

IMPACT OF HEAVY METALS ON HEXOKINASE ISOFORMS: AN IN SILICO STUDY

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Abstract:

Coal mining activities in South Kalimantan produce waste that is very dangerous if not processed wisely. Coal waste produces heavy metals cadmium and mercury that can pollute the environment. Heavy metals that enter the human body will cause negative impacts in the field of health such as the disruption of the glycolysis process in humans. The purpose of this study was determine the interaction of heavy metals which is cadmium and mercury against hexokinase enzymes using hexokinase enzymes type I, II, III with PDB ID : 4F9O, 2NZT, 3HM8 taken from Protein Data Bank and using the molecular docking website MIB: Metal Ion Binding Site Prediction and Docking server. Docking results will be visualized using chimera app version 1.15. Molecular docking of the heavy metals cadmium and mercury can interact with all three types of hexokinase enzymes. Cadmium metal ions bind hydrophobicly to amino acid residues of hexokinase enzymes type I, II, and III, while mercury metal ions bind covalently coordinate with amino acid residues of hexokinase enzymes type I and III. Mercury metal ions bind more strongly than cadmium metal ions. Of the three types of hexokinase enzymes, mercury metal ions bind most strongly with hexokinase enzyme type II because mercury ions bind to the active site of the three amino acid residues of hexokinase enzymes type II.

Keywords: Cadmium ; hexokinase enzyme ; mercury ; molecular docking

INTRODUCTION

South Kalimantan is a province with rich natural resources, especially in the mining sector. In addition to the positive impact obtained from coal mining in the economy, the use of this coal produces waste that can have negative impacts on the environment and health.^[1] The coal washing process produces acidic liquid waste containing hazardous heavy metals.^[2] It is known that the cadmium content in some sediments in South Kalimantan waters has exceeded the quality standard of the governor's regulations that have been set.^[3] The content of other heavy metals, which is mercury (Hg) obtained in the Martapura River is an average of 0.761 ppm. This is when compared with the Hg metal threshold which has a value ranging from 0.02-0.35 ppm, then this is of course the Hg content above the sediment pollution threshold.^[4]

Heavy metals that pollute the environment have a diabetogenic effect on animals and humans.^[5] Experimental animals given high doses of cadmium caused an increase in fasting blood sugar, a decrease in insulin and atrophy of the islets of Langerhans.^[6] Mercury exposure can also interfere with the endocrine function of the pancreas and may increase the risk of insulin resistance.^[7] In diabetes mellitus, carbohydrate metabolism that is impaired is the process of forming energy from glucose through glycolysis. The first step in glycolysis is the phosphorylation of glucose-6-phosphate catalyzed by the enzyme hexokinase.^[8]

In normal body conditions, the hexokinase enzyme has very low levels, but when the body is in a state of type 1 diabetes mellitus, the amount of hexokinase enzyme is known to increase.^[9] The action of the hexokinase enzyme can be affected by exposure to heavy metals, for example, exposure to cadmium will inhibit the work of

the hexokinase enzyme during the glycolysis process in rats. Exposure to heavy metals can inhibit all types of enzymes through certain mechanisms.^[10] This can cause disturbances in carbohydrate metabolism. Related to this, the researchers are interested in examining the interaction of heavy metal bonds between cadmium and mercury on the work of the Hexokinase enzyme

RESEARCH METHOD

Preparation of Ligand and Protein

Cadmium and mercury metal ions are found on the MIB website: Metal Ion-Binding site prediction and docking server (<http://bioinfo.cmu.edu.tw/MIB/>).^[11] The enzymes obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/search>) are hexokinase I (PDB ID : 4F9O), hexokinase II (PDB ID : 2NZT), and hexokinase III (PDB ID : 3HM8).^[12-14] Preparation of ligands and proteins using Chimera version 1.15 (<https://www.cgl.ucsf.edu/chimera/download.html>).^[15]

Analysis and Visualization

Analysis and visualization of docking results using Chimera version 1.15 . Visualization is used to explain the interaction between ligands and receptor protein residues, which is in the form of interacting amino acids and the bond distance between ligands and receptor protein residues.

