mutiara medika
Jurnal Kedokteran dan Kesehatan

Mutiara Medika: Jurnal Kedokteran dan Kesehatan
http://journal.umy.ac.id/index.php/mm

Vol 25 No 2 Page 15-22, July 2025

In Silico Modeling of Glucokinase Mutation Effect on Thermodynamics and Enzymatic Kinetics in Diabetes Mechanism

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DATE OF ARTICLE:
Received: 19 May 2025
Reviewed: 20 May 2025
Revised: 22 Jul 2025
Accepted: 04 Aug 2025

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DOI:
https://doi.org/10.27011/mmjkk.v25i2

TYPE OF ARTICLE:
Research

Abstract: Mutations in the glucokinase (GCK) enzyme can disrupt the glucose metabolism pathway, potentially increasing the risk of diabetes mellitus. This study attempts to examine the impact of GCK mutations in silico, focusing on thermodynamic and kinetic aspects. Four specific GCK mutations (43 R→H, 131 S→P, 160 D→N, 182 V→L) were analyzed at physiologically temperatures (298-313K) and pH 7.4. Computational analysis revealed that all mutations significantly altered Gibbs free energy (ΔG) values, with the wild-type enzyme showing -14.39J compared to substantially reduced negative values (-1.23J to -2.94J) in mutant forms. Enthalpy changes (ΔH) demonstrated significant linear regression relationships ($p<0.05$) for most mutations, indicating thermodynamic destabilization of the enzyme structure. Reaction rate constants showed decreased catalytic efficiency across all mutations (wild-type: 1.614×1027 s-1 vs. mutants: 1.606 - 1.607×1027 s-1). Three-dimensional visualizations confirmed structural perturbations at mutation sites. These findings suggest that GCK mutations impair glucose-sensing capabilities through dual mechanisms: reduced catalytic efficiency and thermodynamic instability, potentially the altered insulin secretion thresholds observed in Maturity-Onset Diabetes of the Young type 2 (MODY2) and other diabetes subtypes. Integrating kinetic and thermodynamic parameters provides valuable insights for developing targeted therapeutics such as glucokinase activators (GKAs), offering a ray of hope for diabetes treatment.

Keywords: Diabetes Mellitus; Enzyme Kinetics; Glucokinase; MODY2; Thermodynamics.

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic hyperglycemia due to impaired insulin secretion, insulin action, or both.[1,2] Based on its etiology, DM is classified into several main types: type 1, type 2, and monogenic forms, such as MODY. The MODY is a monogenic form of diabetes characterized by autosomal dominant inheritance, which results from gene mutations that affect insulin production and are often misdiagnosed as type 1 or type 2 diabetes. Various subtypes exist depending on the

specific gene involved. One of the most studied forms of MODY is GCK-MODY / MODY2, caused by GCK gene mutations.[3] In addition, there is also the clinically important condition glucokinase hyperinsulinism (GCK-HH), which reflects another spectrum of GCK mutations that cause hypoglycemia.[4,5]

Glucokinase, a key enzyme in glucose metabolism, acts as a glucose sensor in pancreatic β-cells and a regulator of glucose flow in the liver. What makes it truly fascinating are its unique kinetic characteristics of low affinity for glucose and its resistance to inhibition by glucose-6-phosphate. This allows GCK to act as a metabolic switch that responds to glucose levels in the blood.[6,7] Mutations in GCK can lead to fundamental changes in enzyme function, which ultimately directly impact the regulation of insulin secretion and blood glucose balance.[8,9]

Kinetically, GCK mutations can alter important enzymatic parameters such as Km (affinity to glucose) and Kcat (catalytic capacity), as well as the Kcat/Km ratio. This ratio, as an indicator of catalytic efficiency, plays a crucial role in understanding enzyme activity.[10,11] In MODY2, mutations are generally loss-of-function, increasing Km and/or decreasing Kcat, causing decreased β-cell sensitivity to glucose and resulting in mild hyperglycemia that is stable since childhood. In contrast, in GCK-HH, mutations are gain-of-function that decrease Km or increase Kcat, causing excess insulin release even at low glucose levels.[10]

It is important to note that not all effects of mutations can be explained from the kinetic angle alone, but also from the thermodynamic aspect. The significance of these analyses cannot be overstated. Thermodynamic analysis provides crucial insight into how mutations can alter protein fold stability and the distribution of active/inactive conformations.[12] Changes in ΔG due to mutations can affect the proportion of active forms of GCK, thus explaining both increases and decreases in enzyme activity without significant changes in Km or Kcat values. The integration of kinetic and thermodynamic data is not just beneficial, but essential for a more thorough understanding of the structure-function relationship of this enzyme.[13]

