UV VIS STUDY IN INTERACTION ASTAXANTHIN AND GLYCATED BOVINE SERUM ALBUMIN (BSA)

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ABSTRACT

Astaxanthin antioxidant activity has been reported to be more than 100 times greater than that of vitamin E against lipid peroxidation and approximately 550 times more potent than that of vitamin E for singlet oxygen quenching. The purpose of this study is to find out the best concentration of astaxanthin to reduce the UV absorbance from Bovine Serum Albumin (BSA) glycated by using UV spectroscopy. Astaxanthin was dissolved in methanol at the concentration (1x10⁻⁹ – 1x10⁻¹⁰ mol/L) different concentrations, then added to glycated BSA 100 mM and 500 mM then observed with UV spectroscopy. From the UV spectroscopy observations at 284 nm wavelengths, the best result for astaxanthin concentration is 0.2x 10⁻⁸ and 1x10⁻¹⁰ mol/L, it showed the best concentration on its effect to reduce the absorbance on BSA conformation.

Keywords: Astaxanthin, Glycated BSA, Spectrum UV

1. INTRODUCTION

Glycation is a nonenzymatic reaction between reducing sugar and an amino group of proteins (Yamagishi, 2013). The underlying stage of chemistry is a condensation reaction between an amino group and an aldehyde. This step is known as the formation of Schiff Base. The bases of Schiff containing additional reactive groups, such as the carbonyl and hydroxyl groups can undergo further reaction and often form yellow-brown products. This reaction is also known as Maillard reaction (Sadowska-Bartosz et al., 2015). Amadori product undergoes auto oxidation on transition metals resulting in free radicals. Auto oxidation of glucose produces ketoamine capable of reacting with proteins to form advanced glycation end Products (AGE) and also produce free radicals such as radical superoxide in the process (Taghavi et al., 2017). Amadori products undergo oxidation of metal catalysts (glycoxidation), producing dicarbonyl compounds that form the AGE (Fang et al., 2002) Amadori products undergo a variation of dehydration reaction and irreversible rearrangement that leads to the formation of an advanced glycated end products (Sakata et al., 2002). The glycation reaction leads to the loss of protein function and tissue elasticity disorders such as blood vessels, skin, and tendons (He et al., 2018). The glycation reaction is highly accelerated by the presence of hyperglycemia and oxidative stress tissues. The glycation implicates the pathogenesis of diabetic and aging complications. There is no known enzyme to be able to eliminate or decrease the product of the application (Roche et al., 2008).

The number of complications in diabetes seems to be correlated with blood glucose concentrations so that it becomes the main cause of tissue damage. Glycation leads to the activation of polyol metabolic pathways that further accelerate the formation of reactive oxygen compounds (Bern et al., 2015). The formation of such reactive oxygen compounds can improve the modification of lipids, DNA, and proteins on various tissues. Molecular modification of these tissues leads to an imbalance between protective antioxidants (antioxidant defenses) and increased free radical production, the beginning of oxidative damage known as oxidative stress (Ott et al., 2014). In diabetic conditions, complications are related to oxidative stress in particular the formation of free radical superoxide. Sources of oxidative stress in diabetes include displacement of redox reaction balance due to
changes in carbohydrate and lipid metabolism that will increase the formation of ROS from the reaction of glycation and lipid oxidation, in addition the source of radical also originated from the glycation process (Treatment et al., 2018).

An important rule of oxidative stress in pathophysiology, caused by a redox imbalance between free radicals and antioxidants, forms the rationale for the therapeutic approach of antioxidants(Kawasaki et al., 2013). To reduce oxidative damage is required antioxidant. Albumin has the potential to increase the protection of endogenous antioxidants in critical pathological interventions (Masuelli, 2013).

