

Water effect on the conformation of glycosylated albumin and blood viscosity in a diabetic patient

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ABSTRACT

Introduction: Water can influence the conformation process of albumin and blood viscosity. The purpose of this study was to determine the effect of water molecules on the viscosity of plasma and plasma albumin conformation in diabetic patients. **Methods:** This study was using *in silico* molecular dynamics method. Molecular dynamics simulation program for a glycosylated albumin was run in various glucose concentrations (5 mg/ml, 11 mg/ml, and 20 mg/ml) and performed using YASARA software. Blood viscosity was measured using design methods with post-test only. The three steps measurement was including sampling, plasma collection, and blood viscosity measurement method. This study was obtained the concentration of glucose in the glycosylated albumin can affect the changes of conformational albumin and secondary structure of the protein albumin. **Results:** The results showed a decrease of plasma viscosity (PV) after addition of water. A decrease in PV showed that the water not only as a solvent but also related to the distribution of electrons contained in the molecule. **Conclusion:** Based on these results, it can be concluded that with the addition of water in the cell plasma may cause a decrease in viscosity. Moreover, the addition of plasma glucose concentrations can cause a conformational change in albumin.

KEY WORDS: Blood viscosity, Glycosylated albumin conformation, *In silico*, Water

INTRODUCTION

The complicity of water at the molecular level has not been fully examined. It is due to the complexity of the water molecule at the cellular level on the tissue and molecular organism. The functions of water for protein are to facilitate the protein folding, maintain the structural integrity, mediate the protein rigidity, and accelerate the enzymatic catalysis. It is important to be studied to determine the dynamics properties of biomolecules related to the biological properties of the water at the molecular level.^[1-4] The addition of 10 μ L water in 6 mL of blood plasma cells may affect the rheology due to differences in the molecular charge, which lead to attraction forces between molecules can affect the rheology, which results in the molecular flow/motion.^[5]

The purpose of blood viscosity examination is to assess the change of blood concentration and blood

component abnormalities such as blood rigidity or erythrocyte deformability. Furthermore, plasma viscosity (PV) examination is used to assess changes in the level of albumin, globulin, and fibrinogen.^[6] Water can affect the changes in protein conformation (albumin). Conformation changes are also caused by the distribution of electron and the bonding of hydrophobic and hydrophilic albumin. Electron distribution causes the dynamics gravitational pull in each molecule. Furthermore, the addition of water on the hydrophobic area causes conformation changes in albumin.^[2,4,5]

Diabetes mellitus (DM) patients with impaired metabolism of carbohydrates, lipids, and proteins have an increased blood glucose levels (hyperglycemia). Hyperglycemia can lead to the increased blood viscosity, either whole blood viscosity (WBV) or PV, which will lead to changes in blood rheology. Hyperglycemia can also result in albumin glycation, which is the non-enzymatic glycosylation process between glucose and albumin.^[7] Another effect of hyperglycemia is an increase in osmotic diuretic that can cause dehydration and results in an increase

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in blood viscosity. Dehydration can influence the conformation changes in albumin.^[8]

Molecular dynamics is a simulation method that allows the computer, as the media, to represent the interaction of atomic molecules within a certain period. Molecular dynamics simulation can examine the structure, dynamics, and thermodynamics of biological molecules and its complexity.^[1] The purpose of this study is to examine the effect of water molecules on blood viscosity and albumin protein conformation using *in silico* analysis with the molecular dynamics simulation, which can be used to determine the influence of water on the relationship between blood viscosity and glycosylated protein albumin conformation in a diabetic patient.

METHODS

Blood Viscosity Examination Method

Blood viscosity examination was carried out through several stages: (1) Blood sampling, before the blood sampling, an informed concern was given to give a brief explanation before the respondent agrees to become a respondent. (2) Plasma collection, the blood sample was placed in a test tube and centrifuged at 1500 rpm for 10 min. Plasma was taken using a pipette and placed into a test tube that has been labeled. (3) Measurement of blood viscosity, tools preparation: Spindle was placed on the shaft player and then viscometer was switched “on” and waited for 5 min to stabilize the tool. Viscosity was measured in two stages: (1) Measurement of viscosity on normal individuals. It aimed to examine the addition of water to the changes of viscosity through *in vitro* method, which was done to determine the water volume and the optimum time to reduce the viscosity. Blood plasma was added into the 10 mL tube, and the viscosity was measured every 5 min for 60 min. (2) Measurement of viscosity in diabetic patients, which was performed to determine the addition of water in the blood plasma of patients with diabetes.

