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Transformation of Glucokinase under Variable Rate Constants and Thermal Conditions: A Mathematical Model

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Abstract

The glucokinase (GK) in cells plays a pivotal role in the regulation of carbohydrate metabolism and acts as a sensor of glucose. It helps us to control glucose levels during fast and food intake conditions through triggering shifts in metabolism or cell functions. Various forms of hypoglycaemia and hyperglycaemia occur due to the transformations of the gene of the Glucokinase. The mathematical modelling of enzyme dynamics is an emerging research area to serve its role in biological investigations. Thus, it is imperative to establish a mathematical model to understand the kinetics of native and denatured forms of enzyme-GK under thermal stress with respect to time. The formulation of the current model is based on the number of non-linear ordinary differential equations with suitable initial and boundary conditions. The transformations of glucokinase were studied using mathematical and computational simulations in order to estimate the concentration of native and denatured enzyme forms with respect to different rate constants and under various thermal changes. The results obtained in this model were verified with the empirical outcome of Sanchez Ruiz et al. and Weinhouse for the validity and efficacy of the formulated model.

Keywords: Enzymes; Glucokinase; Transformation; Model; Denaturation; Ordinary differential equations; Rate constants

MSC 2020 No.: 92F05, 32G34, 35L05

1. Introduction

Glucose, or usually called sugar is a significant energy source that is required by all the cells and organs in the biological systems. Glucose flows in the blood cells as blood sugar to feed tissues and body organs. Glucose is produced by plants and does not occur in its free form, but in the form of its polymers. Carbohydrates like natural products (fruits), bread, oats are well-known sources of glucose. Glucose is phosphorylated to glucose-6-phosphate by glucokinase or hexokinase IV or D, which is the initial step of glycolysis.

Glucokinase is the first enzyme of glycolytic pathway that causes phosphorylation of glucose to glucose-6-phosphate. Most of the glucokinase is found in the liver and pancreas of humans and most other vertebrates. In each of these organs, it plays a significant role in the regulation of carbohydrate metabolism by acting as a sensor of glucose, triggering shifts in metabolism or cell function in response to rising or falling levels of glucose, occur after a meal or when fasting (Satyanarayana; Sweet Ian et al. (2013)). Transformations of the gene for this enzyme can cause abnormal types of diabetes or hypoglycemia (Osbak et al. (2009)).

Glucokinase (GK) is a hexokinase isozyme, related homologously to at least three other hexokinases. The initial step of both glycogen synthesis and glycolysis is that entirety of the hexokinases can intercede phosphorylation of glucose to glucose-6-phosphate (G6P) (Wilson (1995)). Glucokinase is coded by a different gene and its distinctive kinetic properties permit it to serve an alternate set of functions. It has a lower affinity for glucose than the other hexokinases, and its activity is limited to a couple of cell types, leaving the other three hexokinases as more significant prepares of glucose for glycolysis and glycogen synthesis for most tissues and organs. Because of this reduced affinity, the activity of glucokinase, under regular physiological conditions, differs generously as per the concentration of glucose (Matschinsky et al. (2011)). Further GK enzyme is not inhibited by its product G-6-P and that permits proceeded with signal yield, i.e., to trigger insulin discharge in the midst of huge amounts of its product (Colowick (1973)). Such activities allow GK to regulate a supply driven metabolic pathway. This means that the rate of reaction is driven by the supply of glucose and not by the demand for end products (Satyanarayana; Liang et al. (1995)).

