Modelling the Metabolic Pathways

Chanchal K Mitra, Kalyani Korla
University of Hyderabad, Department of Biochemistry, Hyderabad 500 046, India, c_mitra@yahoo.com

The biochemical reactions taking place within a cell follow a particular sequential order called the metabolic pathways. Although all the reactants and products are mixed in a soup, there exists specific substructures (organelles within a cell) that are tasked with carrying out more specialized reactions. One of these specialized organelles is mitochondrion that is present in all eukaryotic cells and is tasked with the production of ATP and other carbon skeletons. It has its own genetic material but needs a host of proteins and other substrates for proper function. A cell can have a rather variable number of mitochondria. In addition, the mitochondrion by itself is rather complex, having a outer and inner membrane, intermembrane space, matrix etc. and much of the processes are membrane bound.

The Krebs cycle (also called the citric acid cycle or tricarboxylic acid cycle) is a series of biochemical reactions in a cyclic pathway. The overall reaction carried out the Krebs cycle is the oxidation of one molecule acetate (as acetyl-CoA) to two molecules of carbon dioxide with concommitant reduction of one molecule of FAD and three molecules of NAD (per rotation of the cycle). The acetyl-CoA is supplied externally by pyruvate (mainly) from glycolysis. On the other hand, the reduced coenzymes are oxidized back in the electron transport chain, that essentially functions in maintaining an electrochemical potential gradient across the inner membrane and reduces dioxygen to water. The electrochemical potential gradient is used to synthesize ATP.

In addition to producing ATP, mitochondria also supply several carbon skeletons to the cytosol in the form of malate and oxaloacetate and supply reducing equivalents of NADH via several shuttles. Indirect transport of reducing equivalents via shuttles reduces any sudden impact on the concentrations of NAD/NADH that are essential for the proper function of the Krebs cycle.

We have simulated all the above steps using a simple kinetic model using an approximate form of the Michealis Menten (MM) equation. We have also considered the effect of the feedback inhibitors. We have made some drastic approximations our results agree rather qualitatively

with experimental results. The Michealis Menten equation, as written below, gives the following rate equation:

$E+S \rightleftarrows ES \rightarrow E+P$

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

and this can be written in a dimensionless format:

$$v/V_{max} = \frac{[S]/K_M}{1 + [S]/K_M}$$

which is ideal for simulation. The Krebs cycle reactions were written in a form suitable for simulation:

$$\text{Acetyl} - \text{CoA} + \text{Oxaloacetate} + H_2O \rightarrow \text{Citrate} + \text{CoA-SH}$$

$$\text{Citrate} \rightarrow \text{Isoitrate}$$

$$\text{Isocitrate} + \text{NAD} \rightarrow \alpha\text{-oxoglutarate} + \text{NADH} + H^+ + CO_2$$

$$\alpha\text{-oxoglutarate} + \text{CoA-SH} + NAD^+ \rightarrow \text{Succinyl-CoA} + \text{NADH} + H^+ + CO_2$$

$$\text{Succinyl-CoA} + \text{GDP} + P_i \rightarrow \text{Succinate} + \text{GTP} + \text{CoA-SH}$$

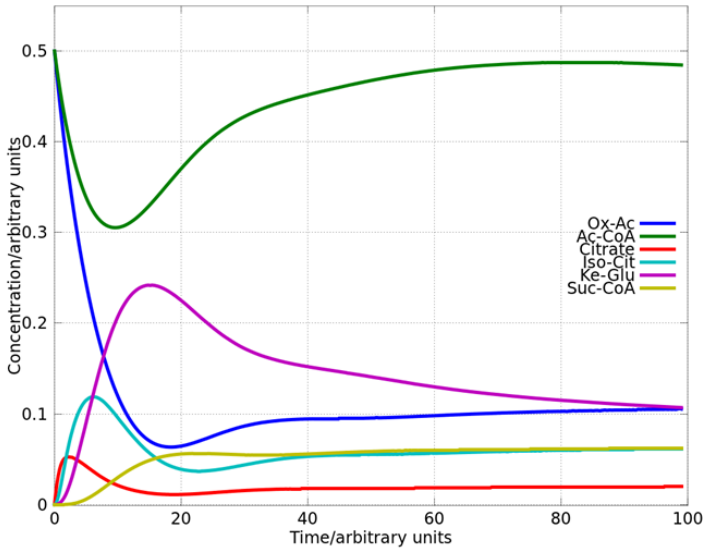
$$\text{Succinate} + \text{FAD} \rightarrow \text{Fumarate} + FADH_2$$