Albumin Protein Conformation Method

Molecular dynamics simulation method was used in this research with the help of YASARA software (1). It aimed to examine the dynamics of all atoms constructing the albumin protein in each time unit and to predict the physical impact caused by the difference in the interaction partner. The stages in YASARA software were HSA and samples were taken from the PDB database, with ID 1AO6. This structure was the result of X-ray method with a resolution of 2.5 Å. Furthermore, the glycosylation was performed on the Cys 34, Arg 410, and Lys 525 sites, in which the most frequent glycosylation site was the Arg410, Lys525, and Cys347. Glycosylation process was done by adding H atoms using the VegaZZ9 program. The

simulation was performed on four different glucose concentrations: 5, 8, 10, and 20 mg/ml within the system limits that have been determined. Furthermore, running simulation was performed with the temperature of 310 K (body temperature). Simulations were performed for 1000 ps. The simulation result presented by root mean square deviation (RMSD) demonstrates the stability of albumin protein that has been glycosylated in the system with different glucose concentrations.

RESULTS

WBV depends on several factors including hematocrit, PV, the aggregation and form of white blood cells, and leukocytes. Meanwhile, PV is used to determine changes in the level of albumin, globulin, and fibrinogen. In this study, WBV and PV in patients with DM were higher than normal individuals [Figure 1]. Diabetic patients showed changes in blood flow, which increased the blood flow.

Furthermore, we analyze the blood viscosity. In this study, water is a molecule that can affect blood rheology, in which, differences in electron charge can result in the flow or movement or rheology of water. Figure 2 showed that research on PV of DM patients has decreased compared to research on normal individuals. 90% of plasma component is water,

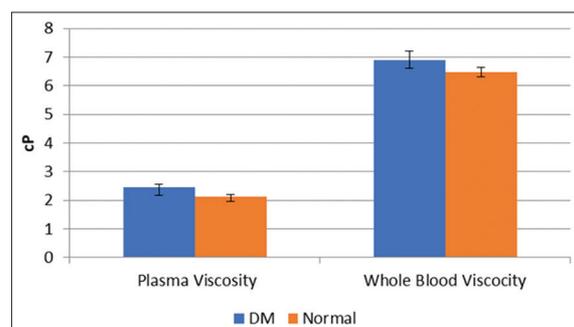


Figure 1: Comparison between whole blood viscosity and plasma viscosity

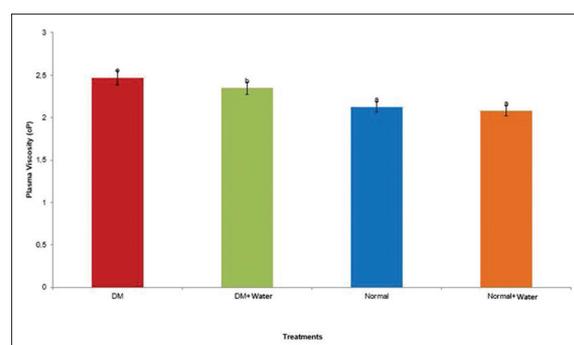


Figure 2: Changes in blood viscosity due to water induction in normal individuals and patients with diabetes mellitus (DM) without complications. Water can affect the viscosity of blood plasma in DM patients and normal individuals

and water has contributed to the stability of protein. Viscosity is the measure of fluid adhesive properties which states the friction size in the fluid. In a liquid, the viscosity is generated by the cohesive forces between the liquid molecules. The addition of 10 μL water can affect the viscosity of blood plasma.

In many diabetic patients, albumin underwent glycosylation. Glycosylation occurs in the following three main amino acids: Arg410, Lys525, and Cys34. If the glycosylation continues, other amino acids would also influence the albumin. Glycosylated albumin has been simulated in different glucose concentrations. It was done to examine the effect of glucose addition in the dynamics of protein albumin. Simulation results showed that the addition of glucose concentration in glycosylated albumin influenced the conformational changes and also changes in the secondary structure of the protein albumin [Figure 3].

