Cadmium binding to antioxidant enzymes: in silico study

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Abstract. Cadmium (Cd) is a heavy metal that can be used for industry. Cd is toxic and can damage the kidneys, neurotoxic, glucose metabolism disorders and others. The reaction of Cd with protein causes oxidative stress, which is an imbalance of oxidants and antioxidant enzymes. Antioxidant enzymes include Superoxide dismutase (SOD), Catalase (CAT), and Glutathione reductase (GSR). There are not many studies that explain the interactions between Cd and antioxidant enzymes. For this reason, this research was carried out using in silico. The structure of the enzymes was obtained from the RCSB Protein Data Bank (http://www.rcsb.org) with the following code SOD (PDB ID 1E9Q), CAT (PDB ID 3RGP), and GSR (PDB ID 1GRT). Cd interactions with these enzymes were used by MIB: Metal Ion-Binding site prediction and docking server (http://bioinfo.cmu.edu.tw/MIB/). The interactions between Cd and amino acids of targeted protein were visualized on UCSF Chimera 1.14. The results of the study identified amino acid residues involved in the mechanism of binding of Cd with antioxidant enzymes. SOD binding sites with Cd were GLU-121 and SER-142; CAT binding sites were amino acid residues of HIS-230, ASP-228, HIS-181, HIS-109 and ASP-178; GSR binding sites were HIS-503 and TYR-502.

1. Introduction

Cadmium (Cd) is a heavy metal located in group 12 period 5 in the periodic system. This metal is widely used in various industries such as the battery industry, metal coating, the paint industry and others [1,2]. Cd is a pollutant that can disrupt ecosystem stability and diversity. The disruption of the ecosystem is determined by factors in the amount of pollutants, toxicity, and bioaccumulation [3].

Cd enters the body through water, food, and air. Furthermore, Cd is metabolized through a biotransformation mechanism involving cytochrome P-450 in the mitochondria. Cd binds covalently to
various ligands, for example -OH, -COO-, -C=O, -SH, -S-S-, -NH₂ and -NH, which are contained in the residues of the amino acids making up proteins and enzymes. Cd bonding with these ligands will inhibit the activity of antioxidant enzymes, so oxidative balance disorders occur [4,5].

Previous studies have suggested that administration of Cd to teeth can cause a decrease in minerals Ca, P, Mg, and Zn [6]. Cd can also affect glucose metabolism in vitro [7,8] and induce neurotoxic [9] by involving oxidative stress. Oxidative stress is an imbalance between oxidants and enzymatic antioxidants, which includes Superoxide dismutase (SOD), Catalase (CAT), and Glutathione reductase (GSR). Superoxide dismutase works by catalyzing superoxide (·O₂) to peroxide molecule, whereas CAT decomposes peroxide acid substrate into water and oxygen [10,11].

Tribowo [12] states that Cd can reduce the activity of SOD and CAT ovary enzymes. However, the research has not explained in detail the pathomechanism. Therefore, it is necessary to explain in detail about pathomechanism and interaction site of antioxidant enzymes that are SOD, CAT and GSR.

2. Materials and methods

This study tested 3 antioxidant enzymes, namely Superoxide dismutase (SOD), Catalase (CAT), and Glutathione reductase (GSR). The structure of the enzymes was obtained from the RCSB Protein Data Bank (http://www.rcsb.org) with the following code SOD (PDB ID 1E9Q), CAT (PDB ID 3RGP) and GSR (PDB ID 1GRT). Cd interactions with these enzymes were used by MIB: Metal Ion-Binding site prediction and docking server (http://bioinfo.cmu.edu.tw/MIB/). The interactions between Cd and amino acid of targeted protein were visualized on UCSF Chimera 1.14 [13].

3. Results and discussion

The SOD enzyme has the PDB code 1E9Q. This enzyme was released on December 3, 2000 with a resolution of 1.75 Å. SOD enzyme from the Boss taurus has sequence length of 151 with the name of the SOD1 gene with E.C: 1.15.1.1 [14]. The CAT used in this study was Catalase with PDB code 3RGP. This enzyme was released on May 11, 2011 with a resolution of 1.88 Å. An enzyme was a catalase from Boss taurus, with sequence length of 499 and has 4 chains, A, B, C and D whose gene name is CAT with E.C: 1.11.1.6 [15]. The GSR enzyme has PDB code 1GRT. This enzyme was released on June 16, 1997 with a resolution of 2.30 Å. GSR originates from Homo sapiens with sequence length of 478 with chain A, with E.C: 1.6.4.2 [16]. Each enzyme is shown in figure 1. Interactions of antioxidant enzymes with cadmium occurred in several amino acid residues. The results of the interaction can be seen as in table 1.