Interestingly, although GCK is not a gene directly involved in type 1 (T1DM) or type 2 (T2DM) DM, recent studies have shown that mutations in GCK can affect the activation threshold of insulin secretion.[14] In T1DM, showing autoimmune damage to pancreatic β-cells, GCK mutations that reduce glucose sensitivity can accelerate β-cell exhaustion and increase susceptibility to metabolic stress. In T2DM, insulin resistance accompanied by GCK mutations can exacerbate early-stage insulin secretion defects, thereby exacerbating hyperglycemia.[15] Research in Sidoarjo, East Java, has found a mutation at point rs7903146 in patients diagnosed with type 2 diabetes in a wound house in Sidoarjo, East Java. Therefore, although not causal, GCK mutations can be a modifier contributor in the pathogenesis of both types of DM.[16]

Research on mutations in GCK associated with kinetics and thermodynamics has been relatively scarce. Therefore, this study, which will analyze GCK mutations affecting enzyme function in terms of kinetics and thermodynamics with a computational approach, is a novel and promising endeavor. The potential development of mutation-based therapies, such as glucokinase activators (GKAs), adds an exciting dimension to this research.

MATERIALS AND METHOD

Instrumentation

The computer used has a 13th Gen Intel(R) Core (TM) i5-1335U 1.30 GHz Processor specification, 16.0 GB RAM (15.7 GB usable), type 64-bit operating system, and an x64-based processor.

Source Glucokinase Enzyme

The glucokinase enzyme sequence was downloaded from https://www.uniprot.org/ with code P35557-HXK4_HUMAN. The enzyme consists of 465 amino acid residues and was downloaded using the FASTA format. [1]

Glucokinase Mutation Analysis

In this study, 4 glucokinase mutation models were used, namely 43 R → H; 131 S → P; 160 D → N; and 182 V → L. These mutations would produce ΔG by entering temperature data (K) 298, 303, 310, and 313 with pH 7.4 on the webserver https://folding.biofold.org/i-mutant/i-mutant2.0.html.[18,19]

Glucokinase Mutation Visualization

The GCK mutations are visualized in 3 dimensions using a free webserver, http://genetics.bwh.harvard.edu/pph2/.[20]

Bioenergetics of Glucokinase Mutations

Bioenergetics calculations used thermodynamic parameters, namely ΔG and enthalpy change (ΔH). The value of ΔH can be calculated from the thermodynamic relationship, namely ΔG = ΔH-TΔS. The relationship can be established using linear regression with the X-axis being T, while the Y-axis is ΔG. The value of ΔH would be calculated on each glucokinase mutation model.[21] If the value of ΔG = negative, the reaction would proceed spontaneously, while if ΔG = positive, the reaction would not proceed spontaneously. Meanwhile, if ΔH = positive, it means that the reaction took place endergonically or required ATP, while ΔH = negative means that the reaction took place exergonically or produced ATP.

Kinetics of Glucokinase Mutation

Kinetics is a parameter that reveals the rate of the glucokinase catalysis reaction to glucose as a substrate. The reaction rate can also be interpreted as the speed of the glucose-catalyzed reaction by glucokinase. The reaction rate can be determined by calculating the value of the reaction rate constant (k). [22,23] The relationship between reaction rate and ΔG can be determined by using the Eyring equation[24] as follows:

$$k = \frac{Kb.T}{h} e^{\frac{\Delta G}{RT}}$$

Description:

k = reaction rate (s^{-1})
Kb = Botzman constant = 1.381 × 10^{-23} J K^{-1}
h = Planck constant = 6,626 × 10^{-34} J s
R = Ideal gas constant = 8,314 J K^{-1}
T = temperature (K)
ΔG = delta Gibbs free energy (Joule)

RESULT

Glucokinase is a key enzyme involved in carbohydrate metabolism. Located in the cytoplasm, this enzyme converts glucose into glucose-6-phosphate. The sequence of this enzyme can be seen in Figure 1.

