A Computational Systems Biology Approach to Decipher Significant Intricacies of Dihydrolipoamide Dehydrogenase Deficiency in Human

Abhishek Sengupta, Sarika Saxena, Gaurav Singh, Priyanka Narad, Ayushi Yadav, Monendra Grover

Abstract- The alpha-ketoglutarate dehydrogenase complex (KGDHC) is elemental in mitochondria, and its deficiency is associated with a number of neurological disorders like Alzheimer's disease, Parkinson's disease and Dihydrolipoamide dehydrogenase deficiency (DLDD). The molecular mechanisms underlying the age dependent loss of brain KGDHC activity remain incomprehensible. In order to disentangle this phenomenon, a kinetic model is developed representing correlations between three aspects namely the reduced AKGDHC activity; mitochondrial ATP generation and increased pyruvate concentration. The kinetic model centralizes on the mitochondrial-derived ATP production and is distributed into cytosol and mitochondria. The model revealed a decline in ATP production with lowered enzyme concentration. With the effect, the concentration of pyruvate was increased resulting in its excretion which is a characteristic feature of DLDD. In agreement with the previous literature the model simulations confirmed the decline in reaction fluxes and NADH level. The finding suggests that reduction of pyruvate is the rate limiting step of the TCAC which is supported by past bibliographic findings. Since ATP production is also affected by NADH production rate hence it can be safely assumed that decrease in NADH also causes Change in ATP production rate. Change in pyruvate concentration on changing the concentration of AKGDH also underpins the importance of the studied enzyme in DLDD. It is clearly indicated by simulations that AKGDH deficiency can cause increase in pyruvate concentration.

Index Terms—Alpha-ketoglutarate dehydrogenase complex, Dihydrolipoamide dehydrogenase deficiency, kinetic model

I. INTRODUCTION

Enzymes participating in metabolic pathways have been known since long time but their control and regulation have not been well understood at the system level. Kinetic modeling in Systems Biology acts as a tool to carry out a control analysis of metabolic systems, determine potential targets for biotechnological alterations, as well as to identify the regulatory importance of different enzymes in a pathway. Mitochondria are essential cellular organelles that play central roles in energy metabolism and apoptosis.

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Mitochondrial dysfunctions plays critical role in neurodegenerative disorders like Alzheimer's disease and Parkinson's disease. Alterations in mitochondrial function have been implicated in age-related neurodegenerative diseases through reactive oxygen species (ROS) hypothesis and have been suggested to have a role in the loss of brain function with healthy aging [1,2]. Mitochondria are the major producer of reactive oxygen species ROS in cells. Respiratory chain (RC) acts as a rich source of mitochondrial ROS. It has been observed that KGDHC generates and is also self-inactivated by ROS, is of paramount importance in neuronal pathology. It is not known if the physical association of KGDHC with complex I play a role in the dual deficiency of these protein complexes in Parkinson's disease. It appears that neuronal pathology is preferentially associated with KGDHC deficiency [3]. DLDD is an autosomal recessive metabolic disorder distinguished biochemically by a combined deficiency of 3 enzymes which are branched-chain alpha-keto acid dehydrogenase complex (BCKDC), alpha-ketoglutarate dehydrogenase complex (AKGDC) and pyruvate dehydrogenase complex (PDC). Clinically affected individuals suffer from neurological deterioration and lactic acidosis due to sensitivity of the central nervous system to defects in oxidative metabolism. E3 deficiency is often associated with increased urinary excretion of pyruvate. Here, we present a kinetic model of mitochondrial metabolic pathways is presented which is simulated to analyze the associated metabolic disorders. Reactions occurring among a set of reactants define a kinetic reaction network. Modeling the kinetics of the processes that describe a biophysical system has long been pursued with the aim of improving our understanding of the studied system. Energy metabolism is a highly coordinated cellular activity in which enzymes are organized into discrete metabolic pathways that cooperate in degrading energy-rich nutrients from the environment [5,6,7]. Biochemical kinetic networks which model cellular activities as a set of reaction processes can be used to analyze and validate the metabolic pathway in an organized and efficient manner. A systematic approach is followed to explore and construct a kinetic metabolic model of TCA in neuronal cells. A comprehensive multidimensional representation of all of the biosynthetic reactions of TCA is done using CellDesigner for designing & Copasi for simulating [8].