Spectrophotometry (UV-Vis) is an instrument used to detect the intensity of absorption of light energy by a molecule at certain wavelengths. Spectroscopy uses an electromagnetic radiation of between 190-800 nm which is divided into ultraviolet areas (UV at 190-400 nm) and range (Vis at 400-800 nm) (Kumirska et al., 2010). The light appears to be a small part of all electromagnetic radiation. The visible light spectrum consists of red, orange, yellow, green, blue and purple which each have a certain distinct wavelength. The unit used to declare wavelength is Angstrom (1 Å = 10^-10 meters). Each molecule has its own unique characteristics based on its ability to absorb electromagnetic energy. Molecules with the same atomic composition but have different bonds will absorb energy at different wavelengths. This molecular trait is used as the basis of molecular characterization using a spectrophotometer or is usually called the UV-Vis spectrum (Yuan et al., 2012).

The mechanism of action of the Spectrophotometer is as follows. The light coming from a polycrystalline deuterium or tungsten lamp is transmitted through the lens to the monochromator in the spectrophotometer and the light filter on the photometer. Monochromator converts polychromatic light into monochromatic light (single). Light files of certain lengths will then be passed on samples containing substances in certain concentrations. The transmitted light will be received by the detector which will then calculate the absorbable light and against the light being transposed by the sample. The absorbed light is comparable to the concentration of the substance contained in the sample so that the concentration of substances in the sample can be known quantitatively. Peak-spectrum albumin on the UV-Vis spectrum is at 220nm (backbone) and 280nm (aromatic chain)(Lange et al., 1996).

Astaxanthin is a carotenoid pigment found in various organisms of bacteria, algae, yeast, crustaceans and several types of fish. Astaxanthin is the most powerful antioxidant, even with higher activity than alpha-tocopherol and other carotenoids (Matthews et al., 2006). Astaxanthin is one of the carotene and red pigments, which are universally distributed in aquatic animals, such as salmon, crustaceans. Various microorganisms and microalgae species can also produce astaxanthin (Chen et al., 2014). In particular, unicellular microalgae Haematococcus pluvialis which can accumulate 0.5-5.0% Astaxanthin from its weight. Previous studies show that Astaxanthin is effective, as an anti-cancer (Sowmya et al., 2017), anti-tumor, increased immunity(Ambigaipalan & Shahidi, 2017), anti-ulcer (Kamath et al., 2008) and protects the function of the eye (Li et al., 2015).

Antioxidants protect cells against free radicals derived from glycation and have been proposed as therapeutic agents. Diabetes can be treated more effectively with a synergistic effect of compounds that offer antioxidants and anti-glycation properties. The increasing role of AGE in diabetic and age-related pathology, it has been suggested that inhibitory formation of AGEs may prevent the development of diabetes complications and slow the aging process (Roche et al., 2008).

Aim of this study is to investigate the conformational changes of protein by using UV spectrum techniques and to know which concentration of astaxanthin can reduce the absorbance of glycated albumin.

2. METHOD

2.1. Materials Preparation

Role of Astaxanthin in the improvement of physicochemical character Bovine serum albumin (BSA) which is exposed to high concentrations of glucose as a model on diabetic conditions. Experiment did by glycation on BSA (SIGMA A3782). BSA Normal and BSA
in a state of high glucose (100 mM & 500 mM) that has been incubated for 21 days is interacted with Astaxanthin at the concentration (1x10^{-9} to 1x10^{-10}). Bovine Serum Albumin 1 mM prepared by dissolving 0.68 g BSA (BSA Fraction V, BioPLUS Chemicals) in 10 ml PBS pH 7.4 (1 L PBS consists of 800 ml ddH2O, 0.2 gr KCl, 8 gr NaCl, 1, 44 gr Na2HPO4). 1 M stock glucose solution in the way of dissolving 9 g glucose (Merck) in 50 ml of PBS. 500 mM glucose is prepared by diluted 500 μl glucose 1 M by adding a 4500 μl of PBS. 500 mM glucose is prepared by diluted 2500 μl glucose 1 M by adding a 2500 μl of PBS. Solution NaN3 prepared by dissolving 0.1 gr powder NaN3 to 9.9 ml PBS.

