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Fine Characterization of Glucose Modified Human Serum Albumin by Different Biophysical and Biochemical Techniques at a Range

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Abstract : Protein modification in diabetes mellitus may lead to early glycation products (EGPs) or amadori product as well as advanced glycation end products (AGEs). Early glycation involves the reaction of glucose with N-terminal and lysyl side chain amino groups to form Schiff's base which undergoes rearrangements to form more stable early glycation product known as Amadori product. After Amadori, the reactions become more complicated leading to the formation of advanced glycation end products (AGEs) that interact with various AGE receptors, thereby playing an important role in the long-term complications of diabetes. Millard reaction or nonenzymatic glycation reaction accelerate in diabetes due to hyperglycation and alter serum protein's structure, their normal functions that lead micro and macro vascular complications in diabetic patients. In this study, Human Serum Albumin (HSA) with a constant concentration was incubated with different concentrations of glucose at 370C for a week. At 4th day, Amadori product was formed that was confirmed by colorimetric method NBT assay and TBA assay which both are authenticate early glycation product. Conformational changes in native as well as all samples of Amadori albumin with different concentrations of glucose were investigated by various biophysical and biochemical techniques. Main biophysical techniques hyperchromacity, quenching of fluorescence intensity, FTIR, CD and SDS-PAGE were used. Further conformational changes were observed by biochemical assays mainly HMF formation, fructoseamine, reduction of fructoseamine with NaBH4, carbonyl content estimation, lysine and arginine residues estimation, ANS binding property and thiol group estimation. This study find structural and biochemical changes in Amadori modified HSA with normal to hyperchronic range of glucose with respect to native HSA. When glucose concentration was increased from normal to chronic range biochemical and structural changes also increased. Highest alteration in secondary and tertiary structure and conformation in glycated HSA was observed at the hyperchronic concentration (75mM) of glucose. Although it has been found that Amadori modified proteins is also involved in secondary complications of diabetes as AGEs but very few studies have been done to analyze the conformational changes in Amadori modified proteins due to early glycation. Most of the studies were found on the structural changes in Amadori protein at a particular glucose concentration but no study was found to compare the biophysical and biochemical changes in HSA due to early glycation with a range of glucose concentration at a constant incubation time. So this study provide the information about the biochemical and biophysical changes occur in Amadori modified albumin at a range of glucose normal to chronic in diabetes. Although many implicates currently in use i.e. glycaemic control, insulin treatment and other chemical therapies that can control many aspects of diabetes. However, even with intensive use of current antidiabetic agents more than 50 % of diabetic patient's type 2 suffers poor glycaemic control and 18 % develop serious complications within six years of diagnosis. Experimental evidence related to diabetes suggests that preventing the nonenzymatic glycation of relevant proteins or blocking their biological effects might beneficially influence the evolution of vascular complications in diabetic patients or quantization of amadori adduct of HSA by authentic antibodies against HSA-EGPs can be used as marker for early detection of the initiation/progression of secondary complications of diabetes. So this research work may be helpful for the same.

Keywords : diabetes mellitus, glycation, albumin, amadori, biophysical and biochemical techniques
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