II. METHODOLOGY

Extensive literature investigation revealed interesting facts and led to the opening up of a plethora of research in this

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field. It was found that TCA plays a central role in catabolism of the fuel molecule and production of ATP. The dynamics of TCA is still an unanswered puzzle and gaining the interest of scientists all over the world. The rate limiting enzyme of TCA, alpha-ketoglutarate, and its role in brain cell metabolism by following a modelling approach was studied and analyzed. A model of TCA was created as depicted in Figure2 using CellDesigner notations (Figure1) by taking reference from several books and journals. The concentrations of various metabolites of the TCA were manually integrated into the model. Rigorous data search was done and information had to be scrutinized to see if it did fit the model or not. SBML squeezer was used to generate the kinetic laws. SBML2LATEX was used to generate the PDF of the model in human readable format. It converts SBML files to pdf format. Once the model was computationally constructed, the simulations were done using COPASI platform to check the predictability of the model generated. Plots were generated by changing the concentration of enzymes. Changes, both minor and major were minutely studied and analysed. The model structure representation after it was exported was shown in Figure 3.

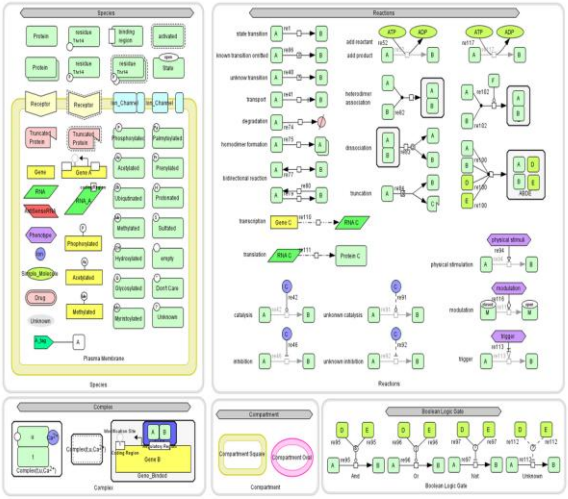

Figure 1: Symbols and notations used in Cell Designer (Source: www.celldesigner.org)

III. RESULTS

A) Change of Succinyl CoA concentration with time.

A simulation plot was generated depicting the change of Succynl CoA (SCoA) in TCA cycle with respect to time as shown in Fig 4(a) and Fig 4(b). A primary graph was plotted with the time period of 1000s. It was observed that concentration of SCoA decreases initially at exponential rate. An increase is seen gradually and within no time the level decreases slightly as the reaction progresses until it acquires a constant rate of reaction. To further observe the correlation with time, the time scale of 400 times lesser than the original one which gives better insight about the phenomenon. SCoA falls as the initial concentration of SCoA is much more than enzyme concentration then it reaches a plateau phase where SCoA concentration declines even below a critical level.

B) Simulated concentration of alpha-ketoglutarate as a function of time.

A plot of simulated concentration of alpha-ketoglutarate (AKG) as a function of time shows initial increase, as it acts as a substrate in the rate limiting step as shown in Figure 5(a). Next, a graph is plotted depicting time course simulation of AKG with time as shown in Figure 5(b); since it is the rate limiting step, concentration of AKG increases with time. Gradually as the time passes, the substrate reaction increases exponentially and rate of conversion of AKG into SCoA also increases, till it reaches an equilibrium state.

C) Correlation between NAD+, AKG and NADH.

