ANALYSIS OF GLYCATED HUMAN SERUM ALBUMIN BY ELECTROPHORESIS AND FLUORESCENCE SPECTROSCOPY

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Abstract: For body's energy requirement we need regular supply of glucose. To transport glucose, human serum albumin (HSA) is important and most abundant plasma protein. During transportation non-enzymatic glycation of protein occurs, which is a multi-step process, and is the first event in case of hyperglycemia. Accordingly, it yields advanced glycation end-products (AGEs), which can severely alter protein structure. Different methods have been used for determination of glycation of proteins and related AGEs like, Enzyme-linked immunosorbent assay (ELISA), High-performance liquid chromatography (HPLC) and mass spectrometry. However, their instrumentation and high cost of analysis limited their routine usefulness in analytical laboratories. Therefore, alternative methods to study glycation for routine use are desirable. Thus, the purpose is to develop rapid, simple, and sensitive methods for the determination of glycation of human serum albumin by using a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) & spectrofluorometric technique. In our study we have observed that, the molecular weight of glycated HSA has increased due to the formation of advanced glycation end products, and hence non-glycated HSA can be compared with properly glycated HSA by using SDS- PAGE. Similarly, fluorescence intensity of glycated HSA has increased using wavelengths 420 nm to 600 nm and excitation at 470 nm.

Keywords: Analysis, Human serum albumin, Glycation, Glucose, Fluorescence Spectroscopy, SDS-PAGE.

I. INTRODUCTION

Human serum albumin is a protein that can transfer different drugs in the blood (27). HSA is important and most abundant plasma protein (12), having 66 kD molecular weight (15). Normal glucose concentration in blood is previously reported to be 100 mg/dL (8, 12), and in case of diabetics it is between175 mg/dL and 400 mg/dL (7). Nearly 20% changes in HSA in diabetic conditions are induced by non-enzymatic glycosylation (10).

Non-enzymatic glycation of protein, is a multi-step process, and is the first event in case of hyperglycemia. This process is important in long-term complications in diabetes. It involves glucose reacting with number of amino acid side chains (such as lysine and arginine residues). Accordingly, it yields AGEs, a class of heterogeneous chemical compounds. These reactions depend on different glucose concentrations and the resultant AGE can severely alter protein structure (11, 14, 18, and 23). Complications of diabetes are caused by structural changes in biomolecules such as HSA; these changes strongly favour severe complications such as retinopathy, nephropathy, and atherosclerosis (1, 4).

The glycated Human serum albumin accumulate in diabetic patients due to presence of very high glucose concentration (6). The change in structure of glycated protein results in impairment of its normal functions. This has been suggested for haemoglobin and human serum albumin (22). But, HSA is most important quantitatively as it is a transport protein in plasma. It is argued that in diabetes nearly 80% of HSA is not glycosylated. Therefore, it is important to study proper conditions under which glycated HSA interacts with number of molecules (25).

Different methods have been used for determination of glycation of proteins and related AGEs like ELISA, HPLC and mass spectrometry. However, their instrumentation and high cost of analysis limited their routine usefulness in analytical laboratories. Therefore, alternative methods to study glycation for routine use are desirable. Thus, the purpose is to develop rapid, simple, and sensitive method for the determination of glycation by using a SDS-PAGE and spectrofluorometric technique.

The technology to obtain analytical separation of proteins is SDS-PAGE (9, 24). Thus, assessment of protein samples, protein expression evaluation, and quantification of proteins utilizes SDS-PAGE (9, 20, 24.). Similarly, Spectrofluorometry is one of the analytical techniques that are convenient because of its low cost, wide availability and simplicity in laboratories.

II. MATERIAL AND METHODS

Human serum albumin and other chemicals were purchased from Sigma-Aldrich or HiMedia.

Preparation of glycated HSA

Reaction mixture: 1.5 mM 500 µl HSA incubated with 100 mM glucose in 0.4 mM phosphate buffer for 30 days at 37° C.

Control: 1.5 mM 500 µl HSA incubated in 0.4 mM phosphate buffer for 30 days at 37° C.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

For the investigation of protein separation and purity, SDS-PAGE was carried out according to the method of Laemmli (13). 0.5 M Tris-HCl, pH 6.8 was used for making the stacking gel. 1.5 M Tris-HCl , pH 8.8 was used to make the resolving gel. 30% 29:1 Acrylamide/Bis-Acrylamide was utilised,10% (w/v) Ammonium persulfate (APS), TEMED & 1% (w/v) Bromophenol blue were utilized.