Results and Discussion

From the result of docking visualization with Chimera application version 1.15, it produces the picturization of exact location and range between bonds of cadmium ion and the amino acid residual of hexokinase enzyme type I, II, and III. The visualization result of docking using presets with interactive type 3, which is hydrophobicity surface with 40% transparency. Next, the binding interaction of cadmium to hexokinase enzyme type I, II, and

III focused to observe closer detail of amino acid residual that is bound and the range of its bond. Cadmium metal's ion is represented by light green circles located among amino acid

residual that are bond together. Exact place where mercury and cadmium interacted with amino acid residual of hexokinase enzyme type I, II, and III will be shown in figure 1.

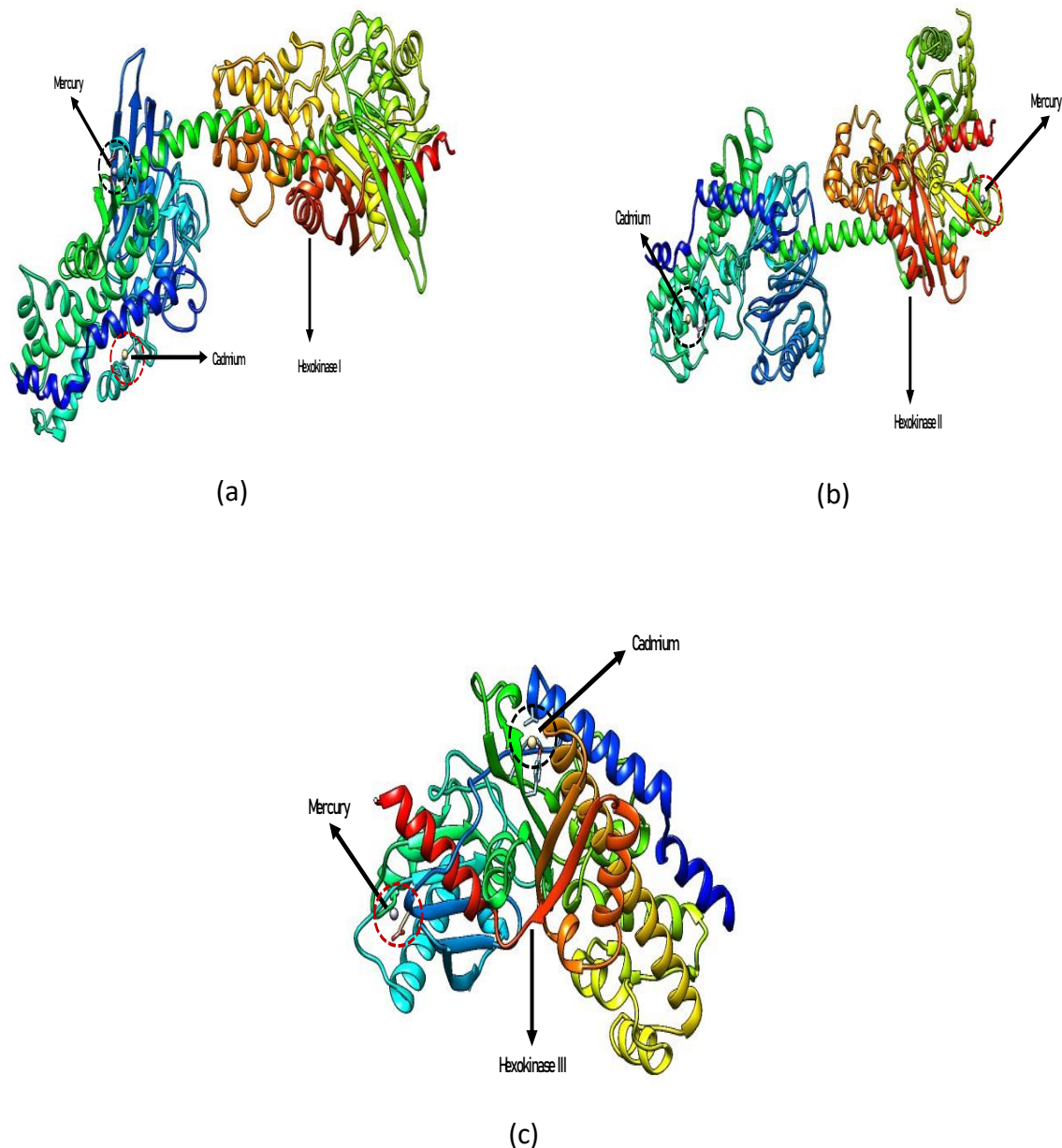


Figure 1. Interaction of Cadmium (Cd^{2+}) and Mercury (Hg^{2+}) against : **(a)** Hexokinase enzyme type I ; **(b)** hexokinase enzyme type II ; and **(c)** Hexokinase enzyme type III

The types of interactions that happened in the bonds of cadmium and mercury to

hexokinase I, II and III enzymes can be obtained as shown in Table 1.

