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Interactions of Heavy Metals on Enzymes in Carbohydrate Metabolism: In silico study on glucokinase and pyruvate kinase

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Abstract: Cadmium (Cd^{2+}) and mercury (Hg^{2+}) are heavy metals, which can cause chronic inflammation, oxidative stress, obesity, hyperglycemia, and diabetes. Exposure to heavy metals cadmium and mercury can interfere with glycolysis metabolic functions through the inactivation of two key enzymes: glucokinase (GK) and pyruvate kinase (PyK). However, pathomechanism is unknown. Based on the background, this research was conducted. Enzyme structures were obtained from the RCSB Protein Data Bank (http://www.rcsb.org) with the following codes, GK (GDP ID: 3IDH) and PyK (GDP ID: 4IP7). The interaction between Cd and Hg with this enzyme was used via the MIB site: Metal Ion Binding site prediction and docking server (http://bioinfo.cmu.edu.tw/MIB/). Next, the target protein's interaction between metal ions and amino acids was visualized on UCSF Chimera 1.15. Based on the research results, the metal ion bond of mercury was more reactive than cadmium based on the number of amino acid residues bound. The bond was stronger based on a lower distance with PyK and GK enzymes. Therefore, mercury and cadmium metal ions were considered to inhibit the glycolysis process by causing the inactivation of the two enzymes.

Keywords: cadmium; mercury; carbohydrate metabolism; glucokinase; pyruvate kinase

INTRODUCTION

Cadmium (Cd) and mercury (Hg) belong to the types of heavy metals that are toxic to the human body despite their low concentrations.[1] Cadmium is a heavy metal with atomic number 40 group 12 period 5 on the periodic table.[2] This metal may interfere with calcium absorption, affect glucose homeostasis and induce hyperglycemia.[3,4]

In addition to Cd, Hg metal is a heavy metal that is still used as a material for making thermometers, amalgam products, and making fluorescent lamps. Mercury has been shown to harm the development and function of pancreatic beta cells (β), forming free radicals, binding enzymes and structural proteins, and thus causing diabetes.[2,5,6]

Experimental-based animal studies revealed that exposure to cadmium positively causes an increase in glucose concentrations in the liver.[7] In addition, epidemiological studies in Suzhou City in China also mentioned that metals, manganese, copper, zinc, arsenic, selenium, and cadmium in plasma are associated with the morbidity of diabetes.[8]However, the mechanism of occurrence of diabetes remains unclear.

Previous research stated that Cd and Hg could be covalently bound to glycolysis enzymes to interfere with glucose oxidation.[9] The protein models used in the previous study were the Catalytic complex of Human

Glucokinase (PDB: 3FGU) and Pyruvate Kinase (GDP: 1ZJH). Meanwhile, in this study, Human pancreatic glucokinase in complex with glucose (PDB: 3IDH) and structure of the S12D variant of human liver pyruvate kinase in complex with citrate and FBP (PDB: 4IP7) were used. The two heavy metals interact by binding to the active and allosteric sides of the enzyme and forming a bond with the amino acid residues contained in the enzyme.[10] Nevertheless, the pathomechanism of the involvement of Cd and Hg as the cause of diabetes is still not fully known.[11] Therefore, this study aims to explore the interaction of Cd and Hg on the enzymes involved in carbohydrate metabolism, namely glucokinase and pyruvate kinase.

MATERIAL AND METHOD

Ligand and Protein Preparation

The interaction between cadmium and mercury ligands against enzymes was performed using MIB: Metal Ion-Binding site prediction and server docking (http://bioinmfo.cmu.edu.tw/MIB/). The enzymes were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/search), namely glucokinase enzyme with PDB code: 3IDH and pyruvate kinase enzyme with PDB code: 4IP7. Proteins were prepared by removing the natural ligand residues in the protein. Ligand and protein preparations were used by the Chimera 1.15 program (https://www.cgl.ucsf.edu/chimera/downl oad.html).

Enzyme Active Site Prediction

The active site of enzymes is where they react between enzymes and ligands. The interaction of amino acid residues contained in the active site causes the inactivation of the enzyme. The active site can be predicted using a server with a link http://sts.bioe.uic.edu/castp.

