Interaction of cadmium-cysteine binding and oxidation of protein causes of blood thrombosis

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Abstract. Thrombosis occurs if the balance between thrombogenic factors and protective mechanisms is disrupted. These disorders can be caused by endothelial disorders due to the presence of heavy metals such as cadmium (Cd). These amino acid residues can bind Cd metal covalently with the -S-S-, -C-S-, -SH, and phenyl groups, which are present in the amino acid residues. This situation causes changes in the structure of the protein, so that it becomes modified and forms a blood clot (thrombosis). However, the spectroscopic interaction of cadmium-cysteine as causes of thrombosis has not been widely studied. Therefore, this research needs to be done. The research conducted was an experimental study with posttest control with group design. This study used healthy blood obtained from PMI Banjar regency. This study used one control group and three treatment groups, namely P0 = control group, blood without Cd; P1 = blood group with Cd 0.003 mg/l; P2 = blood group with Cd 0.03 mg/l; P3 = blood group with Cd 0.3 mg/l; P4 = blood group with Cd 3 mg/l. Furthermore, each group was incubated for 45 minutes at 37°C. Then cloth weight, AOPP levels, and absorbance were measured at wavelengths 253 nm. The results showed that an increase in cadmium levels caused an increase in the rate of thrombosis. This increase is associated with an increase in AOPP levels. Cadmium can bind covalently with cysteine and other amino acids which results in the breakdown of peptide bonds. The study concluded that cd accelerated the rate of thrombosis due to increased protein oxidation (aopp). This oxidation is caused by binding cd to cysteine with kbind = 0.355.

1. Introduction
Thrombosis is the formation of blood clot (thrombus) in vein. Thrombosis occurs if the balance between thrombogenic factors and protective mechanisms is disrupted. These disorders can be caused by endothelial disorders due to the presence of heavy metals interacts with amino acid making up the constituent biomolecules of membranes.

Previous research said that Lead (Pb) heavy metal can interact with cysteine and tryptophan in rats induced by Pb with Spectroscopy enclosure. That research also concluded that Pb²⁺ - Pb interactions had higher affinity than Pb³⁻–tryptophan interactions [1]. Zairin’s research also stated that spectroscopy
study in rats induced by Pb^{2+} can cause Pb^{2+}\text{-}\text{tyrosine interaction in bone. This result in crystal structure becomes wider and bone calcium degraded that can lead to osteoporosis [2].}

Metal Cd enters the body through food, and then absorbed and carried by blood through Ligand–blood protein binding [3]. That interaction is started with covalent binding to the functional group in amino acids of protein constituent, such as cysteine, tryptophan, and tyrosine. The amino acid residues can bind metal Cd covalently with the groups of $-\text{S-S-}$, $-\text{C-S-}$, $-\text{SH}$, as well as phenil, which are present in the amino acid residues [4]. This situation causes changes in the structure of the protein, so that it becomes modified and forms a blood clot (thrombosis). However, the spectroscopic interaction of cadmium-cysteine as causes of thrombosis has not been widely studied. Therefore, this research needs to be done.

2. Materials and Method

2.1. Study design

This research is an experimental in-vitro study for one week in May 2019.

2.2. Experimental section

This study used healthy blood obtained from PMI Banjar regency. This study used one control group and three treatment groups, namely P0 = control group, blood without Cd; P1 = blood group with Cd 0.003 mg/l; P2 = blood group with Cd 0.03 mg/l; P3 = blood group with Cd 0.3 mg/l; P4 = blood group with Cd 3 mg/l.

2.3. Measurement of blood thrombosis

Each 1 mL of vein blood was put in 4 tubes, that had been weighed before (Wo) and noted as P0, P1, P2, P3, and P4. Then, 1 mL of Cd was put in each tubes for its group. After that, they were incubated in 37 °C for 45 minutes. Next, the serum in each tubes was taken and kept for AOPP and cysteine measurement. The tubes consisted with cloth (W1) were weighed.

\[
\text{Thrombosis rate (mg/minutes)} = \frac{(W1 - Wo)}{45}
\]

2.4. Measurement of AOPP

The solution for test was made by mixing 200 µL of blood, 600 µL of phosphate buffer, and 100 µL of KI 1,16 M. Blank solution was made by mixing 800 µL buffer phosphate, 100 µL KI 1.16 M. The test and blank solutions were left for 2 minutes before added by 200 µL of acetate acid, measured its absorbance at $\lambda=340$ nm. AOPPs concentration was stated in $A = \varepsilon b C$ with $\varepsilon = 26$ mM$^{-1}$ cm$^{-1}$ and $b = 1$ cm [5].

2.5. Measurement of absorbance in Cd – cysteine interaction

Cysteine serum that was kept in each groups were measured its absorbance at $\lambda=210$-280 nm.

2.6. Metal-protein bonding constant determination

Interactions between metal (L) and protein (P) were based on reaction equations:

\[\text{Metal + Protein} \rightleftharpoons \text{Metal:Protein}.\]

$K_{\text{bind}}$ calculation used equation of the previous research [1-3]

3. Result and Discussion

Based on spectroscopy data of UV-Vis at wavelength 210-280 nm, resulted absorbance data such in figure 1. Figure 1 shows that the increase of Cd$^{2+}$ can increase serum absorbance. This means Cd$^{2+}$ binds covalently with amino acid that was represented in serum, so that the interaction between ligand and protein occurs. The interaction of Cd$^{2+}$ with protein was due to the covalent binding between a) Cd$^{2+}$ and
N-terminal in amide group (b) Cd$^{2+}$ and N-terminal in imidazole group (c) Cd$^{2+}$ and N-terminal in amin group [6].

![Figure 1](image1.png)

**Figure 1.** Absorbance Cd$^{2+}$-Protein at wavelength 210-280 nm.

Interaction of Cd$^{2+}$-protein can be determined from $K_{\text{bind}}$ value which can be calculated with graphic (figure 2).

![Figure 2](image2.png)

**Figure 2.** Constanta binding in the interaction of Cd$^{2+}$-Cys.

Based on figure 2, $K_{\text{bind}}$ value of Cd$^{2+}$-Cys was resulted ($K_{\text{bind}} = 5.467$). This means Cd$^{2+}$ has tendency to bind with cysteine that has binding of $-\text{S-S}-$. Next, the interaction results in protein oxidized and its level increases (figure 3).
Figure 3. The relation between protein oxidation and \( \text{Cd}^{2+} \)-Cys interaction (\( \lambda=253 \)).

Protein oxidation that increased results in thrombosis rate that also increased along with the increasing concentration of \( \text{Cd}^{2+} \) (figure 4).

Figure 4. The relation between thrombosis rate and \( \text{Cd}^{2+} \)-Cys interaction (\( \lambda=253 \)).

4. Conclusion

Therefore, in this study can be suggested for thrombotic mechanism with the existence of \( \text{Cd}^{2+} \), which starts with covalent binding of –SH in cysteine. This binding causes modification of protein marked by the increase of protein oxidation level, so that resulted in increasing of thrombotic rate exponentially.

References

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