Fluorescence Measurements

Spectrofluorometric experiment was performed by using Jasco FP-6500 spectrofluorometer. For measurement 10 mm quartz cell was used. It consists of two monochromators, which selects two types of wavelengths as excitation and emission (fluorescence). Xenon flash lamp was utilized as a light source. Data analysis was performed using standard software supplied by the manufacturer. Standard software supplied by manufacturer was used for data analysis. In this experiment, wavelengths used were 420 nm to 600 nm and excitation at 470 nm.

III. RESULTS AND DISCUSSION

Many studies discussed the structural changes and characteristics of proteins during the glycation process *in vitro* (2, 3, 5, 15, 16, 18, 19, 21, 26, and 28). In order to determine the difference between glycated and non-glycated human serum albumin, HSA was incubated with glucose for 30 days at 37° C to study the progression of glycation reaction *in vitro*. SDS-PAGE (figure1) and Fluorescence (figure 2) methods were used to confirm the glycation of HSA. Based on the result, HSA was truly glycated considering control sample.

SDS-PAGE analysis:

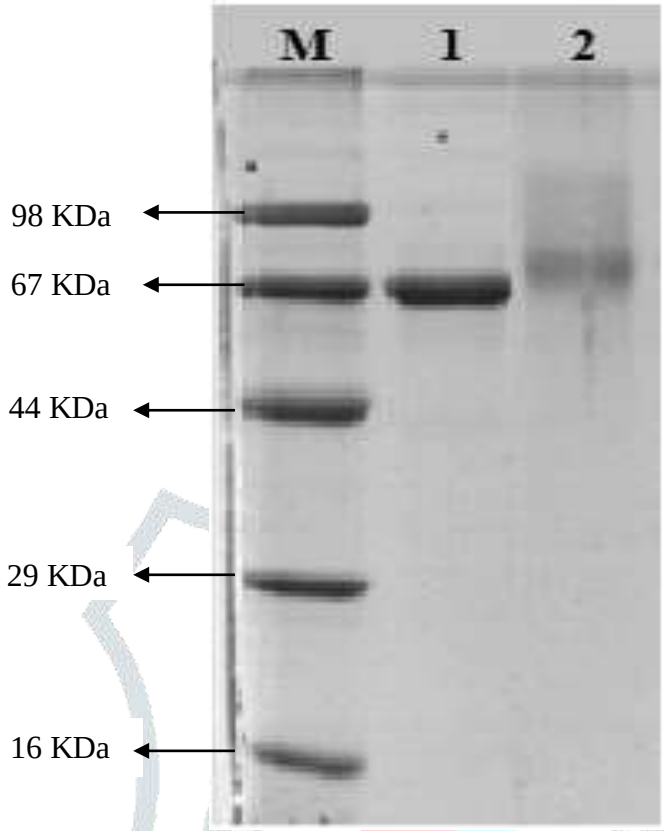

Figure 1: SDS-PAGE electrophoresis of Human serum albumin

(M – Marker, 1 – Normal HSA, 2 – Glycated HSA)

From SDS-PAGE, it is easily seen that molecular weight of glycated HSA has increased due to the formation of advanced glycation end products, hence it is confirmed that non-glycated HSA can be compared with properly glycated HSA by using SDS- PAGE electrophoresis. The SDS-PAGE electrophoresis of HSA in the absence and presence of glucose has been shown in Figure 1. These control & glycated samples were used to investigate their effects on albumin separately, corresponding to the normal condition & diabetic condition, respectively. Increasing the sugar concentration leads to an increase in molecular weight of HSA.

