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Detection of Advanced Glycated End-Products Formed in Human Serum Albumin as a Model for Describing Diabetes Complications

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Reducing sugars react with protein amino groups to form a diverse group of protein-bound moieties with fluorescent and cross-linking properties. These compounds,called advanced glycosylation end products (AGEs), have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes.Samples of 0.6mM of dialyzed HSA against 2.5mM phosphate buffer were incubated with 5.55, 11.11, 16.66, 22.22 and 33.33mM D-glucose for 10 days. At the end of the incubation period, the ambient glucose was removed and the samples were incubated for a further 10 days in glucose-free buffer. At the end of the incubation, the emission fluorescence at 440 nm (following continuous excitation at 370 nm) of glycated HSA samples was measured. The results showed significantly increase in fluorescent activity of samples as the concentration of glucose increased.The fluorescence findings prove the presence of stable AGEs on HSA. It is concluded that AGEs can indeed be formed and detected by this method in HSA subjected to nonenzymatic glycation In vitro. So, similarly as the increased concentration of blood glucose in diabetes mellitus may lead to accelerated AGEs formation, the In vitro model described may be of value in better understanding the pathophysiologic and blochemical mechanisms involved in the development of diabetic cataract, immunopathy, microangiopathy and other diabetes complications.The findings also proved that the AGEs phenomena is a nonstopping process since after reducing the concentration of glucose, even removing glucose from ambient solution the fluorescence activity of glycated HSA developed. It seems that the growth of diabetes complications depend to this fact, that AGEs is irreversible and progressive.So, in the presence of glucose many plasma proteins can be glycated and suffering to AGEs and its consequences complications. It is reasonable to reduce the blood glucose concentration via regimen and health orders.

Keywords: HSA, AGEs, Diabetes, Fluorescence.

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Use of BSA Amyloid Fibrils as a Nanomaterial for Enzyme Immobilization

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Proteins and peptides are prone to form amyloid nano-aggregates when they are mis-folded. These elongated fibrilar protein aggregates are well known for their association with amyloidogenic disorders such as Alzheimer, Parkinson. Their constituent amino acids make them ideally suited as a functional nano-scaffold for enzyme immobilization and introduce them as new bio-nanomaterials. In this study, bovine serum albumin as a predominant serum protein was subjected to physical conditions which are bring about its partial unfolding to develop fibrilar nano-structures. Then, fibrils successfully conjugated with glucose oxidase (GOD) using glutaraldehyde to form catalytic nano-fibers as potentially applied approach.Fibrillation are confirmed using methods including UV/visible spectroscopy, both of intrinsic and extrinsic spectrofluorometry, circular dichroism and transmission electron microscopy and manufactured catalytic nano-fibers were kinetically/functionally characterized for glucose oxidase activity. The immobilized preparation was also featured with its antimicrobial activity using microbial culture.The immobilization of the GOD on amyloid fibrils provides a great 'proof of concept' model for the creation of a catalytic nano-material using a functionalized amyloid fibril nanoscaffold.

Keywords: Protein Aggregation, Amyloid, Glucose Oxidase, Immobilization.