The plots of concentration as shown in Figure 6 depicts the changes with respect to time between NAD^+ (purple), AKG (blue) and NADH (yellow). This integrated plot shows that rate of change of NADH with NAD^+ and AKG. It clearly depicts that rate of NADH formation depends upon AKG.

Next, a plot as shown in Figure 7(a) for varying concentrations of AKGDHC was generated, and a change in slopes was observed. These plots are of reaction AKG → SCoA with time. With decrease in concentration of enzyme AKGDHC, slope also decreases. Nearly at about 20% inhibition the increase in substrate concentration causes the reaction flux to be maintained to an extent. This is because the decrease in enzyme causes increase in AKG concentration which compensates the change in flux, AKG level that activates the remaining dehydrogenase enzyme. This increase can also be taken as marker for AKGDH impairment as it leads to elevation in blood and urine alpha-keto glutarate level. At the 40 per cent decrease, the reaction flux decreases which is clearly visible. At about 60 per cent inhibition curve for reaction flux becomes more flat and hence shows that reaction flux is decreased. At 80 per cent decreases, the slope decreases further. At about 95 per cent the reaction flux decreases drastically and it can be assumed that ATP production falls accordingly hence the energy content of cell decreases. Graph of rate of ATP generation with respect to time as depicted in Figure 7(b) shows the drop in level of ATP concentration with decrease in AKGDHC supports the initial hypothesis that ATP generation is dependent on AKGDHC. Graph between different concentration of AKGDHC and Pyruvate concentration shows relation between DLDD and AKGDHC. Lesser the AKGDHC more is pyruvate which is a diagnostic feature of DLDD.

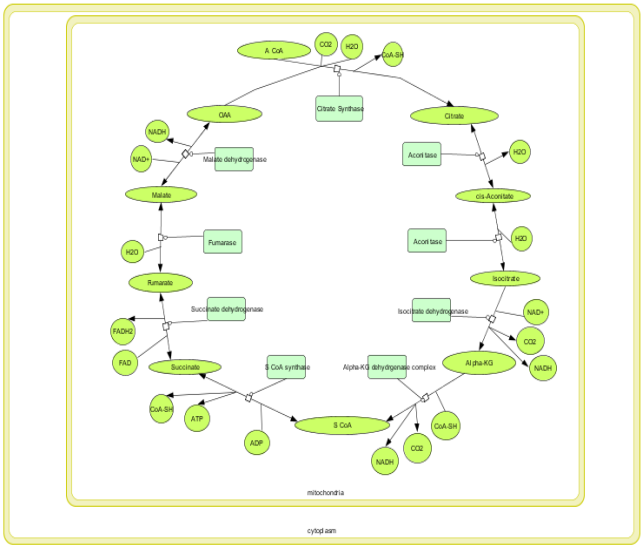

Figure 2: A shematic representation of the model of TCA pathway created in CellDesigner.

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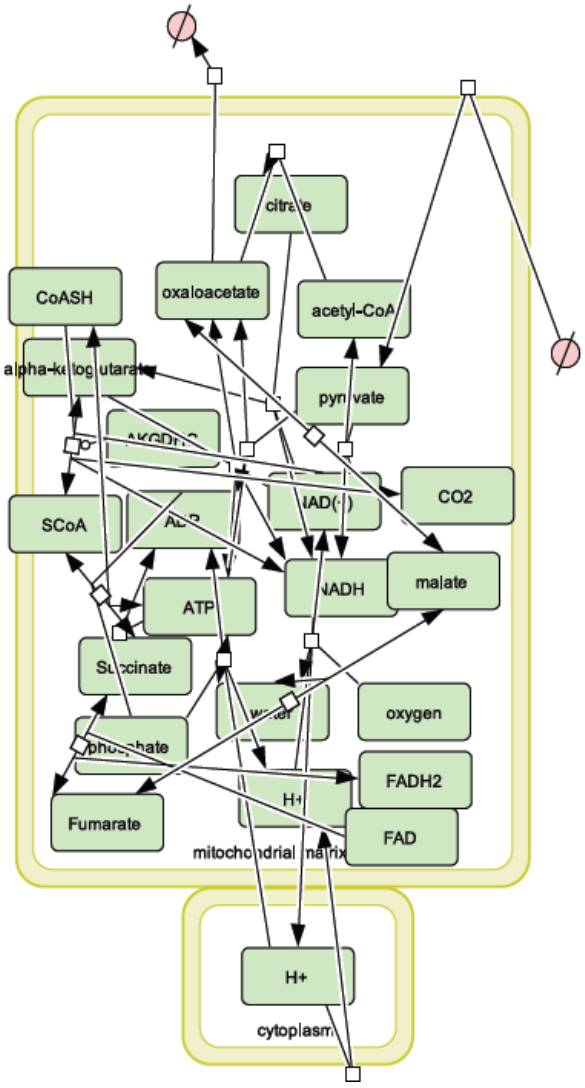