Spectrofluorometric analysis:

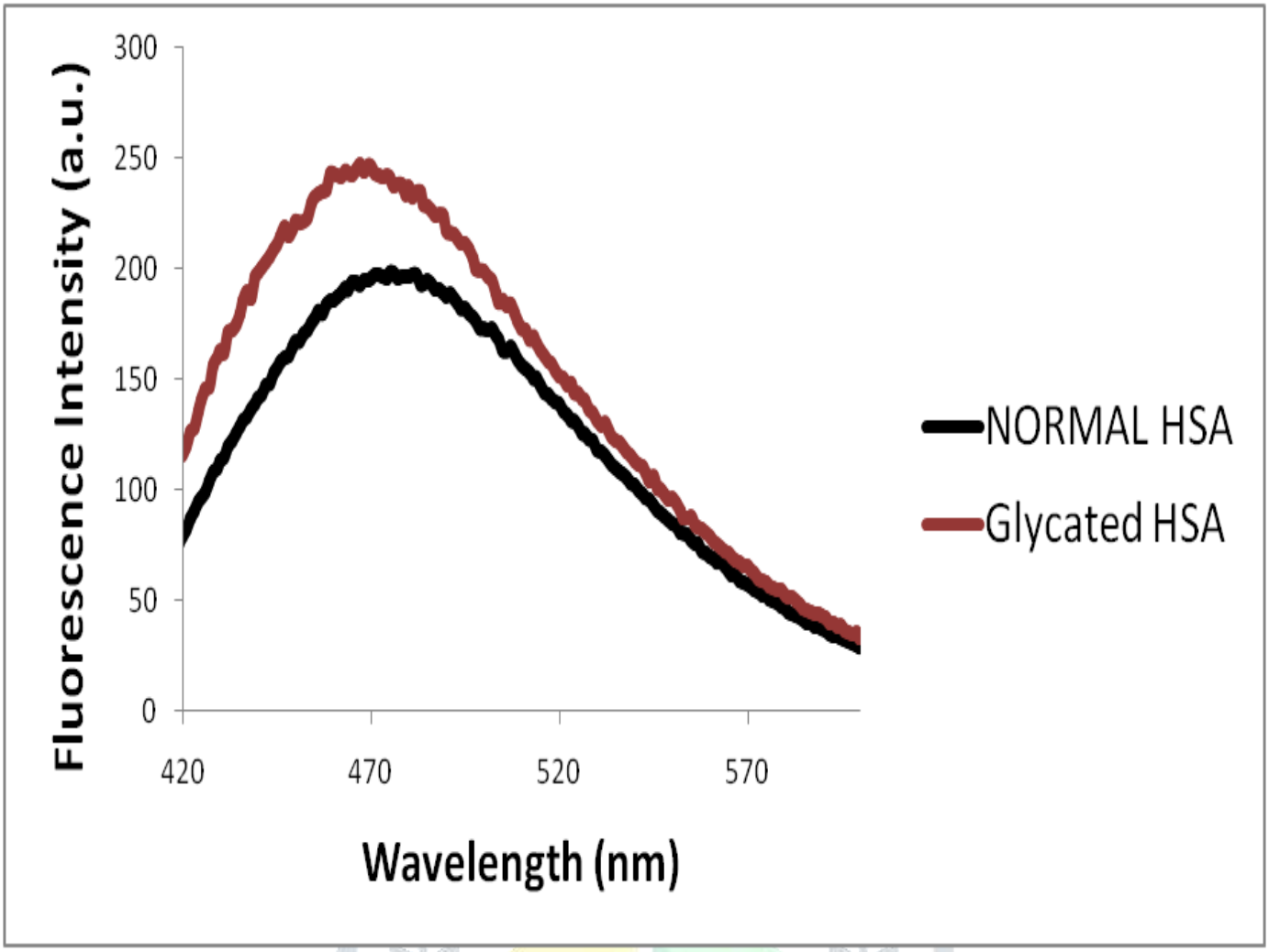

Figure 2: Fluorescence spectrometry of normal and glycated HSA.

Results were analysed by plotting a graph of wavelength (nm) vs. fluorescence intensity (a.u.). From graph we can easily compare normal HSA with glycated HSA where fluorescence intensity of glycated HSA has increased. In previous study (27), it was stated that fluorescence for some AGEs observed with maximum emission wavelengths at 431, 440, 453, and 460 nm at correspondence maximum excitation of 322, 342, 352, 375 and 381 nm, respectively. Fluorescence spectra of non-glycated & glycated HSA is presented on figure 2. , glycated HSA shows increased fluorescence intensity. The glycated sample of HSA in given conditions shows better result comparison of glycated HSA and non-glycated HSA *in vitro* by using wavelengths 420 nm to 600 nm and excitation at 470 nm.

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