Different results were obtained by the addition of 20 mg/mL glucose. The albumin conformation has increased in the beginning of the simulation, which started at 200 ps. There was a drastic increase of RMSD at 780 ps (reached 3.5 Å). However, subsequently, albumin was relatively static. It occurred due to damages in albumin structures and caused inclined static of the dynamics [Figure 4]. Simulation results of the addition of glucose concentration showed that higher concentration of glucose had more effects on albumin conformation and even changed the secondary structure of albumin protein. These significant changes resulted in a change in albumin protein functions, which changed into an antioxidant and mediator drug.

DISCUSSION

Comparison between WBV and PV

WBV contributes to the rheology factors such as the resistance of blood against the blood flow. WBV depends on several factors including hematocrit, PV, the aggregation and form of white blood cells, and leukocytes. Meanwhile, PV is used to determine changes in the level of albumin, globulin, and fibrinogen. Objective clinical examination of blood viscosity is necessary to determine the risk factors for cardiovascular disease, diabetes, and malignancy hematology^[9]. Thus, WBV is expected to be used as the basis for early detection of microvascular complications in DM patients.^[6]

In this study, WBV and PV in patients with DM were higher than normal individuals [Figure 1]. Diabetic patients showed changes in blood flow, which increased the blood flow. Increased blood viscosity of patients with DM is associated with increased hematocrit, blood glucose, osmolarity

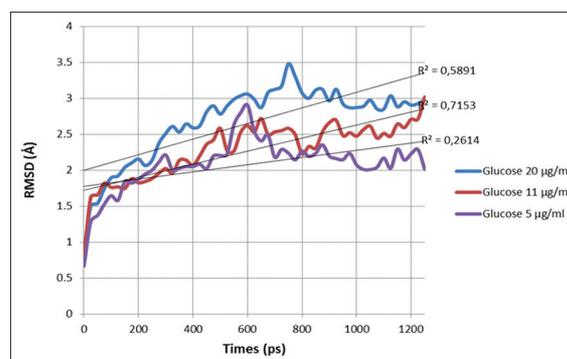


Figure 3: Root mean square deviation values of albumin proteins are simulated in different concentrations of glucose

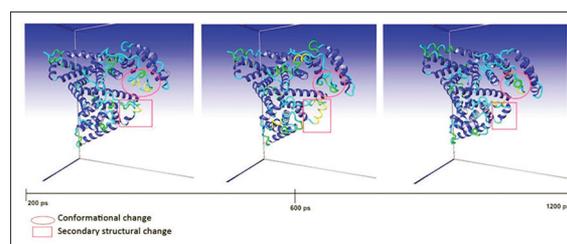


Figure 4: Changes in albumin conformation in glucose concentration conditions 20 $\mu\text{g/mL}$.

levels, and BMI. Increased blood viscosity can cause aggregate erythrocytes, so the blood cells cannot pass through capillaries, which results in decreased blood perfusion. Increased blood viscosity can also interfere with microcirculation. Decreasing the blood viscosity through metabolic control can reduce microvascular complications.^[10]

Treatment Results of 10 μL Water Provision in 6 mL Blood Plasma of Normal Individuals and Patients with DM

The provision of 10 μL water can affect the rheology due to differences in the electron charge. Water has a good effect on PV in diabetic patients and normal individuals. Research on PV of DM patients has decreased compared to research on normal individuals [Figure 2]. A small amount of additional water can affect blood rheology. Rheology is affected by the attractive force between molecules, which increases the dynamics of blood flow. Charge difference between molecules of water and albumin protein causes movement of attraction. It causes the movement or flows.^[8] The addition of 10 μL of blood plasma can decrease the viscosity of DM patients. Rehydration is more focused with volume, but of these results showed that small amount of 10 μL water can reduce the viscosity of blood plasma.^[11]

Results of the Examination of the Effect of Glucose Addition in Various Concentrations of Albumin

RMSD results on glycosylated albumin with the addition of different glucose concentrations suggested that

the conformational changes started on the addition of 11 mg/mL glucose. Simulation results in the addition of 5 mg/mL glucose showed that albumin had good dynamics from the beginning until the end as indicated by its r -value ($r = 0.26$). Changes in RMSD were not significant (ranged from 2 to 2.8 Å). Changes in albumin protein dynamics started on the addition of 11 mg/mL glucose concentration to the simulation system. RMSD results on 11 mg/mL concentration showed significant conformational changes ($r = 0.7$). These results suggested that RMSD increases exponentially through time. In addition, the dynamics pattern of albumin protein was different from the normal conditions. RMSD has increased at 800 ps, and it has decreased in normal conditions (5 mg/mL).

