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Study of Interaction between Cadmium and Bovine Serum Albumin with UV-Vis Spectrocopy Approach

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Abstract. This study aims to explain the interaction of cadmium (Cd) with serum albumin through visible light (UV-Vis) spectroscopy approach. This study is an in vitro experimental study using Cd with several concentrations and Bovine Serum Albumin (BSA). Each solution was then incubated for 10 min at 37 ℃, and measured the absorbance at 220-300 nm. The absorbance data is then presented in graphical form. From the graph, a linear equation will appear to calculate the value of metal binding constants (K) to proteins. Also, in this present study we analsyed the ratio between A220 and A220 to identify changes in the protein region especially tyrosine and peptide bonds. The results show that the addition of Cd in different concentrations could increase the absorbance with a constant value (K) = 1.634. Based on the result, it seems the addition of Cd in different concentrations will lead the reaction to form BSA-Cd. Also, the result shows that the ration of A220/A280 were decreased with the increasing of Cd concentration. In conclusion, the addition of Cd could interact and changes the protein structure in BSA.

1. Introduction

Cadmium (Cd) used since 1950 with a total world production of about 15,000-18,000 tons per year. Cd is a heavy metal that is widely used by humans for the benefit of various industries, for example agricultural industry, paint, aircraft, and others. In addition to beneficial, Cd is also harmful to the environment and adversely affect human health, such as hepatic, ovarian, kidney, and brain damage [1]-[6]. Previous research has also revealed that Cd can lead to increased blood cholesterol and rat endothelial damage [7].

Cd ability to cause oxidative damage and metabolic disorder begins with the binding of Cd covalently with functional groups of amino acids making up the protein, for example -OH, -COO-, -C = O, -SH, -S-S-, -NH2 and -NH. The binding of the Cd with the functional group may form a crosslink of the residue of ditirosin and disulfide bonds in the protein. The cross linking is called Advanced Oxidation Protein Products (AOPPs). Increased AOPP results in changes in protein structure and function [8]-[9]. The results of Arizal et al. [6] have concluded that the administration of Cd could significantly

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increase the levels of AOPP in rats ovarium, resulting in impaired ovarian function. Meanwhile, the results Aflanie et al. [10] also concluded that the increased concentration of Cd will be followed by the increasing level of AOPPs in vitro. Nevertheless, in those studies, there has been no explanation of Cd interactions with amino acids that cause crosslinking of ditirosin and disulfide.

Many methods are used to explain metal interactions with protein. However, the spectroscopic method is a frequently used method. One of them is visible light spectroscopy (UV-Vis). The basic principle of UV-Vis spectroscopy is that the molecule has an electron energy level analogous to the level of electron energy in the atom. The energy level of this molecule is called a molecular orbital. Thus, any change in molecular structure will give different uptake. Zhang et al. [11] had studied spectroscopically the interaction of flavonoid metal divalent Cu2+ and protein. In these study, it was revealed that Cu2+ interacts with flavonoids to form flavonoids-Cu2+ and a change in protein conformation due to flavonoids-Cu2+. Curvale et al. [12] studies have examined the interaction of Arsenic (As) with amino acids in proteins by UV-Vis spectroscopy [12]. In that study, it was revealed that As ionic charge can affect the interaction of its bonding to proteins. The interaction between As (III) and protein is an ionic interaction, but As (V) interaction is a hydrophobic interaction.

The use of bovine serum albumin (BSA) as an interaction model between metal and protein is due to its ability to reversibly bind various ligands. Albumin contains 17 disulfide bridges and thiol groups which are free cysteine (Cys). This residue (Cys 34) and tyrosine (Tyr) are reactive, polarized, and have a high affinity for metals and are very sensitive to the local environment [12]. In this regard, in order to explain the interaction of Cd with protein, a research between Cd in various concentrations with the bovine serum BSA through spectroscopic approach should be done for a deeper understanding.

2. Material and methods

2.1. Research design

This study is an experimental study to explain the interaction of metals and proteins. Thus, the following groups were created: P0 = BSA; P1 = BSA + 0.001 ppm Cd^{2+}; P2 = BSA + 0.01 ppm Cd^{2+}; P3 = BSA + 0.1 ppm Cd^{2+}. Each group was incubated for 10 min at 37^{o}C. Then, in each group sample the absorbance was measured at a wavelength of 220-300 nm. The absorbance data will be presented in graphical form.

