Myoglobin in the utilization of oxygen in animals biology essay

Myoglobin is an oxygen-binding globular protein which is vital in facilitating the acquisition and utilization of oxygen in animals. Myoglobin was isolated and purified from ground water buffalo extract. The Myoglobin was isolated by cation-exchange chromatography, and concentration of Myoglobin is measured by spectrophotometry. Total concentration of protein was determined by performing Bradford protein assay. Iron analysis was performed by atomic absorption spectrophotometry (AAS). Molecular weight of Myoglobin was determined by running SDS-PAGE. A total amount of 144 ug Myoglobin was purified by the cation-exchange chromatography with a relative purity of 25. 9%. The concentration of the purified Myoglobin was determined to be 0. 072 ug/uL. The molecular weight of Myoglobin was determined to be 18204 Da And 0. 423 mg of iron were detected in the acid digested extract

Introduction

Myoglobin is a globular protein which contains a single polypeptide chain of about 153 amino acids and an iron-porphyrin complex, or the heme group (3). And it has a molecular weight of 16700 Da and 153 amino acids (4). The non-covalently bound heme group, which resides in the hydrophobic interior of the native globin chain, is able to unfold under acidic condition and consequently weakens the interaction between the heme group and the globin (5).

Myoglobin is an oxygen-transport protein which can be found in muscle tissues of all mammles. Myoglobin is critical in mammalian cell in that it is responsible for storage and distribution of oxygen, and possibly carrying

energy (4). Diving animals such as seals and whales have excessive amount of Myoglobin that help them travel undersea by storing and transport oxygen (9). It plays a significant role in the physiological function of heart and skeletal muscle (2). Elevated consumption of oxygen during exercise necessitates the production of myoglobin in red muscle and heart cells, and the transportation of oxygen by myoglobin from the sarcolemma to the mitochondria in vertebrate heart and red muscle cells. (1)

Spectrophotometry studies the interaction of electromagnetic radiation with molecules, atoms and ions (10). It can shed light on the physical and chemical properties by measuring the emission or absorption of electromagnetic radiation (10). Besides, it is also used to identify biomolecules from their individual absorption spectrum. In the meantime, spectrophotomoetry can quantitatively measure the concentration of molecules in solution According to the Beer-Lambert law, the fraction of incident light absorbed by a solution at a given wavelength is indicative of the concentration of the absorbing species (10) . Tryptophan and tyrosine can absorb ultraviolet light, which accounts for the characteristic strong absorbance of light at wavelength of 280 nm by most proteins (9). Atomic absorption spectroscopy (AAS)can be used to determine the identity and concentration of chemical elements in the gaseous state by measuring the light radiation absorbed by the elements (10).

Ion-exchange chromatography is a technique for separating biomolecules capable of being involved in electrostatic interactions (10). Molecules can be separated based on their sign and magnitude of net charge at a given pH and formation of electrostatic linkages between thee resin and the protein of

interest (9). Ion exchange separations take place in columns packed with an iron-exchange resin (10). Resins with bound anionic groups are cation exchanges whereas those with bound cationic groups are anion exchangers (9). Selection of ion exchange resin depends on what is to be purified, the pH to be used in the column, and the strength of the functional group (10).

Electrophoresis is the separation of proteins based on the motion of the charged proteins under the influence of an electric field (9). The migration of the protein depends on its shape, size, charge and chemical composition (10). An electrophoretic method, polyacrylamide gel electrophoresis (SDS-PAGE) can be used to estimate the purity and determine the molecular weight of the protein (9). Treatment of the protein by the ionic detergent sodium dodecyl sulfate (SDS) can give it a uniform net charge, and protein can then be separated based solely on its mass (10).

In this experiment, Myoglobin is extracted and purified by cation-exchange chromatography from ground water buffalo using Beffer A (20 nM, pH 5. 6, KH2PO4) and Buffer B (20mM, Tris buffer, pH 7. 5). Absorbance of the eluent fractions is measured at 280nm and 417nm. Total amount of protein and the concentration of Myoglobin are determined using Bradford assay from BSA protein. Molecular weight of Myoglobin was determined by performing SDS-PAGE. Iron content in the acid digested extract is measured by atomic absorption spectrophotometry (AAS). A total amount of 144 ug Myoglobin was purified by the cation-exchange chromatography with a relative purity of 25. 9%. The molecular weight of Myoglobin was determined to be 18204 Da. 0. 757 mmol of iron and 39. 5 nmol of myoglobin were detected in the acid digested extract, with a ration of 20: 1.