Glucokinase is found in vertebrate tissues. It is one of the four glucose-phosphorylating enzymes (Cardenas et al. (1998); Davis et al. (1999)). It is manifested in liver, pancreatic β-cells and certain rare neuroendocrine cells of the brain and gut (Arden et al. (2007); Jetton et al. (1994)). Glucokinase plays an important role in glucose homeostasis. It is regulated by a protein known as glucokinase regulatory protein (Matschinsky et al. (2011)). It plays a crucial role in the regulation of insulin secretion and transform the glucose sensor in pancreatic β- cell (Matschinsky et al. (1968)). Its kinetics permits pancreatic β-cells to change glucose phosphorylation rate over a range of physiological glucose concentrations (4-15 m mol./l) (Weinhouse (1976)). To analytically check the hypothesis that glucokinase controls the rate of glucose metabolism and henceforth the rate of insulin secretion, a mathematical model of glycolysis in the pancreatic β cell was developed (Iynedjian (1993)). The model addresses how the normal β-cell transduces the glucose signal (Matschinsky et al. (2011); Sweet Ian et al. (2013)). It is understandable that changes in the gene of the enzyme-GK can cause both hyper and hypoglycemia (Kara K Osbak et al. (2009); Sogan et al. (2006)). In bio-

chemical genetic investigations of diabetes and hyperinsulinism, functional and structural stability of glucokinase are important factors and equally significant (Fenner et al. (2011); Gloyn A.L. et al. (2003)). The stability and kinetics of glucokinase are influenced by several osmolytes (like glycerol and urea) and activators (like glucokinase activator drug (GKA)) (Sagen et al. (2006); Quinn et al. (2018); Zelent et al. (2012)). Glucokinase cooperativity is exclusively associated with the conformational reorganization that is experienced upon glucose association (Larion et al. (2012)). The results of temperature on enzyme activity are complicated, and can be considered as two forces acting together but in opposite directions. As the temperature is raised, the fraction of molecules and hence the rate of reaction increases but at the same time there is a continuous inactivation due to denaturation of the enzyme. Thermal denaturation is time dependent. So the thermal stability of an enzyme can be decided by first revealing the enzyme to a range of temperatures for a specific time frame and eventually measuring its activity at one favourable temperature (e.g., $25°$C). The temperature at which denaturation becomes significant differs enzyme to enzyme. Generally, it is negligible below $30°$C and starts to become observable above $40°$C. The effect of decreasing temperature on the native enzyme at which denaturation begins to reduce the activity of the enzyme can be investigated to determine activation energy of the reaction (Peter (2015)).

It is considered that the thermal denaturation of glucokinase is occurring in two steps, an initial equilibrium process followed by an irreversible step. The hypothesis is made that denaturation is in principle reversible at least for a significant part of an essential amino-acid (tryptophan fluorescence (TF)) and differential scanning calorimetry (DSC) melting transitions allowing the application of equilibrium thermodynamic analysis (Sanchez-Ruiz (1992); Zelent et al. (2012)).

It is worthwhile to mention here that no meticulous mathematical expressions of molar concentrations of the native form, denatured form and thermal inactivation of Glucokinase at various rate constants k_1, k_{-1}, k_2 have been accounted. The purpose of this mathematical model is to derive simple approximate analytical expression for the non-steady state concentrations for thermal inactivation of glucokinase under different rate constants and at various thermal changes. The two dimensional differential equations can be solved by using the reduced differential transform method (Noori et al. (2019)). The exp-function method can also be used to get the exact solution of some differential equations (Eskandari et al. (2020)).

2. Mathematical Model

2.1 Model Formulation

The two-step mechanism of thermal inactivation of the native form of the enzyme-N (GK), into a denatured form N_{DF} via partially denatured form N_{DP} is given below:

$$N \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} N_{DP} \overset{k_2}{\longmapsto} N_{DF}, \tag{1}$$

where k_1, k_{-1} and k_2 represent the rate constants.

The first order irreversible reaction kinetic model has been widely used for the analysis of en-