Astaxanthin (Astin Plus) as much as two tablets (4 mg) is crushed using mortal, then dissolved with 200 μl ethanol, centrifuged at a speed of 12,000 rpm, a temperature of 40°C for 20 minutes. The the supernatant parts are taken and separated in different tubes then plus 10 ml PBS pH 7.4 As a stock solution (wrapped in a meeting using aluminium foil).

2.2. Glycated Bovine Serum Albumin

A total of 10 ml of BSA 1mM was applied by adding glucose concentrations of 100 (BG 100) and 500 mM (BG 500), during 21 days at a temperature of 37°C and stir by stirrer at 100 rpm. Albumin is assumed to have been glycated when there is increased spectrum absorption compared to albumin without glycation (at a wavelength of 200-300 nm).

2.3. Addition of Astaxanthin on Glycated BSA

Incubation of the 14 days albumin (BG100 14d) and 21 days (BG500 21d), added astaxanthin with centration of 1x10^{-9} to 1x10^{-10} of Astaxanthin incubation was carried out for 7 days. At the end of incubation, samples were made to analyze the UV spectrum profile

2.4. Data Collection

Absorbance Measurement

BSA, and BSA-Astaxanthin with various solvents at several concentrations were observed with UV spectro (Genesys 10 UV) at a wavelength of 200-300 nm. UV absorbance spectra were recorded with spectrophotometer equipped with 1.0 cm quartz cells at room temperature.

3. RESULT AND DISCUSSION

To investigate structural changes UV spectro measurement is a simple and pertinent method can be used. Dynamic quenching affects only the excited states of the fluorophores well accepted, while has a scarce effect on the absorption spectrum, to initially verify the quenching mechanism, the UV–spectro of astaxanthin and astaxanthin-BSA were measured and recorded.

Determination of astaxanthin concentration needs to be done so that the researcher can obtain information, in what concentration does astaxanthin dissolve interact with the maximum with BSA, so astaxanthin can maintain BSA function. In the initial scanning changes of structural conformation of albumin, UV spectro measurement is a simple and relevant method can be used. Glycated BSA with glucose concentration 100 mM (BG 100) and 500 mM (BG 500) are interacted with Astaxanthin with different concentrations. Glycated BSA changed albumin conformation from having 2 peaks absorbance (224 nm and 284 nm) shifted to 224 nm and 248 nm.

Optimal Astaxanthin concentration affecting the basis of the albumin-glycated structure is determined based on the decrease in absorption from the resulting UV spectrum profile (Rajendran et al., 2014) BSA show two absorption peaks at 224 nm and 284 nm. The absorption peak of the backbone of BSA around 224 nm, while the weak absorption peak at around 284 nm resulted from the aromatic amino acids (Trp, Tyr, and Phe). Based on this study, the best astaxanthin concentrations In lowering the absorbance in the glycated albumin is astaxanthin with concentrations of 0.2 x10^{-9} and 1x10^{-10}.

Albumin is composed of 585 amino acids and has a molecular weight of 66 kDa. These highly soluble proteins present in the human plasma at normal concentrations between 35 and 50 g/l. Albumin has several important physiological and pharmacological functions. Albumin transports metals, fatty acids, cholesterol, bile pigments, and drugs. Albumin is a key element in the arrangement of osmotic
pressure and fluid distribution. In normal conditions, the half-life of albumin is about 20 days (Guerin-Dubourg et al., 2012).

Albumin is the primary and dominant antioxidant in plasma. Most of the antioxidant properties can be associated with albumin. Previous research has shown that more than 70% of radical scavenging serum activity is caused by Bovine Serum Albumin (BSA) tested using free radical hemolysis test (Fasano et al., 2005).