Analysis and Visualization

Analysis and visualization of docking results used the Chimera 1.15 program (https://www.cgl.ucsf.edu/chimera/downlod.html). Visualization was used to explain the interaction between ligands and receptor protein residues, namely the interacting amino acids, the type of the interaction, and the bond distance between the receptor protein ligands.[4,9]

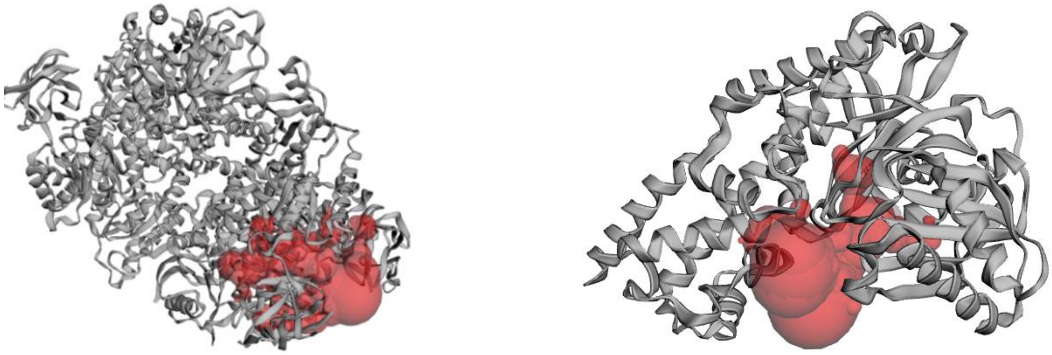
RESULT

The interaction between Cd and Hg on the enzymes glucokinase (3IDH) and pyruvate kinase (4IP7) can be seen in table 1.

Table 1. Interaction of Cd and Hg on the enzymes glucokinase (3IDH) and pyruvate kinase (4IP7)

Metals	Enzyme	Amino Acid Residue	Distances (Å)	Interaction	Binding Score
Cadmium	Pyruvate Kinase (4IP7)	GLU 297	1,589	Coordination Covalent Bonds	7.178
		GLU 294	7,556	Hydrophobic Bonds	7.178
	Glucokinase (3IDH)	GLU 14	4.413	Hydrophobic Bonds	1.745
		GLU 17	4.696	Hydrophobic Bonds	1.745
Mercury	Pyruvate Kinase (4IP7)	CYS 329	2,041	Coordination Covalent Bonds	6.993
		VAL 336	2,398	Coordination Covalent Bonds	4.057
		ILE 302	3,253	Hydrophobic Bonds	2.653
	Glucokinase (3IDH)	SER 411	2.612	Coordination Covalent Bonds	1.389

Meanwhile, the active site area of the Pyruvate kinase (pdb code: 4IP7) and Glucokinase (pdb code: 3IDH) can be seen in figure 1.



(a) (b)

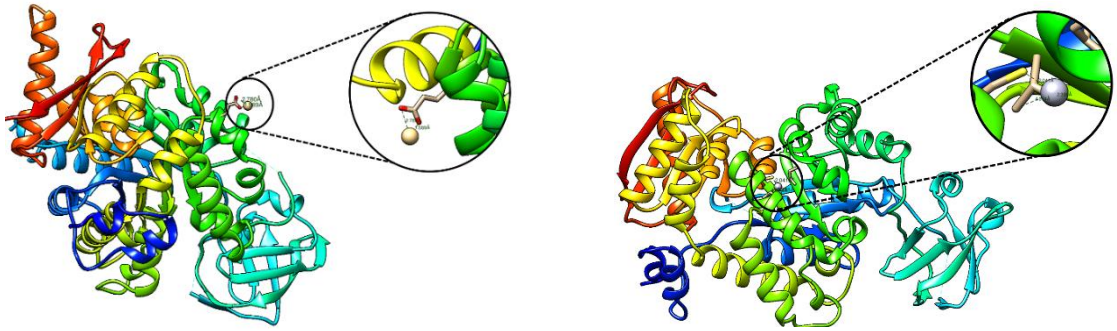
Figure 1. Active site of (a) Pyruvate kinase (pdb code: 4IP7) and (b) Glucokinase (pdb code: 3IDH)

The red color in figure 1 shows the enzyme's active site, where the reaction occurs.[12]

DISCUSSION

Carbohydrate metabolism includes glycolysis, glycogenesis, glycogenolysis, and gluconeogenesis. Glycolysis is the pathway of breaking glucose into pyruvate in an aerobic state or lactic acid in an anaerobic consisting of ten interconnected reaction sequences in providing a substrate and producing products from each series of reactions.[13,14]

Based on table 1, Hg interacts more with pyruvate kinase than Cd. Cadmium and mercury are bound to the other side, outside the active site (figure 2)



