Robust Control in the Carbon Fixation Pathway of C_4 Plants

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***Short Abstract* —We present a putative robust mechanism in the carbon fixation pathway of C_4 plants. We suggest a robust mechanism that is based on avidity of a bifunctional enzyme to its multimeric substrate, and on a product-inhibition feedback loop that couples the system output flux to the activity of the bifunctional regulator. The model provides a context for the peculiar biochemical characteristics of the system and predicts that the system output, PEP formation flux, is insensitive to fluctuations in proteins levels, substrates levels and the catalytic rate of the key enzyme while staying sensitive to the system's input (light levels).**

***Keywords* —Avidity, PPDK, RP, bifunctional enzyme.**

I. Introduction

C_4 plants such as corn and sugarcane use an enzymatic cycle to promote the assimilation of atmospheric CO_2 into biomass. A key step in this cycle is the conversion of pyruvate to PEP by the enzyme pyruvate orthophosphate dikinase (PPDK)[1]. The output of this enzyme is PEP formation flux, denoted F. The activity of PPDK is tuned by light. Light level is encoded in the cell by the concentration of ADP: high ADP means low light, and low ADP means high light [2].

The reactions and regulation of PPDK have the following biochemical features. PPDK catalyzes the conversion of pyruvate to PEP in two steps: The first is auto-phosphorylation of itself at a His residue (denoted $PPDK_1$). The second step is a phospho-transfer reaction that transfers the phosphoryl to pyruvate to produce PEP.

To regulate the activity of PPDK there exists a second phosphorylation/de-phosphorylation cycle. Only the auto-phosphorylated form of PPDK can be phosphorylated again at a Thr residue ($PPDK_2$). This doubly-phosphorylated form is an inactive form of the enzyme. A bi-functional enzyme called Regulatory Protein (RP) catalyzes two opposing reactions, both of which are not of the common type: phosphorylation uses ADP as a substrate and its products are $PPDK_2$ and AMP. Whereas dephosphorylation uses inorganic phosphate Pi to produce PPi, instead of the usual phosphatase hydrolysis reaction that produces Pi.

A recent experimental study indicates that the activity of $PPDK_1$ (the PEP flux) is highly insensitive to variations in PPDK protein levels [3]. Ohta et al. transformed a cold-tolerant PPDK gene into maize. The strains were then measured for PPDK enzyme activity. These measurements show that enzyme activity is nearly insensitive to almost a 6 fold increase in PPDK expression levels (20% change of activity over a 5.7 fold increase. This suggests that the enzyme's activity is regulated in a way to ensure a robust PEP production flux.

The unusual biochemical features in this system triggered our fascination, and we sought to understand them in the context of the behavior of the entire system.

II. Results

We present a putative model for robustness in the PPDK system of the C4 pathway in plants. The mechanism depends on avidity of the bifunctional enzyme RP to its multimeric substrate PPDK, and on a product-inhibition feedback loop that couples the system output flux to the activity of the bifunctional regulator. The resulting output flux, PEP formation, is made insensitive to variations in substrates and protein levels.

Our model also predicts that the robust flux solution F* does not depend on the catalytic rate of PPDK. Thus, the PEP formation flux can be insensitive to temperature effects on PPDK specific activity.

We find conditions for robustness to break down. This occurs at very high or very low light levels, or when PPDK concentration or the concentrations of its substrates are too low to provide the robust flux solution.

Last, we use a model for the spatial arrangement of PPDK subunits that is based on avidity to predict a bimodal distribution of phosphorylated and unphosphorylated tetramers of PPDK.

References

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