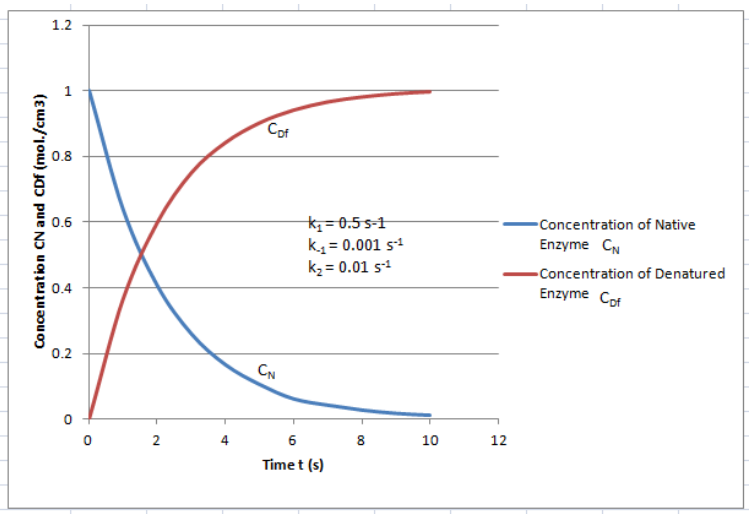

Figure 1. Concentration C_N and C_{DF} (mol/cm^3) vs.time t at $k_1 = 0.5/s$, $k_{-1} = 0.001/s$ and $k_2 = 0.01/s$

zyme denaturation (Dewey (1994); Pearce (2009). The reaction rate of the irreversible process is described by a temperature dependent rate constant (Arrhenius equation),

$$K = A \exp\left(\frac{-E_a}{RT}\right),$$

where A is the frequency factor (s^{-1}), E_a is the activation energy (KJmol^{-1}), R the gas constant. E_a and A are used to describe the kinetics of thermally induced protein denaturation (Zhenpeng et al. (2014)).

Let $C_N(t)$, $C_{DP}(t)$ and $C_{DF}(t)$, respectively, denote the molecular concentrations of native, partially denatured and fully denaturated forms of enzyme-Glucokinase at any time t. Therefore, by the law of mass action, the rate equations from the above reaction can be put in mathematical form as:

$$\frac{dC_N}{dt} = -k_1 C_N + k_{-1} C_{Dp}, \tag{2}$$

$$\frac{dC_{D_P}}{dt} = k_1 C_N - (k_{-1} + k_2) C_{DP}, \tag{3}$$

$$\frac{dC_{DF}}{dt} = k_2 C_{DP}. \tag{4}$$

From Equations (2)-(4), it follows that

$$C_N + C_{DP} + C_{DF} = Constant.$$

Also, the thermal inactivation is governed by the following mathematical expression

$$\frac{dT}{dt} = K_c(T_B - T), \tag{5}$$

where T is the variable temperature, K_c is proportionality factor including the heat transfer coefficient and T_B is the bath temperature.

Further, it is assumed that the bath temperature of the enzyme is $20°C$ and the initial conditions on

the parameters are assumed as

$$C_N = C_{N_0} = 1, \quad C_{Dp} = 0, \quad C_{Df} = 0, \quad T = 20 + T_B, \tag{6}$$

at $t = 0$.

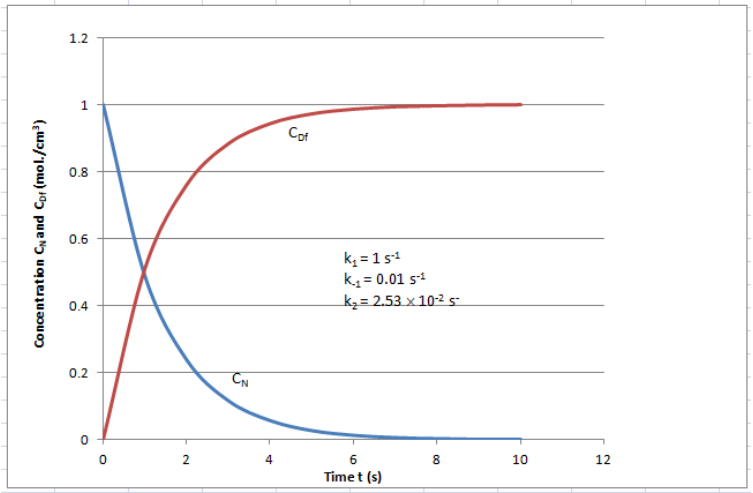

Figure 2. Concentration C_N and C_{DF} (mol/cm^3) vs. time t at $k_1 = 0.5/s$, $k_{-1} = 0.01/s$ and $k_2 = 2.53 \times 10^{-2}/s$