Table 1 Interaction of metal ions cadmium and mercury against hexokinase enzyme type I, II and III

Heavy Metal	Enzyme	Amino Acid Residue	Distance (Å)	Interaction	Binding Score
Cadmium (Cd ²⁺)	Hexokinase Type I (PDB ID : 4F9O)	ASP – 278	5,470	Hydrophobic Bond	6,638
		ASP – 282	5,514	Hydrophobic Bond	
	Hexokinase Type II (PDB ID : 2NZT)	GLU – 276	3,607	Hydrophobic Bond	7,578
		GLU – 280	3,272	Hydrophobic Bond	
	Hexokinase Type III (PDB ID : 3HM8)	GLU – 694	5,099	Hydrophobic Bond	6,932
		GLU – 853	13,926	Hydrophobic Bond	
Mercury (Hg ²⁺)	Hexokinase Type I (PDB ID : 4F9O)	ASP – 221	2,478	Covalent Coordination Bond	4,511
		CYS – 224	4,605	Hydrophobic Bond	
	Hexokinase Type II (PDB ID : 2NZT)	CYS – 517	3,930	Hydrophobic Bond	4,816
		GLY – 701	-----	Not Detected	
		MET – 703	6,828	Hydrophobic Bond	
	Hexokinase Type III (PDB ID : 3HM8)	GLY – 536	-----	Not Detected	3,895
		ASP – 537	2,900	Covalent Coordination Bond	

This study uses the MIB website: Metal Ion-Binding site prediction and docking server as an application to perform the molecular docking process. On the "prediction" page of the MIB website there is a binding score of all amino acid residues listed in the table. The list of amino acid residues that are predicted to bind heavy metals will be marked in blue. On the "docking" page of the MIB website, there are amino acid residues with the highest binding score. Amino acid residues with the highest binding score are the best predictors of metal ion binding with amino acid residues.^[11]

From the docking result, in general cadmium reacted with residual amino acid glutamate (GLU) to hexokinase enzyme type II and III, and asparagine (ASP) to hexokinase enzyme type I. The distance of cadmium bonds with every amino acid residual of hexokinase enzyme type I, II, III is more than 3 Å. Therefore, every reaction happened in every type of hexokinase enzyme is hydrophobic bond.

Meanwhile, the analysis of the interaction between heavy metal mercury to hexokinase enzyme doesn't have dominant amino acid residual in general, unlike the chemical bond happened between heavy metal cadmium to hexokinase enzyme. ASP-221 in hexokinase enzyme (PDB ID: 4F9O) and ASP-537 in hexokinase enzyme type III (PDB ID: 3HM8) has shorter bond range compared to another bond, which are 2,478 Å and 2,900 Å. Both amino acid residual finally created a coordinate covalent bond which make the metal bond become stronger. The narrower the distance of bonds between amino acid residual and the ion of heavy metal mercury, then the metal bond will be stronger and caused the interaction between heavy metal mercury to enzyme becomes stabile. Meanwhile, the longest reaction range is owned by MET-703 amino acid residual to hexokinase enzyme type II (PDB ID: 2NZT), counted 6,828 Å.