![Figure 1](image1.png)

**Figure 1.** The UV spectrum on albumin glycation incubated 21 days with concentration of glucose 100 mM (Figure A) and 500mM (Figure B) was interacted with Astaxanthin at concentrations of $1 \times 10^{-9}$ to $1 \times 10^{-10}$. BSA 1mM as a profile of albumin under normal conditions.
The ability of albumin in free radical-trapping is due to the presence of one cysteine (Cys34) residue where as much as 70-80% of the residue contains sulfhydryl groups (Figure 1). The existence of such residue that makes albumin capable of binding to the hydroxyl radical. Cys 34 is on a subdomain of IA where it located in the crack of a protein surface and not related to a disulfide bridge, but the Sulfhydryng group is able to bind Hg$^{2+}$, Ag$^{+}$, Au$^{+}$ and Pt$^{2+}$ (Wol et al., 1991).

The antioxidant activity owned by albumin is able to protect the ligands from oxidation events. One example is the binding of unsaturated fatty acids. Cys34 in albumin can also reduce thiol in human blood plasma. In addition to the Cys amino acids as a free radical scavenger, there is also a functioning Met in a metal kelator (Taghavi et al., 2017).

According to Melorose et al. (2015) The glycation mechanism initiated from the formation of a reversible and unstable Schiff Base formed from the bond of glucose or its derivation with an amin group of albumin (reversible action lasts for 1-2 weeks), causing residue formation Stable Fruktosamine (also known as Ketoamin). The rearrangement of this compound eventually resulted in an irreversible amadori compound (irreversible, scheduled glycation for 6-8 weeks). The reaction is an early glycation process and is also known as Maillard reaction. Advanced stage Modifications (re-arrangement, oxidation, polymerization, and cleavage) in this early stage glycation product (Amadori product), resulting in an irreversible conjugate called Advanced glycated End Products (AGE) (Ulrich et al., 2001).

On the glycation phenomenon occurs changes in the conformation of BSA structures. Where there is an increase in the backbone (peak 224 nm) and changes in the structure of the aromatic group of BSA glycation (at peak 284 nm). Changes in this aromatic group are characterized by the presence of amino acids (Trp, Tyr, and Phe) (Glazer & Rosenheck, 1962).

The structure of Astaxanthin is similar to lycopene, the double bonds along the backbone of the molecule form a polyene chain that gives this and other similar molecules similar to their unique properties. Alternating single and double bonds allows for the possibility of a number of geometry isomer (Coral-Hinoestroza & Bjerkeng, 2002). Naturally, most carotenoids exist in all trans configurations and such as Astaxanthin. Light exposure is known to isomerise the parent carotenoid molecule, which can affect the shape of the molecules with the form of a trans structurally more rigid. This confirmation change can affect the solubility and absorption of the molecules. Forms of trans carotenoids also tend to be easier to crystallize or aggregate so that they are less easily absorbed. Most carotenoids are hydrophilic and are only soluble in non-polar organic solvents (Sowmya & Sachindra, 2012).

Astaxanthin is an exception and it has the ability to dissolve itself in polar and non-polar solvents. The presence of conjugated double bond systems with the associated delocalized electrons provides carotenoid molecules such as astaxanthin that have unique electrochemical properties. These highly-delocalized electrons only require small energy to promote them into the state of being enstated. As a result the light seems energetic enough to carry an electronic transition with molecules. Carotenoids such as astaxanthin are susceptible to oxidative dehydration during storage and processing and therefore special treatment should be performed when addressing to prevent photobleaching damage and oxidative damage (Zhu & Row, 2013).

4. CONCLUSION

Glycated BSA showed two peak absorbancies at 224 nm and 284 nm. Peak Absorbance in BSA’s backbone is around 224 nm, while the weak absorption peaks around 284 nm are produced from the aromatic group (Trp, Tyr, and Phe). At BSA glycation and Astaxanthin interactions showed the best abortions in the concentration of Astaxanthin 0.2 x10$^{-9}$ and 1x10$^{-10}$ although not close to the normal albumin spectrum.

5. ACKNOWLEDGEMENT

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6. REFERENCES