![Figure 1. Antioxidant enzymes: (a) Catalase, (b) Superoxide dismutase and (c) Glutathione reductase.](image-url)
The SOD enzyme showed that 2 amino acid residues interacted with cadmium. GLU-121 and SER-142 residues formed metal bonded with Cd with bond lengths of 2.326 Å and 2.421 Å (table 1). These metal bonds were very strong and caused Cd to be strongly bound by enzymes. These 2 amino acid residues formed the binding site of the SOD enzyme.

**Table 1.** Amino acid interaction with Cd.

| Antioxidant Enzyme | Amino acid residues | Interactions   | Distances |
|---------------------|---------------------|---------------|-----------|
| SOD                 | GLU-121             | Metal interaction | 2.326 Å  |
|                     | SER-142             | Metal interaction | 2.421 Å  |
| CAT                 | HIS-230             | Metal interaction | 2.510 Å  |
|                     | ASP-228             | Metal interaction | 2.327 Å  |
|                     | HIS-181             | Metal interaction | 2.312 Å  |
|                     | HIS-109             | Hidrophobic interaction | -- |
|                     | ASP-178             | Hidrophobic interaction | -- |
| GSR                 | HIS-503             | Metal interaction | 2.264 Å  |
|                     | TYR-502             | Hidrophobic interaction | -- |

The binding site of Cd to SOD and amino acid residues which were formed in bond formation is presented in figure 2. Cd was bound to the surface of enzymes which were generally dominated by polar amino acid residues.

**Figure 2.** Interactions of Cd and the SOD enzyme: (a) Cd was bound to the surface; (b) amino acid residues bound to Cd.

Table 1 shows that 5 amino acid residues in the CAT interacted with Cd. HIS-230, ASP-228, and HIS-181 residues formed metal bonds with Cd of 2.510 Å; 2.327 Å; and 2.312 Å respectively. These metal bonds were very strong and caused Cd to be strongly bound by enzymes. HIS-109 and ASP-178 residues formed ionic bonds with Cd. This bond supported the strength of existing metal bonds, so that the interaction of Cd and enzymes became very stable. These 5 amino acid residues formed the binding site of CAT. The place where Cd was bound to CAT and the amino acid residues that were involved in forming bonds is shown in figure 3. Cd was bound to the surface of enzymes which were generally dominated by polar amino acid residues.
Figure 3. Interactions of Cd and catalase enzymes: (a) Cd bound to the surface; (b) amino acid residues bound to Cd.

Table 1 also shows the 2 amino acid residues of the GSR enzyme that bound to Cd. HIS-503 residue formed a metal bond with Cd with a bond length of 2.264 Å and TYR-502 residue formed an ionic bond with Cd. These two residues became the binding site of the GSR enzyme to act with Cd. Figure 4 shows that Cd was bound to the surface of an enzyme that was dominated by polar amino acid residues.

Figure 4. Interactions of Cd and GSR enzyme: (a) Cd bound to the surface; (b) amino acid residues that bind to Cd.

In the enzyme with PDB ID 4EVD, Yokohama [17] illustrates that Cd generally interacts with HIS, GLU and ASP residues. While, Leinala [18] in PDB ID 1NP8 shows that amino acid residues that bind to Cd include: ASP, GLU, THR, HIS, ALA and LYS. This is generally a polar amino acid.

4. Conclusion
The results of the study identified amino acid residues involved in the mechanism of binding of cadmium with antioxidant enzymes. SOD binding sites with Cd were GLU-121 and SER-142; CAT binding sites were amino acid residues of HIS-230, ASP-228, HIS-181, HIS-109 and ASP-178; GSR binding sites were HIS-503 and TYR-502. Then, the in silico study can predict the oxidative mechanism of Cd against
antioxidant enzymes. Therefore, it can explain the occurrence of oxidative stress due to the interaction of Cd with SOD, CAT, and GSR.

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