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Gold Nano Particle as a Colorimetric Sensor of HbA0 Glycation Products

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Abstract : Type 2 diabetes mellitus (T2DM) is a very complex and multifactorial metabolic disease where the blood sugar level goes up. One of the major consequence of this elevated blood sugar is the formation of AGE (Advance Glycation Endproducts), from a series of chemical or biochemical reactions. AGE are detrimental because it leads to severe pathogenic complications. They are a group of structurally diverse chemical compounds formed from nonenzymatic reactions between the free amino groups (-NH2) of proteins and carbonyl groups (>C=O) of reducing sugars. The reaction is known as Maillard Reaction. It starts with the formation of reversible schiff's base linkage which after sometime rearranges itself to form Amadori Product along with dicarbonyl compounds. Amadori products are very unstable hence rearrangement goes on until stable products are formed. During the course of the reaction a lot of chemically unknown intermediates and reactive byproducts are formed that can be termed as Early Glycation Products. And when the reaction completes, structurally stable chemical compounds are formed which is termed as Advanced Glycation Endproducts. Though all glycation products have not been characterized well, some fluorescence compounds e.g pentosidine, Malondialdehyde (MDA) or carboxymethyllysine (CML) etc as AGE and α-dicarbonyls or oxoaldehydes such as 3-deoxyglucosone (3-DG) etc as the intermediates have been identified. In this work Gold NanoParticle (GNP) was used as an optical indicator of glycation products. To achieve faster glycation kinetics and high AGE accumulation, fructose was used instead of glucose. Hemoglobin A0 (HbA0) was fructosylated by in-vitro method. AGE formation was measured fluorimetrically by recording emission at 450nm upon excitation at 350nm. Thereafter this fructosylated HbA0 was fractionated by column chromatography. Fractionation separated the proteinaceous substance from the AGEs. Presence of protein part in the fractions was confirmed by measuring the intrinsic protein fluorescence and Bradford reaction. GNPs were synthesized using the templates of chromatographically separated fractions of fructosylated HbA0. Each fractions gave rise to GNPs of varying color, indicating the presence of distinct set of glycation products differing structurally and chemically. Clear solution appeared due to settling down of particles in some vials. The reactive groups of the intermediates kept the GNP formation mechanism on and did not lead to a stable particle formation till Day 10. Whereas SPR of GNP showed monotonous colour for the fractions collected in case of non fructosylated HbA0. Our findings accentuate the use of GNPs as a simple colorimetric sensing platform for the identification of intermediates of glycation reaction which could be implicated in the prognosis of the associated health risk due to T2DM and others.

Keywords : advance glycation endproducts, glycation, gold nano particle, sensor
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