2.2. Spectroscopic analysis

Each solution from each group was diluted with normal saline at concentration of 5 µL/mL. Then, the spectra were scanned in the wavelength 220 and 280 nm. Amide chain of proteins present in the blood absorbs strongly at 220 nm, and absorption of tyrosine and tryptophan is at around 280 nm. Then, the ratio between A220 and A280 was analsyed which is the result of absorbance at 220 and 280 wavelength in each experimental group.

2.3. Data analysis

The interaction between the metal (L) with protein (P) is based on the reaction equation [13]:

Metals + Proteins ⇆ Metals : Protein

So the bonding constant (K) is formulated:

$$K = \frac{[\text{Metal:Protein}]}{[\text{Metal}][\text{Protein}]} \quad (1)$$

If it is assumed [Metals: Protein] = $C_{b,}$ then

$$K = \frac{[Cb]}{[Cl-Cb][Cp-Cb]} \quad (2)$$

Under Lambert-Beer's law, then

$$Cl = \frac{Ao}{\varepsilon l.b}$$

$$Cp = \frac{Ao}{\varepsilon p.b}$$

By entering a value of C_l and C_p in equation (2) then obtained equation (3):

$$\frac{Ao}{A - Ao} = \frac{\varepsilon l}{\varepsilon b} + \frac{\varepsilon l}{\varepsilon b.K} \cdot \frac{1}{Cl}$$

If n = εb/εl, then the equation (4) is as follows:

$$\frac{1}{A - Ao} = \frac{1}{n} + \frac{1}{n.K} \cdot \frac{1}{Cl}$$

By using graph linear curve between the $\frac{1}{A-Ao}$ and $1/C_L$ will be obtained the values of n, the number of the active site binding proteins and metal on metal-protein binding constants (K).

3. Results and discussion

Based on the observations, the addition of Cd concentration causes an increase in absorbance. This shows that there is an interaction between Cd and BSA, as shown in Figure 1.

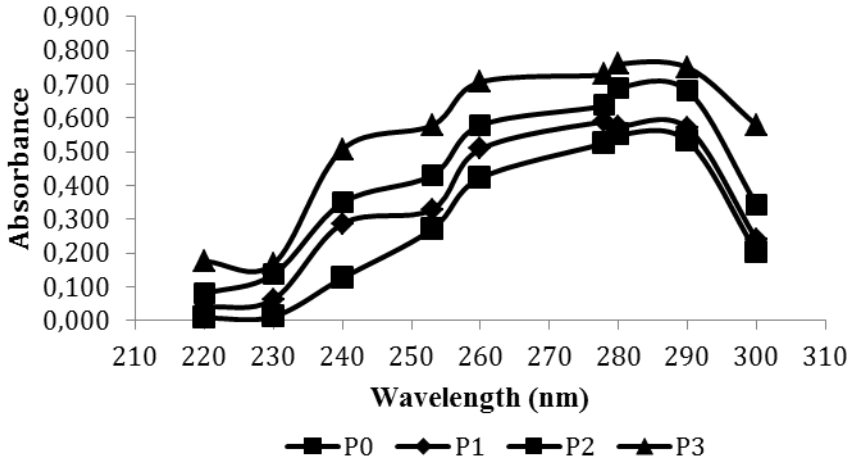


Figure 1. Graph of wavelength relation with absorbance. P0 = BSA; P1 = BSA + 0.001 ppm Cd^{2+}; P2 = BSA + 0.01 ppm Cd^{2+}; P3 = BSA + 0.1 ppm Cd^{2+}

Figure 1 shows that an increase in Cd concentration causes absorbance band change at 220-300 nm wavelength. The alteration is thought to be due to the covalent bonding of Cd with the N-terminal amide group, the N-terminal of the imidazole group, and the N-terminal of the amine group. This results in changes in the structure of protein molecules, which lead to changes in absorbance bands.

The results are consistent with research Dieaconu et al. [14] that was indicated that the metal binding can affect the absorbance band at the BSA by UV-Vis spectroscopy. This study is also consistent with the results of research Curvale et al. [12]. In these study, it was revealed that BSA absorbance band-As^{5+} higher than the BSA-As^{3+}. This implies that the binding interactions BSA metal-As^{5+} more powerful than the BSA-As^{3+} at a wavelength of 200-300 nm.

