. Currently, we are identifying individual aptamer clones by DNA sequencing. Individual identified RNA aptamers will be further characterized.

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