Results

A total of five samples were collected from a solution of 10. 02g thawed ground buffalo mixed with 20. 0mL, 20mM, pH 5. 6, KH2PO4 (Buffer A): 1. 0 mL of crude extract (sample C), 1. 0 mL of filtered extract (sample D), 2. 0 mL of Buffer A sample solution (sample A), 2. 0 mL Buffer B sample solution (sample B) and 5. 0 mL acid digested extract solution. Chromatogram for the purification of myoglobin carried out by cation-exchange chromatography shows a large peak from fraction number 5 to fraction number 12. (Figure 1). The figure also indicates another peak at fraction number 23, with smaller peaks at fraction number 19 and fraction number 25 (Figure 1). Fraction number 23 has the highest 417 nm/ 280 nm (3. 766) and the highest absorbance 90. 278) at 280 nm (Figure 1).

Discussion

When myoglobin was separated by cation-exchange chromatograph, purity of the myoglobin in the eluent fractions collected at specific volumes was examined by spectroscopy. Absorbance of Myoglobin, specifically, was measured at 417 nm and other proteins was measured at 280 nm, due to the presence of Tyrosine and Tryptophan Most proteins absorbs at a wavelength of 280 nm (10). Phophyrin has an absorbance spectrum of 414nm to 418 nm, and intensity and wavelength of the absorption can be influenced by the peripheral substituents on the porphryin and the protonation state of the nitrogen atoms (6). Sincemyoglobin consists of a iron-containing heme prosthetic group with an iron-contained porphyrin ring (9), it can absorb at a wavelength of 417.

Myoglobin has a PI value of 7, so it will have a net positive charge when pH is below its PI and a net negative charge when pH is above its PI. When myoglobin is positively charged when buffer A (pH= 5. 6) is used to wash the column, it binds to a column containing negatively charged beads in cation-exchange chromatography. The positively charged Myoglobin can then be eluted by washing the column with buffers having higher pH value than 7 (Buffer B, pH= 7. 5). Raising the pH of the mobiles phase buffer renders the Myoglobin less protonated and thus negatively charged. As a result, the Myoglobin is not be able to form an ionic interaction with the negatively charged stationary phase and then elutes from the column (10).

144 ug Myoglobin was recovered form the column, which accounts for 10. 7% of the myoglobin that was loaded onto the column. Loss of Myoglobin could be attributed to diffusion spreading of Myoglobin and other contaminant proteins within the mobile phase, as a result of the increase in time length (9). Recovery of myoglobin can be improved by using narrow columns, longer column (7).

The molecular weight of Myoglobin (18204 Da) obtained from the SDS-PAGE was similar to the literature value, 16700 Da (4), which indicated that the purified protein in the Buffer B sample was Myoglobin. The clear band generated from Lane B migrated the same distance as the band from the Myoglobin standard, which further confirms that the purified protein was Myoglobin (Figure 3).

The stoichiometric relationship between iron and hemoglobin is 20: 1 instead of 1: 1 according to the fact each molecule of Myoglobin has only one

molecule of ion. The excessive amount of Fe might be present in oxygen-carrying protein such as hemoglobin, which contains four heme prosthetic groups. Electron carriers in the mitochondrial respiratory chain have Fe incorporated in their prosthetic group, and examples of them include cytochomr c, ubiquinone and cytochrome oxidase. Besides, another hemeprotein, ferritin might also be present in the protein sample. Hemeprotein functions to stores and release iron atoms in biologically available form for use in heme and nonheme proteins and biochemical reactions (8).

In conclusion, a ratio of 1: 20 for Myoglobin and iron was obtained from the ground water buffalo. 144 ug Myoglobin was purified from the filtered extract with a relative purity of 25. 9%. According to SDS-PAGE, the molecular weight of Myoglobin was determined to be 18402 Da.