Figure 3: SBML model of the TCA cycle in organic layout when exported from CellDesigner.

IV. DISCUSSION

To understand the effect of reduced AKGDHC on mitochondrial energy metabolism by TCA cycle, a comprehensive SBML model was generated. This model is advancement over the previous available models of TCA and can be used for studying effect of AKGDH on the above mentioned cycle and its substrates. In agreement with the experimental findings, the model simulations confirmed a decline in reaction fluxes and NADH level. The finding suggests that it is the rate limiting step of the TCA cycle which can be supported by literature evidence. Since ATP production is also affected by NADH production rate hence it can be safely assumed that decrease in NADH also causes change in ATP production rate.

Decrease in AKGDH also correlates with loss of glutameric neurons as seen in Alzheimer's and Parkinson's disease. The simulation reveals the deviations from normal course which can lead to find the steps which are responsible for the disease. Moreover change in pyruvate concentration on changing the concentration of AKGDH also underpins the importance of the studied enzyme in DLDD. It is clearly indicated by simulations that AKGDH deficiency can cause increase in pyruvate concentration.

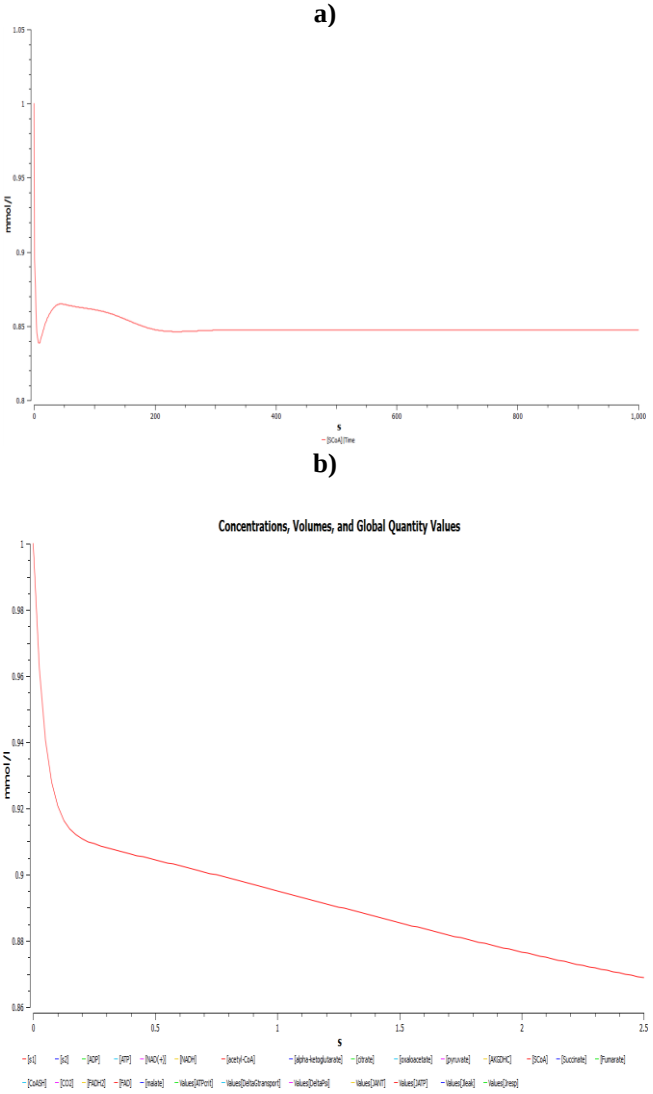
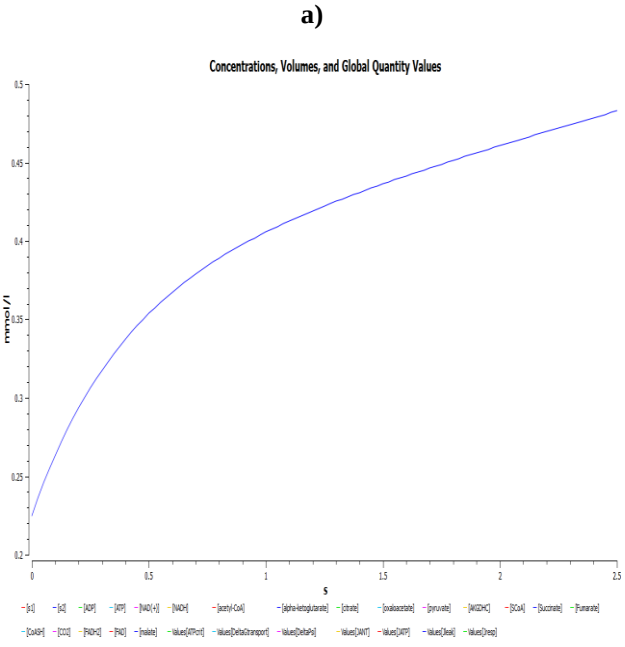


Figure 4: (a) A plot of change of Succinyl CoA concentration with time for a time period of 1000 seconds (b) A plot of Succinyl CoA concentration with time for a time period of 2.5 seconds.



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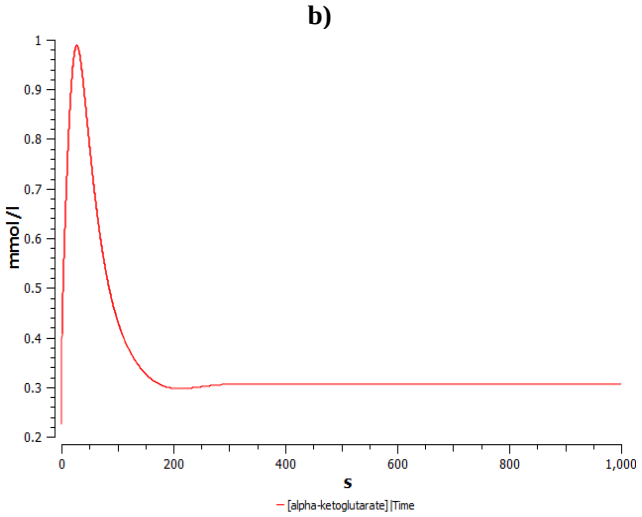


Figure 5: (a) Simulated concentration of alpha ketoglutarate as a function of time, increases initially as it is a substrate in rate limiting step of TCA. (b) Complete graph showing time course simulation of model for AKG