>sp|P35557|HXK4_HUMAN Hexokinase-4 OS=Homo sapiens OX=9606 GN=GCK PE=1 SV=1

10	20	30	40	50	60	70	80
MLDDRARMEA	AKKEKVEQIL	AEFQLQEEDL	KKVMRRMQKE	MDRGLRLETH	EEASVKMLPT	YVRSTPEGSE	VGDFLSLDLG
90	100	110	120	130	140	150	160
GTNFRVMLVK	VGEGEEGQWS	VKTKHQMYSI	PEDAMTGTAE	SFPVRHEDID	HKKLPLGFTF	MLFDYISECI	SDFLDKHQMK
170	180	190	200	210	220	230	240
KGILLNWTKG	FKASGAEGNN	VVGLLRDAIK	RRGDFEMDVV	AMVNDTVATM	ISCYYEDHQC	EVGMIVGTGC	NACYMEEMQN
250	260	270	280	290	300	310	320
VELVEGDEGR	MCVNTEWGAF	GDSGELDEFL	LEYDRLVDES	SANPGQQLYE	KLIGGKYMGE	LVRLVLLRLV	DENLLFHGEA
330	340	350	360	370	380	390	400
SEQLRTRGAF	ETRFVSQVES	DTGDRKQIYN	ILSTLGLRPS	TTDCDIVRRA	CESVSTRAAH	MCSAGLAGVI	NRMRESRSED
410	420	430	440	450	460		
VMRITVGVDG	SVYKLHPSFK	ERFHASVRRL	TPSCEITFIE	SEEGSGRGAA	LVSAVACKKA	CMLGQ	

Figure 1. glucokinase sequence enzyme with P35557 · HXK4_HUMAN.

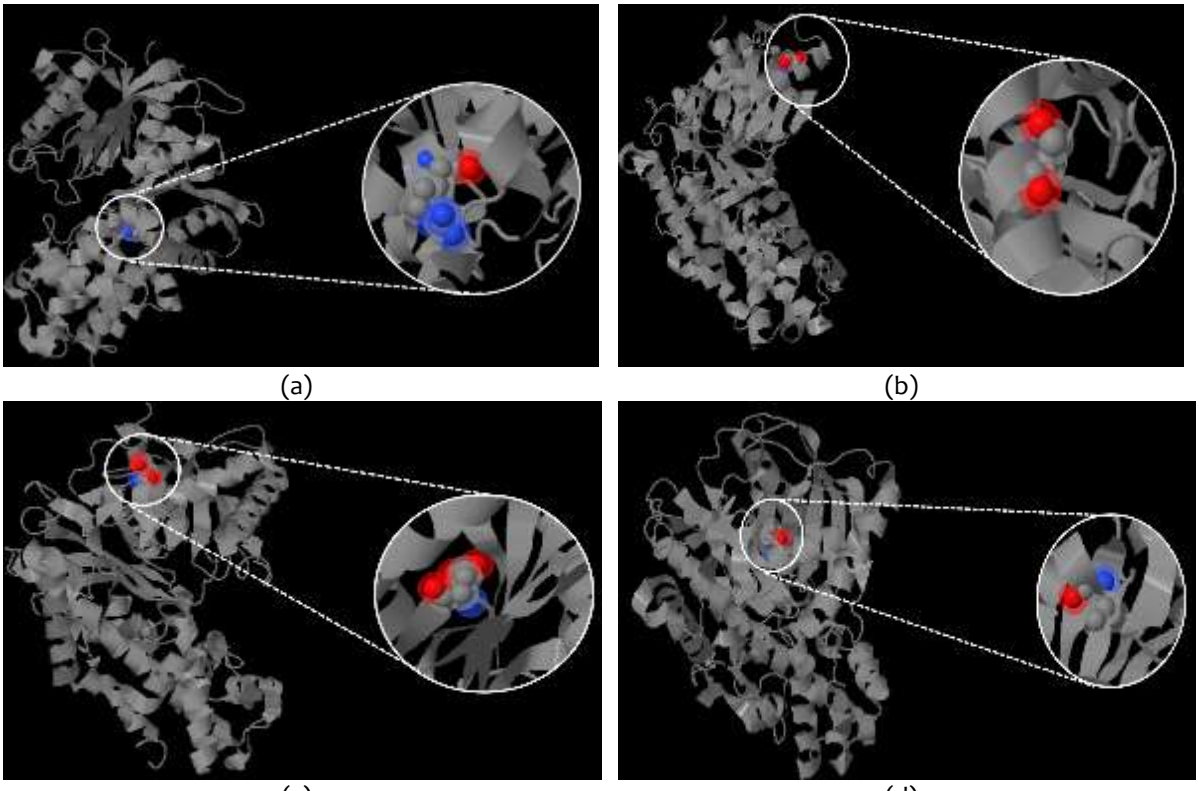
The sequence in Figure 1 was then modified by mutation using the help of https://folding.biofold.org/i-mutant/i-mutant2.0.html at various temperatures and pH = 7.4, resulting in the value of ΔG as in Table 1.