Figure 4 showed conformational changes during the simulation, in which changes in albumin secondary structure were found from the coil into alpha-helix. The transition process of secondary structural changes was highlighted in yellow in Figure 4. Secondary structural changes influenced the function of the protein. Analysis results on albumin protein potential energy during simulation showed that albumin at 11 mg/mL glucose concentration has the lowest energy, followed by 20 mg/mL glucose and the 5 mg/mL glucose concentration has the most positive potential energy.

The Effect of Glucose Addition in Concentrations 20 mg/m on Glycated Albumin Conformation

Changes in protein conformation are determined by the bindings and glucose. This is the main reason that glycation can change the function of a protein. Glycosylation is the attachment of glucose (glycan) on the peptide chain. In general, covalent bond happens between carbohydrate and protein. The purpose of this process is varied. The role of glycosylation process is to determine the folding of a protein. If there is an error in the glycosylation process, the protein might also experience an error in folding or conformation that is not in accordance with the normal conditions. In addition, the glycated protein makes protein to be more stable. In this study, albumin protein glycation was done to suit a mobile condition.^[9,10]

High blood sugar condition can cause the albumin protein to experience multiple glycation processes, which leads to the advanced glycation end-products condition. Glycation process causes changes in the function of albumin protein. High glucose concentrations can cause albumin to undergo excessive glycation process or a condition where glucose directly affect the dynamics of protein albumin. *In vitro* experiment showed that incubated albumin within glucose at physiological conditions can undergo glycation process. This process is

known as non-enzymatic glycosylation. This process occurs when many glucose molecules interact with protein molecules so the interaction between atoms emerges.^[10,12]

Atoms of glucose molecules and amino acids molecules can cause conformational changes in albumin protein that is not in accordance with normal conditions. Higher concentration of glucose will provoke more interaction between glucose molecules and resulted in changes in the albumin protein conformation. Albumin protein conformational changes that influenced the presence of glucose are presented in Figure 4.

Correlation between Albumin Conformational Changes and Blood PV

Conformational change of protein is a normal dynamic process. An abrupt change may lead to unfolding or denaturation process. Changes in protein secondary structure can also be seen in the analysis results of this study. It was due to a drastic conformational change, so a lot of hydrogen bonds were used to maintain the secondary structure of proteins such as an alpha-helix and beta-sheet. If the hydrogen bond is broken, the secondary structure changes. Changes in secondary structure caused changes in protein functions, which resulted in adverse impact on albumin protein. Therefore, normal conformational change is determined by environmental conditions.^[4]

Protein conformation is primarily maintained by non-covalent interactions. Forces that determine and maintain the protein conformation are produced by non-covalent interactions between amino acid residues and the surrounding medium. The involved non-covalent interactions are the hydrophobic interactions. This interaction leads to a decrease in free energy when the hydrophobic side chains are removed from the aquatic environment by folding it into the protein interior. Van der Waals bond between hydrophobic groups contributes to protein stability, in which there are many interactions between molecules. In addition to hydrogen bonds, these groups form H-bonds with one another or with water molecules in protein exterior. H-bond is the strongest bond when three atoms are in a parallel position.^[9]

Conformational changes are followed by changes in electron distribution. An electron of a water molecule with a positive charge will pull electron with a negative charge. This attractive force causes rheological dynamics. A little change in the amount of water can affect the dynamics of water or albumin. Thus, the dynamics of water or protein (albumin) are correlated with viscosity. It means that an increase in water dynamics will decrease PV and vice versa.

Blood viscosity is also known to be affected by albumin protein conformation. A change in protein conformation occurs if the water is reduced, which means that the viscosity increases, but if the amount of water is increased or back to normal, then the viscosity decreases. Therefore, the water addition process in diabetic patients is expected to restore the albumin dynamics to normal conditions.^[4,10]

The albumin protein dynamics were strongly influenced by a number of water molecules. Changes in 20% water molecules caused a conformational change in albumin protein. The dynamics of glycated albumin protein were influenced by glucose concentration. The concentration of 11 mg/mL glucose demonstrated that proteins undergo conformational and secondary structures changes, which resulted in several changes on protein functions. Molecular dynamics simulation results showed that the addition of water and changes in glucose concentration affected albumin protein within blood plasma. Blood viscosity was strongly influenced by albumin protein conformation. A change in protein conformation occurs if the water is reduced, which means that the viscosity increases, but if the amount of water is increased or back to normal, then the viscosity decreases.

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