2.2 Solution and interpretation of the model

Since partially denatured reaction goes to a quasi-quilibrium or quasi-steady state in which the concentration of partially denatured enzyme form barely changes. Therefore, we have

$$\begin{aligned} \frac{dC_{DP}}{dt} &= 0, \\ C_{DP} &= \frac{C_N}{K_m}. \end{aligned} \tag{7}$$

The analytical expression of molar concentrations of C_N, C_{DP}, C_{DF} and temperature dynamics are calculated using the method of separation of variables:

$$C_N = C_{N_0} e^{-Kt}, \tag{8}$$

$$C_{DP} = \frac{C_{N_0} e^{-Kt}}{k_m}, \tag{9}$$

$$C_{DF} = \frac{C_{N_0} k_2}{K k_m}(1 - e^{-Kt}), \tag{10}$$

$$T = T_B + 20e^{-K_c t}, \tag{11}$$

where $K = k_1 - \frac{k_{-1}}{k_m}$, $k_m = \frac{k_{-1}+k_2}{k_1}$.

The equations (8) - (11) represent the analytical expression of the molar concentrations of the native enzyme, partially denatured enzyme, fully denatured enzyme and temperature for various values of the parameters k_1, k_{-1}, k_2, T_B and K.

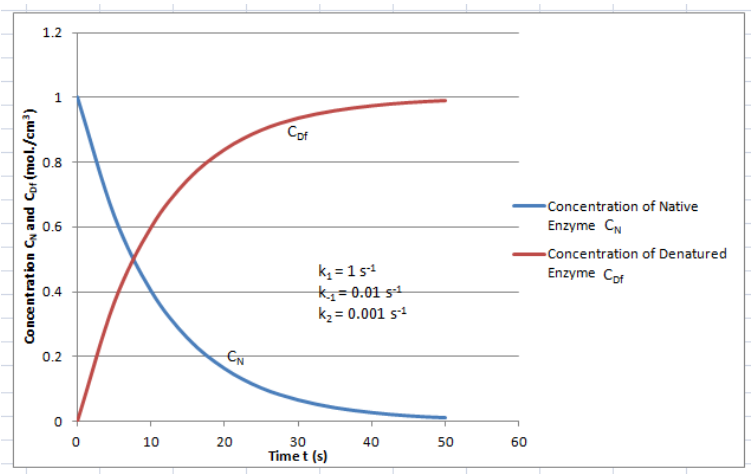

Figure 3. Concentration C_N and C_{DF} (mol/cm^3) vs. time t at $k_1 = 1/s$, $k_{-1} = 0.01/s$ and $k_2 = 0.001/s$

The separation of variables method has been used to establish the solution of equations $(2, 3, 4, 5)$, i.e.,

$$\begin{aligned} \frac{dC_N}{dt} &= -k_1 C_N + k_{-1} C_{DP}, \\ \frac{dC_{DP}}{dt} &= k_1 C_N - (k_{-1} + k_2) C_{DP}, \\ \frac{dC_{DF}}{dt} &= k_2 C_{DP}, \\ \frac{dT}{dt} &= K(T_B - T). \end{aligned} \tag{12}$$

Using Equation (7) in (2), we have

$$\begin{aligned} \frac{dC_N}{dt} &= -k_1 C_N + \frac{k_{-1}}{k_m} C_N, \\ &= -(k_1 - \frac{k_{-1}}{k_m}) C_N, \end{aligned}$$

