Physiologic Glycated-Bovine Serum Albumin Determination using Spectrum-UV

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Abstract. Albumin is not native phase, but it physiologically-binding with another compound which conclude the functions as transporter and scavenger. The general non-enzymatic reaction within proteins, which has a significant impact on their physical and functional properties by reducing sugar, known as glycation. The study investigated the effective composition to glycate the bovine serum albumin (BSA) by UV-spectrum. Five BSA concentrations (750, 500, 100, 10 and 1 mM) was prepared in PBS pH 7.4. The glycation carried out using glucose concentrations (2M, 1.5 M, 1 M, 500 mM, and 100 mM) before and after incubation for seven days. Depending on concentration, BSA 1 mM and 10 mM showed the best UV spectrum of protein that two peaks, 220 and 280 nm. Hence, the glycation by high concentration of glucose would be made a conformational change of BSA which is marked by the UV-spectrum of BSA configuration. Glucose 100 and 500 mM was effective to glycate BSA.

Keywords: Bovine serum albumin, glucose, glycation, UV spectrum

1. Introduction

Albumin is great known proteins of plasma and has a normal concentration around 35–50 g/l. This characters make albumin the most abundant protein with a wide variety of physiological functions [1]. An individual albumin presents approximately 50% of the normal plasma protein and low molecular weight (67 kDa) [2]. Due to its low molecular weight, albumin promotes in osmotic pressure maintenance of plasma, compared with other globulin of plasma. Also, it has weak isoelectric point, this protein has a global negative charge at physiological pH. The structure of albumin allows protein to bind and transport diverse metabolites such as metal ions, fatty acids, bilirubin and drugs. This structures makes an albumin roles as transporter and scavenger, not stand alone, but it physiologically-binding with another molecules. About 12–18% of circulating proteins (including albumin) are glycated in vivo in normoglycemic blood [3].

Protein is a subject to a several enzymatic and non-enzymatic modifications. The general non-enzymatic reaction within proteins, which has a significant impact on their physical and functional properties by reducing sugar, such as glucose and fructose, known as glycation Maillard reaction [4]. The sugars in addition to amine groups of protein leads to the formation of a reversible Schiff base [5,6]. It rearranges to form over a period of days to produce ketoamine or Amadori product. This products undergo rearrangements followed by other reactions (cyclization, oxidation and dehydration) to form more stable advanced glycation end products (AGEs). Glycation transforms the structure and function of protein, managing to protein dysfunction. These changes could be related to pathological problem.
such as diabetes [7,8]. Albumin is one of protein which has a high probability of glycation mechanism. Previous studies have focused albumin as a glycation target due to it acts as a transporter and scavenger. The glycation mechanism of albumin occurs at tryptophan, tyrosine and phenylalanine residues sites. Thus, the albumin conformational changes are caused by modification on binding sites of albumin when glycation happened [1]. BSA is a protein which rich in lysine (59; 10.1%) and arginine residues (23; 3.9%). Besides, in vivo, albumin is glycated at the arginine, lysine and cysteine residues. The albumin structural modifications induced by glycation included an increased in molecular weight and higher exposure of hydrophobic sites to the solvent of albumin [6,9].

Spectroscopy is a simple and significant analysis used to investigate structural changes and to explore complex formation such as glycation mechanism of albumin [9]. Spectroscopy is a measurement and interpretation of electromagnetic radiation absorbed or emitted when a molecules of sample moves from one energy state to another. Electrons absorb the energy thereby molecules undergo transition from ground state to excited state and its occurred at wavelength of 200-400 nm [10,11].

The study of protein have done with many modifications and purposes. Since bovine serum albumin (BSA) may express as a modelling protein in in vitro experiments. This study investigated the effective composition of BSA and glucose to glycate BSA and keep to maintain its conformational structure by UV-spectrum.

2. Materials and Methods

2.1. Reagents

BSA (BioWORLD, purity of 98%, cat#L18122302) concentration in 750, 500, 100, 10, and 1 mM were dissolved in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Glucose (Sigma-Aldrich) concentrations in 2 M, 1.5, 1, 500, and 100 mM were used as glyating agents and were dissolved in 0.1 M PBS, pH 7.4. The samples were preserved by 0.1% natrium azide (Sigma-Aldrich).

2.2. Conditions of glycation

BSA with addition of glucose and natrium azide was dissolved in PBS and was incubated in sterile vial on laminar air flow for seven days stirred-incubation at 120 rpm and a temperature of 37 °C. The parameters of glycation were measured in before incubation (BG2000 0 day; BG1500 0 day; BG1000 0 day; BG500 0 day; BG100 0 day) and after seven days incubation (BG2000 7 days; BG1500 7 days; BG1000 7 days; BG500 7 days; BG100 7 days).

2.3. UV spectrum absorbance of protein

UV absorption spectra were recorded with a Thermo Spectronic (Genesys 10 UV) spectrophotometer (Rochester, USA). Mode of survey scanned protein were calculated at UV wavelength of 200-300 nm. Five BSA concentrations, five glucose concentration, glycated-BSA (before and after seven days incubation) was kept in their concentrations. UV absorption spectra of BSA and glycated BSA were investigated both backbone and aromatic amino acid chains as blue and red shifts also they configuration.
3. Results and Discussion

3.1. UV spectrum of BSA and glucose

UV absorption assay is well accepted which dynamic quenching affects occurred the excited states of the chromophores [9]. Figure 1 shows the UV absorption spectra acquired for BSA at pH 7.4 in various concentrations at wavelength of 200-300 nm. Regularly, with the concentration enhancements of BSA, indicating the saturated of BSA dissolved in sodium phosphate buffer, no more showed the basic UV spectra of protein. BSA possessed two absorption peaks at 230 nm as backbone spectra (blue shift) and 278 nm as aromatic amino acid chain spectra such as Trp, Tyr, and Phe (red shift). BSA 1 mM and 10 mM able to maintain the albumin conformation. Besides, the higher concentrations of BSA (100, 500 and 750 mM) are undetermined as BSA UV absorption spectra. The spectra which undetermined as one of protein has undergone configuration and function changes.