Table 1. Gibbs Free Energy (ΔG) and Entalphi Changes(ΔH) in T = 310 K; pH=7.4

Mutation	ΔG (J)	ΔH (J)	Linear Regression	p
Non	-14.39	1.173.57	ΔG= 0.0007 – 15.2115 ΔH	0.0022*
43 R→ H	-1.23	36.64	ΔG= 0.0078 – 1.515 ΔH	0.0013*
131 S →P	-1.30	28.40	ΔG= 0.0005 – 1.3142 ΔH	0.3119
160 D → N	-2.94	19.366	ΔG= 0.0041 – 3.0914 ΔH	0.0006*
182 V → L	-1.30	42.185	ΔG= 0.0049 – 1.4827 ΔH	0.0034*

*) p < 0.05 = significant linear graphic

Table 1 illustrates that the DG value tends to lead to positive values, signifying a decrease in enzyme structure stability. This decrease in stability has a profound impact on the reaction's spontaneity, causing a significant slowdown in the conversion of glucose by glucokinase. The structure of the mutated enzyme, a key focus of this current research, is presented in Figure 2.



(a) (b)

(c) (d)

Figure 2. (a) Mutation 43 R→ H (b) 131 S →P (c) 160 D → N (d) 182 V → L (red and blue color indicates where the mutation occurred)

Figure 2 depicts the mutations that can cause changes in the stability of the protein structure that can disrupt the enzyme's active site. This disruption results in a decrease in the rate of binding to the substrate. This can be shown in Table 2.

Table 2. Reaction rate based on the Eyring equation

Mutation	ΔG (J mol^{-1})	k (10^{27} s^{-1})
Non	-14.39	1.614
43 R→ H	-1.23	1.606
131 S →P	-1.30	1.606
160 D → N	-2.94	1.607
182 V → L	-1.30	1.606

DISCUSSION

Research on GCK mutations has been conducted to understand the important role of this enzyme as the main glucosensor in pancreatic β-cells and hepatocytes, regulating blood glucose levels, especially in individuals with metabolic disorders such as MODY2.[25] The GCK catalyzes the phosphorylation reaction of glucose to glucose-6-phosphate, the first stage in glucose metabolism. Research by Li et al. [26] states that mutation studies on the GCK gene show that specific amino acid changes can decrease the enzyme's affinity for glucose and its catalytic rate, increasing the glucose threshold for insulin secretion. This explains the symptoms of chronic mild hyperglycemia in patients with MODY2.[25]

The research by Gay et al. [27] has delved into the intricate world of GCK mutations, identifying mutations such as G261R, V62M, and T228M that cause instability of the enzyme structure and disrupt the dynamics of the active domain in vitro and silico. The meticulous kinetics analysis revealed that these mutations reduced the Kcat value and increased Km, thereby lowering the catalytic efficiency. Furthermore, the thermodynamic simulations unveiled that the mutation increased ΔG and ΔH, shifting the reaction from a spontaneous to an endergonic state. These mutations also weaken crucial hydrogen interactions and cause the enzyme structure to become more flexible and unstable in physiological environments.[12, 28]

This study, with its significant findings, showed that the mutation of the enzyme GCK caused a decrease in the catalytic rate.[10,29] This indicates that the mutation reduces the reaction speed and decreases the enzyme's affinity for glucose, thus disrupting the physiological function of GCK as a glucose sensor in pancreatic β-cells.[29]

In biochemical systems, the rate of enzymatic reactions is strongly influenced by the enzyme's ability to stabilize the transition state. This study revealed that GCK mutation plays a significant role in disrupting the stabilization of the transition state, leading to an increase in ΔG and a decrease in the enzymatic reaction rate. [30, 31]

Research by Xie et al. [32] GCK has shown that mutations in GCK, a key enzyme in glucose metabolism, can significantly affect the thermodynamics of the glucose phosphorylation reaction. These mutations can disrupt the conformational transition of the enzyme from inactive to active state and reduce the stability of the enzyme-substrate complex during the transition phase. An increased ΔG value from normal conditions indicates that the system becomes thermodynamically unfavorable to carry out the reaction. Under normal physiological conditions, converting glucose to glucose-6-phosphate (G6P) by GCK is spontaneous and requires ATP. This reaction thermodynamically reflects that the system naturally moves towards product formation by requiring additional external energy input. However, the results of this study revealed that mutations in GCK caused a decrease in the ΔH value. This situation is interpreted as a decrease in ATP utilization, resulting in a decrease in glucose in the phosphorylation reaction.[21, 33]