or,

$$\frac{dC_N}{C_N} = -K dt,$$

$$C_N = C_{N_0} e^{-Kt}. \tag{13}$$

Therefore, from Equations (7) and (8), we have

$$C_{DP} = \frac{C_{N_0} e^{-Kt}}{k_m}. \tag{14}$$

Also,

$$\frac{dC_{DF}}{dt} = \frac{k_2 C_{N_0 e^{-Kt}}}{k_m},$$

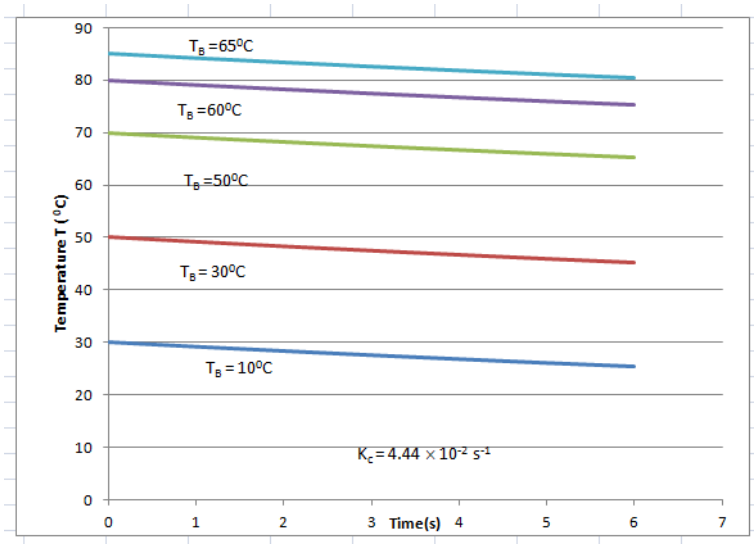

Figure 4. Temperature T vs. time t at various bath temperatures, where $k_c = 4.44 \times 10^{-2}/s$

or,

$$C_{DF} = \frac{C_{N_0} k_2}{K k_m}(1 - e^{-Kt}). \tag{15}$$

Also, from Equation (5), we have

$$\begin{aligned} \frac{dT}{dt} &= K_c(T_B - T), \\ \frac{dT}{T_B - T} &= K_c dt, \\ log(T_B - T) &= -K_c t + a, \\ T_B - T &= a_1 e^{-K_c t}. \end{aligned} \tag{16}$$

Using initial conditions, it implies $a_1 = 20$,

Therefore,

$$T = T_B + 20e^{-K_c t}, \tag{17}$$

where $K_c = 4.44 \times 10^{-2} S^{-1}$.

Since temperature is a time dependent function and the graph between time and temperature at different bath temperatures is shown in Figure 4.

Thus, from the model equations (13)-(15) and (17) obtained above, one can easily determine the concentration of native, partially denatured, fully denatured forms of enzyme (GK) and also thermal effect on the enzyme (GK) respectively.

Table 1. Values of the parameters used in this paper (Illeova et al. (2003); Ananthi et al. (2013); Zelent et al. (2012))

Parameter	Range	Figures 1, 2, 3	Figure 4	Figure 5, 6
$k_1(s^{-1})$	$(8.51 \pm 0.29) \times 10^{-1}$	$0.5, 1$		$0.25 - 4$
$k_{-1}(s^{-1})$	$(8.36 \pm 0.28) \times 10^{1}$	0.01		0.001
$k_2(s^{-1})$	$(1.74 \pm 0.79) \times 10^{-2}$	$0.001 - 0.0253$		0.01
$K_c(s^{-1})$	$4.440000000 \times 10^{-2}$		4.4400×10^{-2}	
$T_B\ (^0C)$	$10.00\ ^0C - 60.00\ ^0C$		$10.00\ ^0C - 65.00\ ^0C$	

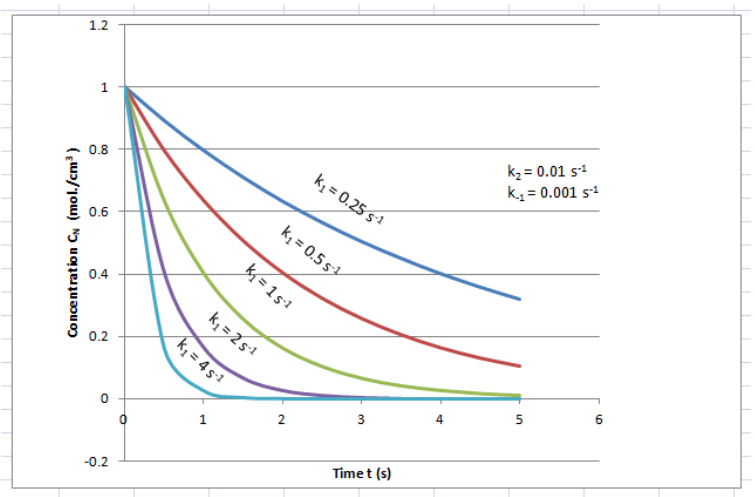

Figure 5. Concentration $C_N(mol/cm^3)$ vs. time t at different values of $k_1, k_{-1} = 0.001/s$ and $k_2 = 0.01/s$

