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Biological Interpretation of Model-Reference Adaptive Control in a Mass Action Kinetics Metabolic Pathway Model

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Abstract

The usefulness of control theory to model robustness in metabolic pathways is limited because controller properties and their implications on pathway regulation are unclear. Using sphingolipid biosynthesis in response to single-gene overexpression as a case study, we apply model-reference adaptive control (MRAC) to model regulation in a mass action kinetics pathway model and report on its properties. Tracking error between treated cells (plant) and wild type (reference) is reduced in 9 of 10 system variables compared to using mass action kinetics only. This result is robust when system parameters are perturbed. Furthermore, we interpret control dynamics to infer potential regulatory interactions. Some observations are consistent with independent studies on the effects of the same experimental treatment, while others represent novel hypotheses that may be tested to yield additional biological insight. The usefulness and interpretation of MRAC to model metabolic pathway regulation is shown where plant dynamics approach the reference.

Introduction

Robust behavior in metabolic pathways suggests that biological systems may be reverse engineered using control theory [1], e.g., in bacterial chemotaxis [2], heat shock response [3], and amino acid regulation [4]. However, the usefulness of control theory to model robustness in metabolic pathways is still limited because properties of specific controllers and their implications to model pathway regulation are not well understood. Here, we use feedback control, namely model-reference adaptive control (MRAC), as a model of metabolic pathway regulation, and report on its efficacy and robustness in terms of tracking error. As a case study, we use sphingolipid *de novo* synthesis in human embryonic kidney (HEK) cells in response to single-gene overexpression, specifically SPT that codes for serine palmitoyl-transferase.

This report extends previous work that demonstrated the original use of MRAC to infer potential regulatory interactions [5]. Specifically, we (a) assess the efficacy and robustness of the proposed controller using MRAC in terms of tracking error, i.e., how closely the plant dynamics follow the reference dynamics, and (b) interpret the control and feedback dynamics in the context of relevant literature for potential insight into sphingolipid *de novo* synthesis pathway regulation.

A. Sphingolipid de novo synthesis

Sphingolipids are involved in key eukaryotic cell functions such as membrane structure, signaling, and metabolism. Regulation of sphingolipid levels through synthesis is important because its pathway intermediates are highly bioactive (Fig. 1). Alterations in sphingolipid synthesis, storage, and metabolism are implicated in human diseases [6].

B. Model-reference adaptive control (MRAC)

The goal of model-reference adaptive control (Fig. 2) is to regulate an unknown system of interest (plant) using adaptive feedback such that it behaves identically to a known model (reference) [7].

Materials and Methods

A. Sphingolipid data acquisition

Sphingolipids, i.e., sphingoid bases and (dihydro)N-acyl species, are extracted from samples of cultured human embryonic kidney (HEK) cells and quantified by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) as described previously [8]. Measurements from four technical replicates are taken at hour intervals from 0–6 hours inclusive.

B. Reaction kinetics

The equations of motion, i.e., biochemical reactions, can be written as a (linear) system of ordinary differential equations (ODEs) using mass action kinetics, e.g.,

$$\dot{S}_a(t) = k_{in,Sa} PalcoA + k_{SaP,Sa} S_aP + k_{DHCer,Sa} DHCer - (k_{Sa,SaP} + k_{Sa,DHCer}) S_a \quad (1)$$

where $k_{a,b}$ indicates the rate constant for the reaction from a to b (see [5] for details of other pathway reactions). Rate constants are estimated using data from the C 16 dual-chain labeled species. The genetic algorithm was implemented for to estimate these system parameters based on minimizing least-squares error [9].