Many studies have revealed the association of changes in absorbance bands with some diseases. Kumar et al. research, [15] has identified absorbance bands in IgG of Duchene Muscular Dystrophy (DMD) patients using UV-Vis spectroscopy. In these study, it was found that in patients with DMD the absorbance of protein were decreased. Research of Gunasekaran et al. [16] also reveals that the UV-Vis absorbance band wer found lower in jaundice patient's than normal in the 200-250 nm regions. Some researchers have used the A280/A220 ratio as a parameter to identify changes in the protein region. It is based on that A280 is the absorbance of tyrosine and tryptophan residues that are sensitive

to change. Meanwhile, A220 is the absorbance of protein-peptide bonds. Changes in the A280/A220 ratio mean that protein structures undergo many changes, especially in the amino acid tyrosine and peptide bonds (figure 2) [17].
In this study also measured the ratio of A280 / A220 to know the molecular changes of protein region. The calculation results are presented in Figure 2.

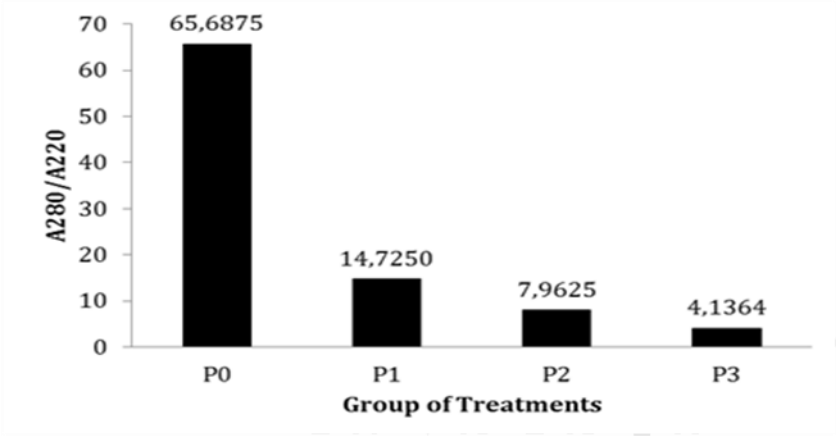


Figure 2. Ratio of A280 / A220 in different group of treatments. P0 = BSA; P1 = BSA + 0.001 ppm Cd^{2+}; P2 = BSA + 0.01 ppm Cd^{2+}; P3 = BSA + 0.1 ppm Cd^{2+}

Figure 3 shows that the increasing of Cd concentration will decrease the A280/A220 ratio. By many researchers, the use of A280/A220 ratio has been used for the identification of several diseases, such as cancer and inflammation [19]. This is evidenced by research Gunasekaran et al that the ratio of A278/A210 leukemia patients were significantly decreased compared to healthy people [19]. In addition, the study also revealed that A278/A210 blood of healthy people is higher than those suffering from anemia and hepatic sirosis.
In addition to calculating the ratio of A280/A220, this study also measures the value of the BSA-Cd dictation constant. The graph of the measurement results can be seen in figure 3.
Based on graph 3, we can calculate the equilibrium constant value of BSA-Cd binding based on equation formula (4). The calculation result shows that the constant of BSA-Cd dictation is K = 1,634. This value means that the reaction between BSA and Cd tends towards the formation of BSA-Cd.

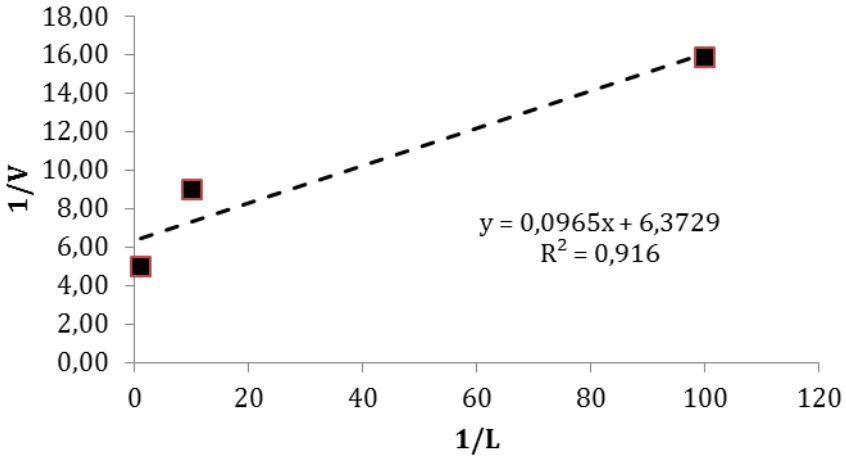


Figure 3. Graphs between 1 / V with 1 / L to determine the value of K

4. Conclusion

Based on the results of the study, it was concluded that the addition of Cd in different concentrations will increase the abosorbance which indicates the covalent binding of Cd in the amino acid protein constituents. The lower A280 / A220 ratio means that the protein structure changes a lot. Meanwhile, the BSA-Cd binding constant value is 1.634.

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