$$\text{Fumarate} + H_2O \rightarrow \text{Malate}$$

$$\text{Malate} + NAD^+ \rightarrow \text{Oxaloacetate} + \text{NADH} + H^+$$

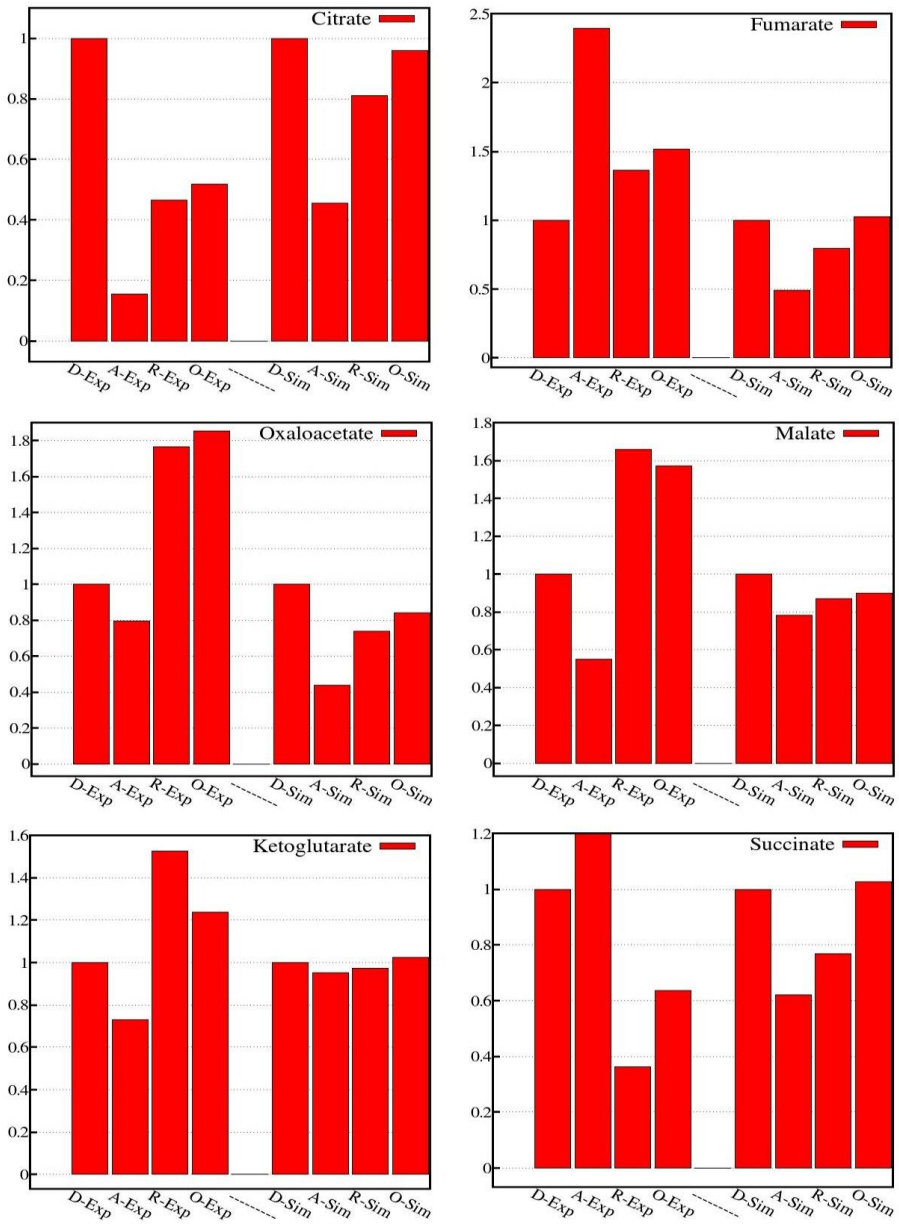
$$\textbf{Acetyl-CoA} + 2\ \textbf{H}_2\textbf{O} + 3\ \textbf{NAD}^+ + \textbf{GDP} + \textbf{P}_i + \textbf{FAD} \rightarrow 3\ \textbf{NADH} + 3\ \textbf{H}^+ + \textbf{FADH}_2 + \textbf{GTP} + \textbf{CoA-SH} + 2\ \textbf{CO}_2$$

The last equation is the overall reaction (sum of the above ones) and we can see that the Krebs cycle acts like a giant catalyst that oxidizes acetate (in the form of Ac-CoA) to two molecules of carbon dioxide (with the production of reducing equivalents and GTP). We have used octave (www.gnu.org/octave), a free software that can solve ordinary differential equations. We have written a simple script to achieve this task. We see some of the results of simulation in the following graph.



Simulation curve for selected components of the Krebs cycle. (Ox-Ac= Oxaloacetate, Ac-CoA= Acetyl CoA, Iso-Cit= Isocitrate, Ke-Glu= α-Ketoglutarate, Suc-CoA= Succinyl CoA). The graphs after time 50 essentially reflect steady state behavior.

It is usually difficult to get experimental data for several metabolites determined under identical conditions but we could find a report by Maner (1973). The experiments were conducted on an untransformed mouse mammalian cell line with inhibitors of oxidative phosphorylation, viz. Antimycin A, rotenone and oligomycin. The x-axis represents the four conditions abbreviated as D=DMSO, A=Antimycin A, R=Rotenone and O=Oligomycin, for two datasets, Exp=experimental values and Sim=Simulation values. The y-axis indicates the fold change calibrated using the concentration under DMSO condition, which was scaled to 1 and the other concentrations were denoted with the same reference. The four bars on the left hand side represent experimental results whereas the four bars on the right hand side present our data. We of course did not expect 100% agreement but the pattern or trend can be clearly seen.



Comparisons of the experimental and simulation results are seen in this set of bar diagrams. Six key components of the Krebs cycle are compared with experimental and simulated values. The arbitrary concentrations have been converted to relative values with respect to the first column (all the bars on the left and right hand panels in all the six graphs have unity values). Although the differences can be seen, the trend is very clear.