Hexokinase enzyme is the enzyme that first works in glycolysis process. Generally, in mammals, hexokinase enzyme has four

isozymes, which are hexokinase type I, II, III, and IV. Hexokinase type I is found in mammals tissue that has important role in process of glucose entrance in the cell in order to produce ATP.^[16] Hexokinase enzyme type I is expressed in brain and kidney, meanwhile hexokinase type II is found in heart and skeleton muscles. Hexokinase enzyme type III is found in almost every tissue, but in very low rate.^[17] Hexokinase enzyme has similarity in active side. (Wiley, 2001). N-Terminal domain of hexokinase enzyme type II is active in catalytic way, but in hexokinase enzyme type I and III, it is inactive. Helix- α 13 that connects N-Terminal domain to C-Terminal domain from hexokinase enzyme type II has important role in maintaining catalytic activity from half of N-Terminal.^[18] The function of hexokinase enzyme can be hampered if it is exposed to heavy metal. Ramirez explained that the exposure of heavy metal can hamper the work of enzyme in certain mechanism.^[10] Heavy metal cadmium and mercury can interact with various amino acid residual in all three types of hexokinase enzyme. Most of amino acid residual that is contained in hexokinase enzyme form a coordinate covalent bond with heavy metal cadmium and mercury. The metal bond makes heavy metal trapped in hexokinase enzyme, hence the metal is hard to be unattached.^[19]

Heavy metal mercury interacts stronger with hexokinase enzyme because it is binding more amino acid residual of hexokinase enzyme in general. In half of C-Terminal domain of hexokinase enzyme type I, amino acid residual between ASP – 532 — LYS – 558 and between MET – 596 — PHE – 623, each is estimated as domain that bind ATP and glucose. ASP-657, GLU-708, and GLU-742 are estimated to interact with hydroxyl glucose group.^[18] Mercury metal ion binds amino acid residual ASP-221 and CYS-224 owned by hexokinase enzyme type I. Meanwhile, cadmium metal ion binds amino acid residual ASP-278 and ASP-282. Both cadmium and mercury metal ion don't interact with active

side of hexokinase enzyme type I, but tied with allosteric side of hexokinase enzyme type I. The bonding of metal ion to allosteric side of enzyme will induce the change of protein conformation, so it can affect the work speed of hexokinase enzyme type I.^[20] But the interaction of heavy metal mercury to hexokinase enzyme type I is stronger compared to cadmium because it has narrower bond range between amino acid residual and mercury metal ion.

In hexokinase enzyme type II, it has active side in amino acid residual MET-474 — ARG-917.^[18] The interaction of heavy metal mercury binds three amino acid residuals, which is amino acid residual CYS-517, GLY-701, and MET-703. Meanwhile in cadmium metal ion, it only binds two amino acid residuals, GLU-276 and GLU-280. Mercury metal ion binds three kinds of amino acid residuals of hexokinase enzyme type II in active side of the enzyme. It can trigger inactivity of enzyme. Hence, it can hamper the speed of hexokinase enzyme type II.^[20] Meanwhile in cadmium metal ion, it binds the amino acid residual of hexokinase enzyme type II in its allosteric side.

In hexokinase enzyme type III, materials used is crystal structure from C-Terminal domain of hexokinase enzyme type III. C-Terminal domain of hexokinase enzyme type III has catalytic side, but in N-Terminal domain, catalytic feature doesn't happen.^[21] Amino acid residual of hexokinase enzyme type III that is bound by mercury metal ion are GLY-536 and ASP-537, meanwhile in cadmium metal ion, these are GLU-694 and GLU-853. Both metal ion bind the amino acid residual in catalytic side of hexokinase enzyme type III and ASP-537 residual; the one that bound by mercury metal ion has the narrowest range of bond, hence make it the strongest bond. The bond of heavy metal mercury interacted stronger with hexokinase enzyme type III compared to cadmium metal bond. This is proven by the result of mercury metal ion that

binds the amino acid residual in active side of hexokinase enzyme type II. It can trigger the inactivity of hexokinase enzyme type II. According to the research result, mercury is more reactive to hexokinase enzyme type I to III compared to cadmium. In conclusion, amino acid residual of hexokinase enzyme type II binds more mercury than cadmium.

Conclusions

According to the result of the research, it can be concluded that interaction between heavy metal mercury to hexokinase enzyme is stronger compared to heavy metal cadmium. From three types of hexokinase enzyme, mercury metal ion binds strongly to hexokinase enzyme type II because it binds with three amino acid residuals in active side of hexokinase enzyme type II. The result of molecular docking in heavy metal mercury that produces coordinate covalent bond to hexokinase enzyme type I and III, meanwhile in interaction between cadmium metal ion produces hydrophobic bond to amino acid residual of hexokinase enzyme type I-III. Hydrophobic interaction shows that cadmium metal ion produces weaker bond compared to mercury metal ion.

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