In a typical system, one ATP molecule efficiently donates a phosphate group to glucose. However, when a mutation disrupts the affinity for glucose or the enzyme's ability to stabilize the transition state, the efficiency of ATP utilization decreases. This can lead to an "ATP-wasting effect," a condition where ATP is consumed, but the reaction does not proceed optimally or even undergoes a reverse reaction. This finding has significant implications, suggesting that more ATP molecules may be required to produce the same amount of glucose-6-phosphate under normal conditions.[32]

Thermodynamically and kinetically occurring glucokinase mutations have the potential to cause conformational changes at the enzyme's active site, which could affect molecular binding with regulatory proteins or small molecule ligands, such as glucokinase activators. This potential, based on the results of previous research using molecular docking analysis, underscores the significant role this current research can play in drug development. These studies have shown that new drugs used for type 2 diabetes, such as glucokinase activators like dorzagliatin and RO-28-1675, work by binding to the allosteric site and stabilizing the enzyme's active site (Chow et al., 2023; Mehra et al., 2024).[34-35]

Comparison with dorzagliatin, a dual-acting GKA recently approved in China, showed that wild-type GCK has higher catalytic efficiency and binding stability with the activator than specific GCK mutants. The reduced catalytic rate (from 1.614×10^{27} s^{-1} to ~1.606×10^{27} s^{-1} in the mutant) and thermodynamic instability (a reduced ΔG value) observed in this current study underscore the urgent need to understand the effects of mutations on drug efficacy. This understanding could explain why GKA exhibits varying efficacy depending on the mutation context.[36]

This research uncovers a complex implication of higher metabolic load on pancreatic β-cells and hepatocytes regarding cellular bioenergetics. The revelation that ATP, a crucial component of insulin secretion, calcium signaling, and ion homeostasis, is also required for other important processes adds a layer of complexity to our understanding. The inefficient increase in ATP consumption by GCK due to the mutation disrupts the intracellular energy balance, potentially leading to metabolic dysfunction. This is particularly relevant in diabetes, as both β-cells and the liver play a key role in maintaining blood glucose levels.[33]

This thermodynamic phenomenon, as revealed by this current research, reinforces previous studies that glucokinase mutations affect kinetics and cause structural destabilization and disruption of energy homeostasis at the molecular level. The defect may lead to a higher insulin activation threshold in individuals carrying this mutation, as β-cells require a greater glucose concentration to induce insulin release. This understanding could pave the way for more effective treatments for hyperglycemia in patients with MODY2.[37]

The Glucokinase mutations not only decrease the enzyme's ability to recognize and process glucose kinetically but also cause changes in the thermodynamic properties of the reaction, making it more dependent on energy input (ATP). Loss-of-function GCK mutations can affect enzyme performance through two main mechanisms: (1) decreased catalytic efficiency due to impaired substrate interaction and (2) structural and thermodynamic instability that makes the reaction endergonic and biologically unfavorable.[38] Understanding these two aspects is crucial in designing molecular interventions that restore structural stability or enhance enzymatic activity through pharmacological or gene therapy approaches.

CONCLUSION

The results of these present studies reveal new insights into the kinetic and thermodynamic effects of GCK mutations via an extensive computational analysis. Our findings elucidate the molecular underpinnings of glucokinase failure in diabetes by illustrating how particular mutations affect catalytic efficiency and energy dynamics. These findings not only improve our comprehension of the pathophysiology related to GCK but also provide support for the development of targeted glucokinase activators (GKAs) as a promising therapeutic approach. In the end, this study establishes the foundation for tailored diabetes treatment strategies that target specific mutations.

ETHICAL CONSIDERATIONS

This study employed computational methods and publicly available data for in silico modeling of glucokinase mutations. No human subjects, animals, or biological samples were used in this research. Therefore, formal ethical approval was not required for this study.

ACKNOWLEDGEMENT

The author wishes to thank Lambung Mangkurat University.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest that might have influenced the work reported in this paper. This includes no financial and non-financial interests and relationships, no employment (whether full or part-time) in private sector or nonprofit boards and advisory panels, no paid or unpaid services that might affect the presentation of this study, and no personal, religious or political beliefs relevant to the topic discussed.

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