Mathematical Modeling and Computer Simulation of *Trypanosoma cruzi* [Ca^{2+}] Response to Mammalian Host Cell Dynamics

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Abstract

A system of two Ordinary Differential Equations (ODE's) was developed to simulate the increase of intracellular [Ca^{2+}] in the parasite *Trypanosoma cruzi*, specifically the infective CL strain. When binding to and invading a host cell, in this study host cells are HeLa cells, the parasite has a measurable change in intracellular [Ca^{2+}] which is known to directly affect the success of parasite invasion of cells. This response is mediated by three major parasite glycoproteins; gp90, gp82, and gp35/50. This model has been set up in such a way that gp90 is not considered in terms of its effect on calcium response due to the fact that it is not expressed in the CL strain. Rates and calcium levels have been determined via analysis of published literature which focused on quantifying the levels of [Ca^{2+}] during an invasion event with respect to time. A future, more general, model of *Trypanosoma cruzi* will include gp90 in its calculations thereby allowing for simulation users to define "strains" of the parasite by varying the expression levels associated with each protein. As of this writing the model is still a work in progress, with several rates still to be defined before large scale testing can be done to assess the biological accuracy of the system. The equation system is given by the following system, variables are defined in the appendix:

$$C_{para}(t) = \{K_{gp82}C_{gp82}'(t) + K_{gp35/50}C_{gp35/50}'(t)\} * t + C_{p}$$

$$[C_{para}](t) = \frac{C_{para}(`}{Vo\iota_{para}}$$

$$1 = K_{gp82} + K_{gp35}$$

$$\frac{dC_{gp82}(t)}{dt} = \rho_{gp82} * (C_{gp82}^{\max} - C_{gp82}(t)) - \mu_{gp82} * C_{p}$$

$$\frac{dC_{gp35/50}(t)}{dt} = \rho_{gp35/50} * (C_{gp35/50}^{\max} - C_{gp35/50}(t)) - \mu_{gp35/50} * C_{para}(t)$$

Fig. 1: Equation System Skeleton

Introduction

It has been shown that successful invasion of nucleated mammalian cells by *Trypanosoma cruzi* is linked to the expression of specific glycoproteins[1][2][3][7]. These glycoproteins cause increases in intracellular [Ca^{2+}] in both the host and the parasite during a binding and invasion event by signaling pathways which release ions from intracellular stores. Proteins identified as significant to the parasite's internalization are; gp90, gp82, and gp35/50. In the parasite, these proteins trigger different responses due to the fact that

they trigger different pathways and release calcium ions from different stores. The ones shown to increase infectivity are gp35/50 and gp82, while gp90 is shown to decrease infectivity by binding to the host but not signaling any release of calcium in the parasite.

Different strains of *T. cruzi* express these three main proteins at different levels[7]. This differential expression leads to the various differences in invasion success, and calcium signaling. An accurate model which could predict the parasite's calcium activity would provide researchers with a tool that could give some insight into the affect that changes on the parasites calcium response system will have on its infectivity.

In this study we focus on building an introductory model of this system. It will focus on the infective CL strain of *T. cruzi*, specifically the infective metacyclic life cycle stage. This strain was chosen because of its high rate of invasion success, as well as the fact that immunoblot tests show that it does not express gp90. This simplifies the model, which will be described in terms of gp82 and gp35/50 only.

Methods

Scientific literature was used as the main source of information needed for the model. The calcium response of various strains of the parasite, and various pathways within each strain, has already been quantified[4]. Information regarding these pathways will be used to develop approximating equations that represent the change in calcium response with regards to time. For this project we chose to use ordinary differential equations. The system will be expressed in Mathematica, a computational tool useful for solving complex integrals and mathematical systems.