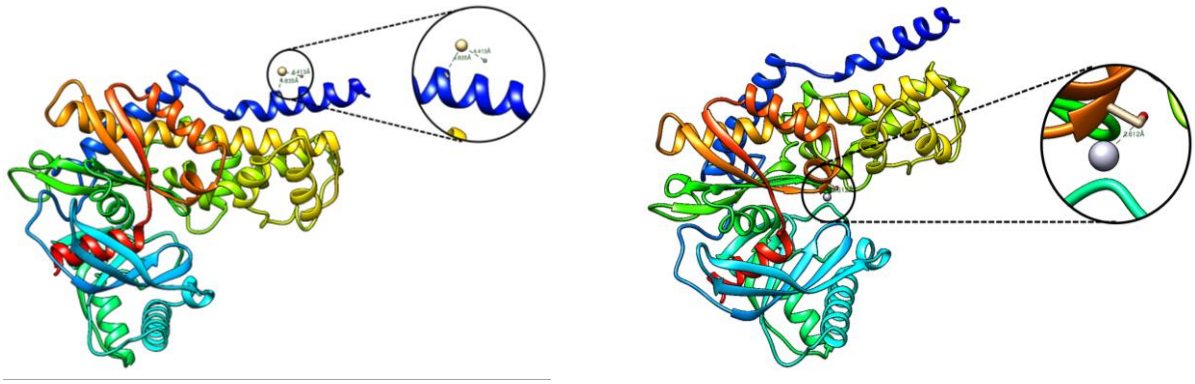
(a) (b)

Figure 2. Binding of pyruvate kinase enzymes by (a) Cd and (b) Hg

Cadmium and mercury bind to amino acid residues located on the allosteric site of the enzyme pyruvate kinase. The enzyme pyruvate kinase plays a role in glycolysis's final stages, converting phosphoenolpyruvate into pyruvate. Through the computational method that has been carried out by Schorman et al.,[15] this enzyme structure is known to have three main domains called "lid domains", namely domains A, B, and C. As a former of the active side of the pyruvate kinase enzyme, it consists of two arginine residues (Arg) and one lysine residue (Lys), as well as two aspartate residues (Asp) and two glutamate residues (Glu). Meanwhile, the other side is called the effector side, which can bind allosterically to enzymes.

In this study, Cd and Hg interacted with residues outside the active site of the enzyme pyruvate kinase, which has been known to involve five active residues that bind to hydrogen, namely arg50, Lys239, Thr297, Gly264, and Asp265 residues in the gap formed by domains A and B of the pyruvate kinase enzyme. Thus, the interaction of Cd and Hg will bind the allosteric side of enzymes containing the sulfhydryl group of cysteine residues covalently with metals. The high affinity of Cd and Hg for the sulfhydryl group of the enzyme catalytic site is the main motive commonly known in enzyme inactivation due to exposure to Hg. According to the research of Sabir et al.,10, metals have a high affinity for electrons, so they can affect the structure of enzymes.[16]

In addition to the enzyme pyruvate kinase, Cd and Hg interact with the enzyme glucokinase, as seen in figure 3.



(a) (b)

Figure 3. Glucokinase enzyme binding by (a) Cd and (b) Hg

Glucokinase enzyme converts a glucose substrate into its product, namely glucose – 6 – phosphate.[17] Glucoskinase has one active side for glucose and one for ATP. This side is on the small domain and the main domain.[17] The active side circle of glucokinase is at residue 151-180, an irregular structure.[19]

In this study, Cd and Hg were known to interact with residues which were the inactive sides of the glucokinase enzyme. It has been known that the active residue of the glucokinase enzyme was residue 151-180. Thus, the interaction of Cd and Hg would bind to the allosteric site of the enzyme. Cd would bind to glutamate residues hydrophobically with the metal. Meanwhile, Hg would bind to serine residues covalently with the metal. The interaction of glucokinase and metal enzyme residues results in enzyme inactivation. According to the research of Sabir et al.,[10] interactions with metals can change the configuration of enzymes resulting in active sites changing and then no enzymatic activity.[15] Previous studies also concluded that Cd and Hg could affect carbohydrate metabolism by inactivating hexokinase,[20] glycogen synthase, and phospofructokinase.[21]

CONCLUSION

Based on the study's results, it can be concluded that Cd and Hg interacted with amino acid residues outside the active site of the enzyme pyruvate kinase and the enzyme glucokinase. The two metals interacted with the structure of the proteins constituting the enzyme and bind allosterically to change the conformation structure of the active site of the enzymes glucokinase and pyruvate kinase, which were thought to inhibit enzyme performance. The interaction by Hg metal was stronger in binding to both enzymes, as evidenced by the presence of three different amino acid residual bonds at a low distance compared to Cd metal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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