C. Model-reference adaptive control (MRAC)

The state space is defined as a 10×1 vector:

$$\vec{x}(t) = [S_a(t), S_aP(t), DHCer(t), DHGC(t), DHSM(t), Cer(t), GC(t), SM(t), So(t), SoP(t)] \quad (2)$$

The state dynamics for the **reference** (wild type) and **plant** (treated cells) are:

$$\vec{x}_m(t) = A_m \vec{x}_m(t) + \vec{b}_m r(t) \quad (3)$$

$$\vec{x}(t) = A \vec{x}(t) + \vec{b} u(t) \quad (4)$$

Where $\vec{x}_m(t)$ represents sphingolipid amounts in the reference and $\vec{x}(t)$ in the plant. The system parameters (reaction rate constants $k_{a,b}$) are contained in the matrices A_m and A . The scalar input $r(t)$ represents the system input palmitoyl-coA (PalCoA). $\vec{b}_m$ and $\vec{b}$ are actuation vectors. The scalar controller $u(t)$ is the sum of two components, input PalCoA and state feedback:

$$u(t) = \vec{h}_x(t)^T \vec{x}(t) + h_r(t) r(t) \quad (5)$$

where $\vec{h}_x(t)$, $h_r(t)$ are adaptation gains associated with state feedback and system input respectively.

Using the Lyapunov synthesis method, the rate of change of these adaptation gains are defined:

$$\dot{\vec{h}}_x(t) = -\theta_x \vec{x}(t) \vec{e}(t)^T P \vec{b} \quad (6)$$

$$\dot{h}_r(t) = -\gamma_r r(t) \vec{e}(t)^T P \vec{b} \quad (7)$$

Potential regulatory pathway interactions may be inferred from these adaptation gain dynamics, where $\vec{e}(t)$ is the difference between plant and reference states:

$$\vec{e}(t) = \vec{x}(t) - \vec{x}_m(t) \quad (8)$$

and θ_x , γ_r are control parameters to be specified. P satisfies the Lyapunov equation:

$$A_m^T P + P A_m + Q = 0 \quad (9)$$

and can be solved by specifying Q . Finally, the initial conditions may be chosen by matching:

$$A + \vec{b} \vec{h}_x^* T = A_m \quad (10)$$

$$\vec{b} \vec{h}_r^* = \vec{b}_m \quad (11)$$

to approximate the ideal constant gains. The model is implemented in MATLAB.

Tracking error between the plant (treated cells) and reference (wild type) dynamics is quantified using the L2-norm, or root-sum-square error (RSS). In addition, robustness is also tested when system parameters are perturbed by varying the reaction rate constants individually, i.e., $k_{\text{perturbed}} = k_{\text{original}} * \{10^{-1}, 10^1\}$.

Results and Discussion

Experimental data and simulated pathway dynamics are shown in Figs 3 (wild type), 4 (treated cells, mass action only), and 5 (treated cells, with adaptive feedback). Simulated dynamics are accurate if they fall clearly and consistently within the range of experimental data. Overall, using linear ODEs to describe individual reaction kinetics produces mixed results in simulated pathway dynamics.

For the wild type (Fig. 3), simulated dynamics are accurate for So, SoP, Cer, and GC, but not for DHCer, DHGC, DHSM, and SM. Data is limited for DHCer, DHGC, and DHSM; no data is available for Sa and SaP. For treated cells using mass action only (Fig. 4), simulated dynamics are accurate for SaP, SoP, DHGC, GC, and SM, but consistently underestimated for Sa, So, DHCer, DHSM, and Cer. With adaptive feedback (Fig. 5), simulated dynamics are accurate for So, SoP, DHGC, and DHSM, but not for Sa, SaP, GC, and SM.

B. Tracking error

Fig. 6 shows tracking error between treated cells and wild type. Tracking error is smaller in 9 of 10 sphingolipids, except SM, using MRAC compared to mass action only. When rate constants are varied, the mean tracking error is also smaller in 9 of 10 sphingolipid (statistically significant for Sa, SaP, and DHGC), except SM, using MRAC compared to mass action only.

C. Control and feedback dynamics

Fig. 7 shows control dynamics in treated cells in terms of two key components: (a) system input PalCoA , $u_r(t)$, and (b) pathway feedback, $u_x(t)$, and Fig. 8 shows feedback dynamics from individual pathway metabolites, in terms of changes in adaptive gain, $h_x(t)$. In both figures, the numerical values are dimensionless, where magnitude and sign indicate activity in pathway regulation, i.e., larger magnitude indicates more activity, sign indicates enhancement (+) or inhibition (-).