Defining Rates of Change

Ordinary differential equations are being developed by analyzing graphs available in published literature, i.e. Figure 7 of (Ruiz *et al.*, 1998) showing a quantitative measure of the parasite's intracellular calcium response over a period of time. Data points will be determined and used to recreate the graph from the paper, at which point a curve will be fitted to the data. All equations generated will hold time as the independent variable and intracellular Ca^{2+}, in moles, dependent. Data has been stored in an Excel spreadsheet where initial analysis has occurred. Once equations for the lines have been obtained it is a simple mathematical operation to take its derivative and get its rate of change.

Defining Volume of Parasite

In this model we use the cylindrical definition of the parasite seen in the Virtual Parasite Project[6]. The volume of the parasite is easily computed from the provided radius and length of the cylinder. For simplicity all parasites are assumed to have the same volume.

Determining Initial Conditions

The same data that was used to define rates of change was also used to determine the initial value of calcium within the parasites. This value varies from experiment to experiment. In order to take this variation into account, the calcium content at time zero is not a single number. Instead each time the system runs a random number is generated within the range of observed starting values.

Results

Although the model still needs values filled in, some key rates have been defined. Rates that have been determined so far are; the total rate of change of the parasite when bound to and invading a host cell, the rates of change of both the gp82 and gp35/50 pathways, as well as the rate of uptake by the gp35/50 calcium store. The values and data sources of each are shown below, all units are mol/sec:

$$C_{para}'(t) = 9.02 * 10^{-24}$$

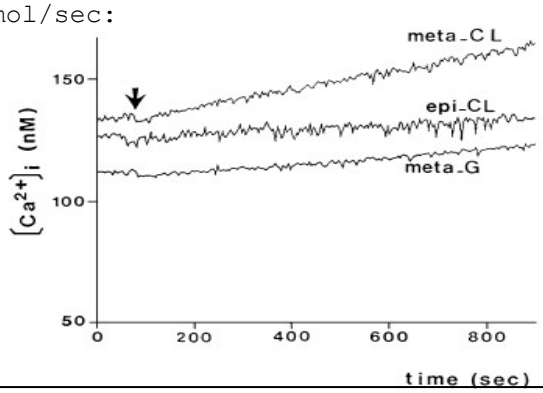


Fig. 2: Rate of total parasite change determined by analyzing Figure 6, trace 'meta_CL' of [4]

$$C_{gp82}'(t) = -3.77 * 10^{-28} t^2 + 3.02 * 10^{-25} t - 2.52 * 10^{-23}$$
$$C_{gp35/50}'(t) = 1.62 * 10^{-23}$$

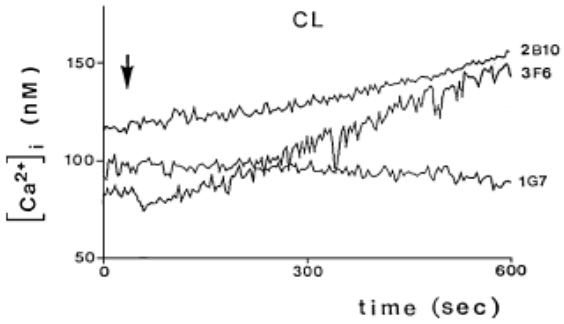


Fig. 3: Rates determined by analyzing Figure 7 traces '3F6' and '2B10' for gp82 and gp35/50, respectively of [4]

$$\mu_{gp35/50} = S(1.71 * 10^{-11})$$

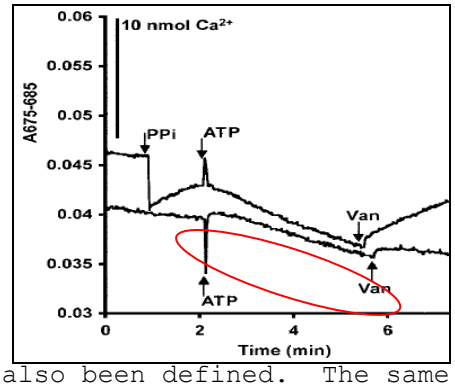
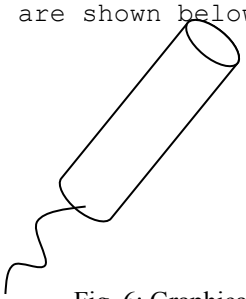