3. Numerical Illustrations

A mathematical model based on ordinary differential equations together with appropriate boundary conditions has been formulated to understand the dynamics of enzyme - glucokinase and its transformations under various temperatures and at different rate constants. The solution of the formulated model equations was obtained using analytical approach and the simulation of results was carried out using mathematical software. The concentration profiles of native form, partially denatured form and fully denatured form of enzyme - glucokinase was established and is shown in Equations (13)-(15). Also, the effect of temperature can be computed based on the formula given in Equation (17). The results obtained in this model are at par with the empirical results obtained by Sanchez Ruiz et al. (1988) and Weinhouse (1976).

In this work, we obtained the analytical solution of transformed enzyme concentrations in terms of

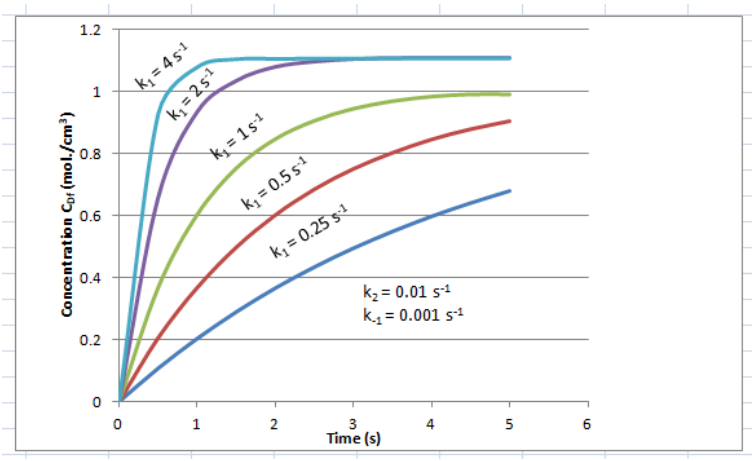

Figure 6. Concentration $C_{DF}(mol/cm^3)$ vs. time t at different values of $k_1, k_{-1} = 0.001/s$ and $k_2 = 0.01/s$

rate constants k_1, k_{-1}, k_2, bath temperature T_B, and coefficient in the enthalpy balance K. The non -linear ordinary differential equations have been solved analytically for the estimation of molar concentrations of C_N, C_{DF} and temperature T in a closed domain.

4. Conclusion

The Equations (8) and (10) represent the explicit solution of an analytical expression of concentrations of native form and denatured form of enzyme for all values of parameters. In Figures 1 - 6, the analytical results for various values of parameters are noted. Figures 1, 2, and 3 represent the molar concentrations for various values of rate constants. From these figures, it is concluded that, when time increases the concentration of the native form of enzyme C_N decreases, whereas the concentration of the denatured form of enzyme C_{DF} increases. The time taken to reach the maximum value of C_D is the same as the time taken to reach the minimum value of C_N. Figure 5 represent the molar concentration of N versus time t for various values of forward rate constant k_1 and it is observed that, C_N decreases when k_1 increases, whereas C_{DF} initially increases and reaches the steady state value when $t \geq 4$. So, it is evident from the graphs that concentration C_{DF} increases when k_1 increases. From Figure 6, it shows that when $k_1 = 4s^{-1}$, the steady-state reaches quickly as compared to when $k_1 = 0.25s^{-1}$. So, it is straightforward that as we increase the rate of forward reaction, the steady state reaches very quickly. Figure 4 represents the temperature representation T versus time t for various values of T_B. When T_B increases, the value of temperature also increases. The results can be further extended by incorporating other relevant parameters like pH, concentration, etc.

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