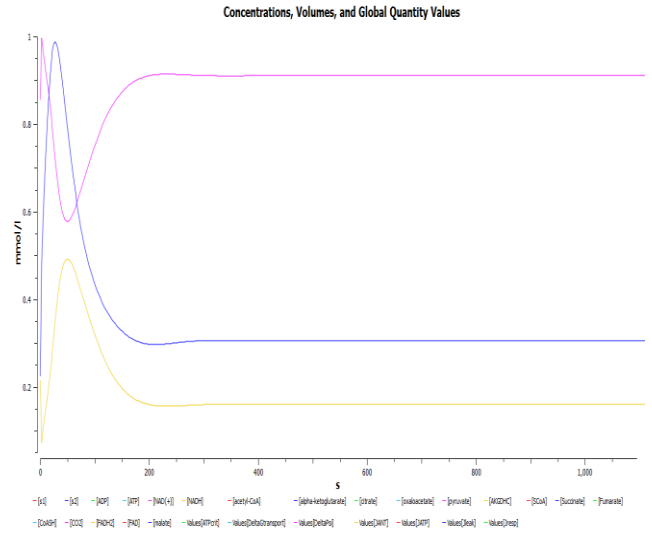
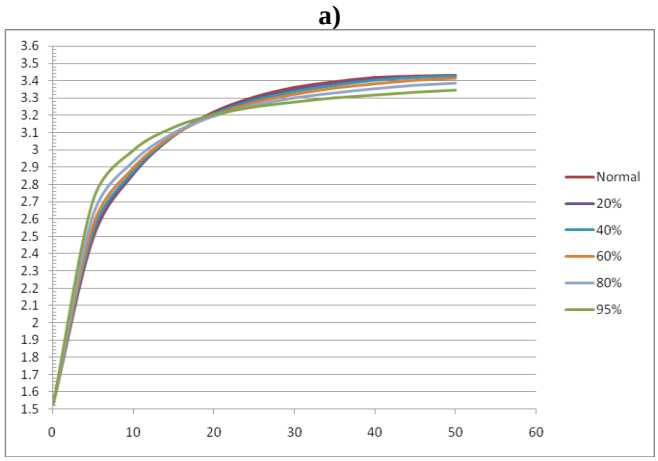


Figure 6: Correlation between NAD+, AKG and NADH. The plots of concentration changes with respect to time between NAD^+ (purple), AKG (blue) and NADH (yellow), this integrated plot shows that rate of change of NADH with NAD^+ and AKG. It clearly depicts that rate of NADH formation depends upon AKG.



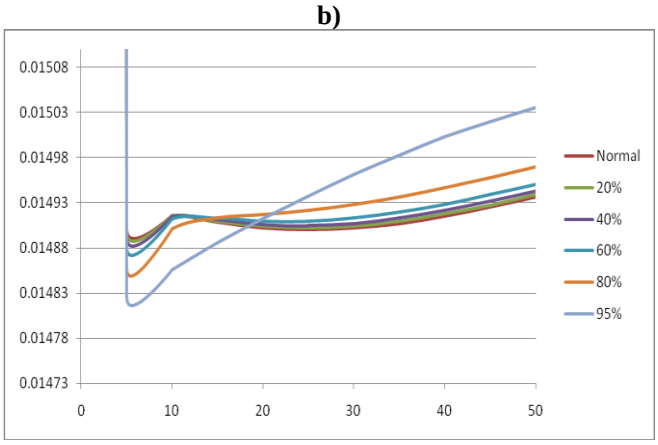


Figure 7: Graph between different concentration of AKGDHC and (a) rate of ATP generation with respect to time and (b) Pyruvate concentration with time.

V. CONCLUSIONS

The model re-constructed can be further extended to study other diseases, course of metabolic cycles in normal and diseased condition. For instance inclusion of pyruvate dehydrogenase complex and branched-chain alpha-keto acid dehydrogenase complex can help to identify the extent of effect of single enzyme which can be used as target for curing the DLDD disease.

It is to be noted that AKG and glutamate level is in equilibrium. With increased level of AKG Glutamate poisoning can also occur. With the help of the model and graphs generated, an insight about Glutamate poisoning and its effects can be inferred. The inclusion of respiratory chain and fatty acid metabolism in the present model can generate a comprehensive analysis of energy production in mitochondria.

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