Fig. 4: Rate of uptake of $gp_{35/50}$ determined by analyzing Figure 6, circled region of [5]

Information regarding the parasite has also been defined. The same experiments used to approximate the Ca^{2+} response of the whole parasite, the gp82, and gp35/50 pathways, were also used to define a starting amount of calcium for the parasites. The volume of the parasite has also been defined. We are using the cylindrical definition of *T. cruzi* seen in the Virtual Parasite Project[6]. Values are shown below:

$$2.04 * 10^{-20}\ \text{mol} \leq C_{para}(0) \leq 3.54 * 10^{-20}\ \text{mol}$$

Fig. 5: Range of values that will be used to determine $C_{para}(0)$



Radius: 2×10^{-4} cm
Length: 20×10^{-4} cm
Volume: $8\pi \times 10^{-14}$ L

Fig. 6: Graphical representation of computational parasite with dimensions and volume

With all of this information taken into account the system as it stands now is as follows:

$$C_{para}(t) = \{K_{gp82}C_{gp82}'(t) + K_{gp35/50}C_{gp35/50}'(t)\} * t + \{2.04*10^{-20} \leq C_{para}(0) < 3.54*$$

$$[C_{para}](t) = \frac{C_{para}(t)}{8\pi*10^{-14}}$$

$$K = K_{gp82} + K_{gp35/50}$$

$$\frac{dC_{gp82}(t)}{dt} = \rho_{gp82} * (C_{gp82}^{max} - C_{gp82}(t)) - \mu_{gp82} * C_{para}(t)$$

$$\frac{dC_{gp35/50}(t)}{dt} = \rho_{gp35/50} * (C_{gp35/50}^{max} - C_{gp35/50}(t)) - (S*.0171) * C_{para}(t)$$

Fig. 7: System of equations with all defined variables

An attempt was made to model the system in a different formation. It simply used the total rates of change for the pathways shown in the table on the previous page, specific uptake and release rates of the calcium stores was not taken into account. What was also ignored was the expression constants associated with each pathway, in addition it was designed in such as way that the parasite was assumed to have no initial cytosolic Ca^{2+}. The system is shown below with the output produced:

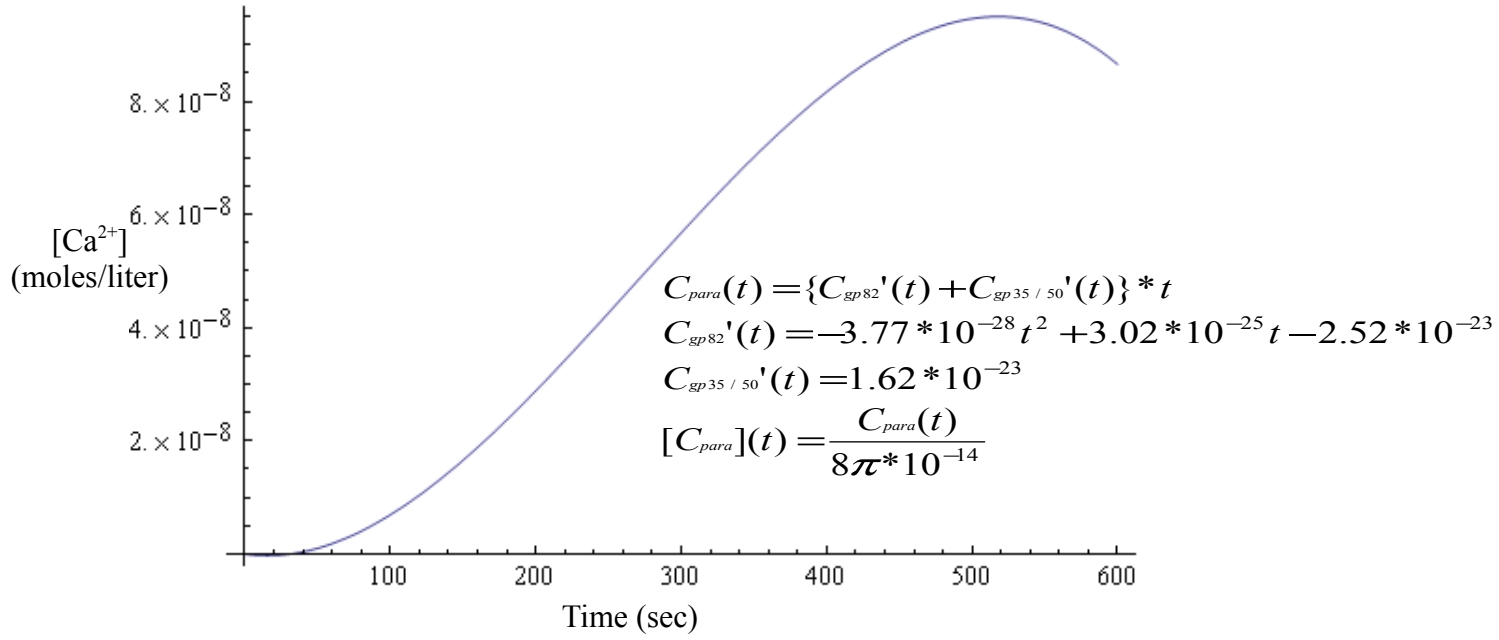


Fig. 8: Plot of the parasite's calcium ion concentration as a function of time with equation system shown

Discussion

Although the system is at a point where it cannot be tested to asses its biological accuracy, some important things have been determined. Most importantly, we see that we are not over complicating

the model. This can be seen by comparing the graph shown in Fig. 8, obtained by running a simplified version of the system, to the actual experimental data shown in trace 'meta_CL' of Fig. 2. The failure of this simplified model tells us that there are important factors at work that a simple rate of change of the whole parasite glosses over. The more complete version of the system makes strides towards accounting for this by taking into account the specific release and uptake rate of each calcium source in relation to the amount of calcium available to each system, as well as the relative expression of each protein with regards to one another.

Finding the data needed to compute these rates, values, and expression constants, has been the largest difficulty. The large majority of published literature regarding the glycoproteins that are involved in *Trypanosoma cruzi* invasion focus on describing the signal pathway in more detail, or look at the affect on total invasion success when certain proteins are inhibited. The data we need to further the model, the quantitative measure of calcium change with regard to time, is scarce in the published literature. Harder to find still is quantitative analysis of the parasite's two calcium stores for gp82 and gp35/50, the endoplasmic reticulum and acidocalcisome organelles, respectively. This is primarily a result of the inability to affectively trigger calcium release from these stores using what are believed to be the second messengers of the respective pathways.

Although difficult to obtain, this data is needed to define the remaining variables while taking on as little assumptions as possible in order to maintain accuracy.

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Appendix

Variable	Description
$C_{para}(t)$	Total amount of calcium ions in the parasite at time t
$[C_{para}](t)$	Calcium ion concentration at time t
Vol_{para}	Volume of the parasite
K_{gp82}	Expression constant of gp82, decimal between 0 and 1
$K_{gp35/50}$	Expression constant of gp35/50, decimal between 0 and 1
ρ_{gp82}	Release rate of calcium ion from Endoplasmic Reticulum
μ_{gp82}	Uptake rate of calcium into Endoplasmic Reticulum
$\rho_{gp35/50}$	Release rate of calcium ion from Acidocalcisomes
$\mu_{35/50}$	Uptake rate of calcium ion into Acidocalcisomes
C_{gp82}^{max}	Maximum amount of calcium ion that can be released from the Endoplasmic Reticulum
$C_{gp35/50}^{max}$	Maximum amount of calcium ion that can be released from the Acidocalcisomes
$C_{gp82}'(t)$	Amount of calcium ions released per unit time from the gp82 pathway
$C_{gp35/50}'(t)$	Amount of calcium ions released per unit time from the gp35/50 pathway

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