Fig. 7 shows that the amount of input PalCoA that enters the pathway is first quickly moderated at the onset, then gradually inhibited over time. Pathway feedback is a lesser factor at first, but is later enhanced. This suggests that pathway regulation may occur at the source of perturbation by moderating the reaction substrate (PalCoA). In terms of control design, such a regulatory strategy is highly efficient and requires the least relative effort to implement. This strategy may also explain how pathway feedback is not a significant factor at the onset.

The possibility that PalCoA may be moderated in this case may also be a result of using only data on labeled sphingolipids such that labeled PalCoA only appears to be moderated relative to the total intracellular pool. This may be tested by tracking unlabeled PalCoA as well. On the other hand, if the amount of available PalCoA is indeed moderated biologically in response to treatment, future studies may focus on elucidating molecular mechanisms that could accomplish this. For instance, possible mechanisms could slow the rate of uptake of extracellular palmitate [10], reduce the rate of converting palmitate to PalCoA, or increase the rate of other metabolic reactions that act on PalCoA as a substrate.

In Fig. 8, relative to other metabolites, feedback from Sa, SoP, DHCer, DHGC, and DHSM change significantly and also exhibit similar trends, i.e., to increase or decrease quickly from zero initial condition and then remain constant over time. This suggests that these metabolites play more active roles in pathway regulation. Some pathway metabolites are also signaling molecules that activate opposing responses. For example, Sa and SoP reverse growth inhibition [11] and activates growth stimulation [12], while So and Cer activate growth inhibition [13]. Hence, taken together, these feedback dynamics suggest that SPT overexpression at these levels leads to growth stimulation in HEK cells, which is consistent with independent studies on the same experimental treatment [14].

Conclusion

Using a case study pathway, we first verified that the property of MRAC is to reduce the tracking error between the plant (treated cells) and reference (wild type). This feature is robust when system parameters are perturbed. Then, we interpreted control and feedback dynamics to infer interactions that may be responsible for regulation. Some observations are consistent with independent studies on the effects of the same experimental treatment, while others represent novel hypotheses that may be tested to yield additional biological insight. Thus, this report demonstrates the usefulness and biological interpretation of MRAC to model metabolic pathway regulation in cases where plant dynamics approach the reference.

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Figure 1. Sphingolipid de novo synthesis pathway: serine palmitoyl-transferase (SPT) catalyzes the initial reaction, i.e., condensation of PalCoA and L-serine to sphinganine (Sa), and is artificially overexpressed in treated HEK cells

Figure 2. Model-reference adaptive control: the reference (top) exhibits a model response to input (left), differences between the plant (middle, right) and reference dynamics are used as adaptive feedback (bottom)

Figure 3. Wild type data (scatter plot, median and range) and simulated dynamics (broken lines): y-axis-sphingolipid amounts (pmol/mg protein), x-axis-time (hours)

Figure 4. Treated cells data (scatter plot, median and range) and simulated dynamics (broken lines): mass action only; y-axis-sphingolipid amounts (pmol/mg protein), x-axis-time (hours)

Figure 5. Treated cells data (scatter plot, median and range) and simulated dynamics (broken lines): with adaptivefeedback; y-axis-sphingolipid amounts (pmol/mg protein), x-axis-time (hours)

Figure 6. Tracking error between treated cells and wild type: MA-mass action only (solid bars, blue), MRAC-with adaptive feedback (hollow bars, red); height-mean, error bars-standard deviation; difference in means is statistically significant ($p < 0.05$, two-tailed t-test) for Sa, SaP, and DG

Figure 7. Control dynamics in treated cells, in terms of system input PaICoA, $u_r(t)$ (blue), and feedback from pathway metabolites, $u_x(t)$ (red)-x-axis: time (hours), y-axis: magnitude (dimensionless), error bars: standard deviation

Figure 8. Feedback dynamics in treated cells, in terms of adaptive gain for individual pathway metabolites, $h_x(t)$ —x-axis: time(hours), y-axis: magnitude (dimensionless), error bars: standard deviation