![Figure 1. UV absorption spectra of various concentrations of BSA. The 1 mM and 10 mM of BSA concentrations have determined an effective concentration to maintained BSA conformation.](image)

Depending on the UV absorption spectra, the effective of BSA concentration is 1 mM and 10 mM. In this experiment, we continued glycated BSA 1 mM using various glucose concentrations. The concentrations of glucose have screened and concluded that glucose with 100 mM and 500 mM is effective to glycate the BSA approaching the basic spectra of protein. Glucose generates non-enzymatic glycation of proteins also plasma proteins (albumin) as a reducing sugar. Significances of glucose bind to albumin lead to conformational changes of the native albumin structure and through loss of its binding properties and induce the signaling cascades transduced by glycated receptor [8].

Glycation of BSA and glucose have done with BSA 750 mM and three glucose concentrations (2M, 1.5 M and 1 M) also BSA 1 mM and two glucose concentrations (500 mM and 100 mM) before and after incubation for seven days. Figure 2 showed that the BSA 750 mM sustain a conformational change. The UV absorption spectra indicated that the absorption value was too high (absorbance of 2.0 – 3.0) deviated to the BSA 750 mM. Absorption peak of backbone spectra (230 nm) has gone and aromatic amino acid spectra too high (more than 0.5 in absorbance). Higher concentration of glucose confirmed to make a conformational change of BSA so, lower concentration of glucose was effective to elevate the glycation.
Figure 2. UV absorption spectra of glycated BSA 750 mM with glucose 1000 mM (a), glucose 1500 mM (b) and glucose 2000 mM (c). The glycation of BSA by three glucose concentrations showed the conformational spectrum of BSA and it could not maintain the native spectra of BSA. The BSA structure was ruined and could not produce the physiological glycated BSA.
3.2. *UV spectrum of physiologic glycated BSA*

The lower of BSA and glucose concentrations were indicated the effective composition of compound which could glycated the BSA and maintain the conformational structure of protein. It was determined by composition of BSA 1 mM with 100 mM and 500 mM. Based on before and after seven days incubations, it made a fabulous UV absorption spectra and it almost consistent both 100 mM and 500 mM concentration of glucose as shown as figure 3. When it compared with peak of BSA 1 mM, both BG100 and BG500 showed a stable peak especially at 230 nm and 280 nm, but it fluctuated in other wavelength. The conformational changes caused by intercalation of glucose. The concentrations of glucose (500 and 100 mM) was given an effective stimulation of BSA to glycate the BSA and keep to maintain its conformational structure. Although, the previous study show more significantly result to produce the physiologic glycated albumin, this study found that the time of exposure of reducing sugar could be shorten, no more weeks to make a glycated protein [5].

The glycation mechanism eventuating in organism body be decided by several characteristics (e.g. glucose concentration, other arrangement of reducing sugar in plasma, revealing time of reducing sugar and local environment) and it is difficult to reproduce to in vitro experiment. Despite the fact that, there is no equalized model of glycation, researchers apply in vitro glycation for primary research. There is two tryptophan residues of BSA molecule (Trp134 and Trp213), while human serum albumin has a tryptophan residue (Trp214), but Trp134 is detected far from the hydrophobic cleft of BSA. However, all data regarding the 230 nm backbone spectra of BSA 1 mM which consistent with the majority of previous study. UV spectra showed a strong quenching at 280 nm for sample which incubated after seven days. It accompanied with an increasing concentration of glucose indicates that binding to BSA receptors. Perhaps, the receptors change the microenvironment of amino acid residues, such as tyrosine which apparently derives from glycation mechanism of neighboring arginine or lysine residues [8,12].

Glycation transforms a function and structure of protein, managing to the protein dysfunction. Surprisingly, this study presents in vitro protein glycation at physiological glucose concentrations and could glycate BSA within seven days incubation approaching the basic spectra of protein. This study and some present research suggest several important avenues for future protein glycation research. It is important to question that some research accepted pathophysiological mechanisms if they could not be explained in vivo at physiological concentrations reducing agent. The evidence of this study clarify that lower concentration of reducing sugar and low BSA concentration plays an early role in physiologic glycated BSA as a novel and it explains a gap in the some of literature that have compared. The duration or time of exposure of the reducing sugar need to be further investigated. Some of previous study declared that the effective incubation is 4-7 weeks, but it might be shorter [8,10,12,13].
Figure 3. UV absorption spectra of glycated BSA 1 mM with glucose 100 mM (A) and glucose 500 mM (B). Both glucose concentration showed the conformational spectrum of BSA and they could change the aromatic amino acid chains spectrum (at wavelength around of 280 nm).

4. Conclusions
The effect of BSA concentration is 1 and 10 mM to glycate BSA without made a conformational change of protein structure. Higher concentration of BSA and glucose showed more chromophore spectra. But, lower concentration of BSA and glucose have no significant effect to produce more chromophore spectra. So, the conformation based on UV spectra was more stable. To know protein glycation function and conformational change of its structure need further analyzed such as with Electron Spin Resonance, Fourier Transform Infrared Spectroscopy, viscosity and spectrofluorometry.

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