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| ***Interactions of Heavy Metals on Enzymes in Carbohydrate Metabolism: In silico study on glucocinase and piruvate kinase***  **Akmal Rizky Harun1, Siti ratna Jinan F1, Bambang Setiawan2, Dona Marisa3, Noer Komari4, Eko Suhartono2**  *1Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia*  *2Department of Biochemistry and Biomoleculer, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, Indonesia*  *3Department of Biomedic, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, Indonesia*  *4Department of Chemsitry, Faculty of Mathematics and Natural Science, Lambung Mangkurat University, Banjarmasin, Indonesia* | |
| **DATA OF ARTICLE:**  Received: ….  Reviewed: ….  Revised: ….  Accepted: …  **\*CORRESPONDENCE:**  esuhartono@ulm.ac.id  **DOI:**  ……….  **TYPE OF ARTICLE:**  Research | **Abstract:** *Cadmium (Cd2+) and mercury (Hg2+) are heavy metals, which can cause chronic inflammation, oxidative stress, obesity, hyperglycemia, and diabetes. Exposure to heavy metals cadmium and mercury could interfere with glycolysis metabolic functions through the inactivation of two key enzymes, namely, glucokinase (GK) and pyruvate kinase (PyK). However, the pathomechanism is not clearly known. Therefore, 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 is used via the MIB site: Metal Ion Binding site prediction and docking server (http://bioinfo.cmu.edu.tw/MIB/). Then the interaction between metal ions and amino acids of the target protein was visualized on UCSF Chimera 1.15. Based on the research results, the metal ion bond of mercury is more reactive than cadmium based on the number of amino acid residues that are bound and the bond is stronger based on a lower distance with PyK and GK enzymes. Therefore, mercury and cadmium metal ions are thought to inhibit the glycolysis process by causing inactivation of the two enzymes.*  **Keyword:** Cadmium; Mercury; Carbohydrat Metabolism; Glucokinase; Piruvat 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 have an adverse effect on the development and function of pancreatic beta cells (β), the formation of free radicals, the binding of enzymes and structural proteins and binding to enzymes thus causing diabetes.2,5,6

Experimental based on 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.8However, the mechanism of occurrence of diabetes, remains unclear.

Previous research stated that Cd and Hg can be covalently bound to glycolysis enzymes so as to interfere with the glucose oxidation process.9 The two heavy metals interact by binding to the active and allosteric sides of the enzyme, and form 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 will explain the interaction of Cd and Hg on the enzymes involved in carbohydrate metabolism, namely the enzymes glucokinase and pyruvate kinase.

**MATERIALS AND METHOD**

**Ligand and Protein Preparation**

The interaction between cadmium and mercury ligands against enzymes were 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 are prepared by removing the natural ligand residues present in the protein. Ligand and protein preparations were used by the Chimera 1.15 program ([https://www.cgl.ucsf.edu/chimera/downl oad.html](https://www.cgl.ucsf.edu/chimera/downl%20oad.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. To find out the active site, it can be predicted using a server with a link http://sts.bioe.uic.edu/castp

**Analysis and Visualization**

Analysis and visualization of docking results using the Chimera 1.15 program (https://www.cgl.ucsf.edu/chimera/downlod.html). Visualization is used to explain using illustrated images the interaction between ligands and receptor protein residues, namely the interacting amino acids, the type of the interacion, 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.

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| 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 |

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

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

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| (a) | (b) |

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

The red color in figure 1 shows the active site of the enzyme, which is where the reaction takes place.

**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 anaerobic consisting of ten interconnected reaction sequences in providing a substrate and producing products from each series of reactions.12,13

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

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| |  |  | | --- | --- | | Diagram  Description automatically generated | Diagram  Description automatically generated | | (a) | (b)  **Figure 2. Binding of pyruvate kinase enzymes by (a) Cd and (b) Hg** | |  |
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Cadmium and mercury bind to amino acid residues located on the allosteric side of the enzyme pyruvate kinase. The enzyme pyruvate kinase plays a role in the final stages of glycolysis that converts phosphoenolpiruvate to pyruvate. Through the computational method that has been carried out by Schorman et al,14 this enzyme structure is known to have three main domains or called "lid domains", namely domains A, B, and C. As a former of the active side of the pyruvate kinase enzyme, consisting of two arginine residues (Arg) and one lysine residue (Lys), as well as two aspartate residues (Asp) and two glutamate residues (Glu). While the other side is called the effector side which is able to bind alosterically 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 sufhydryl 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. This is in accordance with the research of Sabir et al,10 which states that metals have a high affinity for electrons so that they can affect the structure of enzymes.15

In addition to the enzyme pyruvate kinase, Cd and Hg also interact with the enzyme glucokinase. This can be seen in figure 3.

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

Glucokinase enzyme is an enzyme that converts a glucose substrate into its product, namely glucose – 6 – phosphate.16 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 which is an irregular structure.18

In this study, Cd and Hg were known to interact with residues which are the inactive sides of the glucoskinase enzyme, it has been known that the active residue of the glucokinase enzyme is residue 151-180. Thus, the interaction of Cd and Hg will bind to the allosteric side of the enzyme. Cd will bind to glutamate residues hydrophobically with the metal. Meanwhile, Hg will bind to serine residues covalently with the metal. The interaction of glucokinase and metal enzyme residues results in enzyme inactivation. This is in accordance with the research of Sabir et al,10 which states that interactions with metals can change the configuration of enzymes resulting in active sites changing then no enzymatic activity.15 Previous studies also concluded that Cd and Hg can affect carbohydrate metabolism by inactivating hexokinase,19 glycogen synthase, and phospofructokinase.20

**CONCLUSION**

Based on the results of the study, it was concluded that Cd and Hg interact with amino acid residues outside the active site of the enzyme pyruvate kinase and the enzyme glucoskinase. The two metals interact on 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 are thought to inhibit enzyme performance. The interaction by Hg metal is 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 that no conflict of interest.

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