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Autoantibodies in type 1 diabetes mellitus sera recognize peroxynitrite modified HSA

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Diabetes mellitus is a metabolic disorder of considerable concern because a significant population of the world has already contracted the disease. It is characterized by hyperglycemia, either due to insulin deficiency or insulin resistance. Both experimental and clinical studies have suggested role of oxidative stress in the onset of diabetes mellitus and related complications. The rise in oxidative stress in diabetes mellitus has been attributed to imbalance between oxidants/antioxidants. Peroxynitrite, is an inorganic oxidant of immense biological importance, that is produced from union of superoxide and nitrogen monoxide. It has potential to modify variety of biomolecules but posses high affinity for tyrosine residues in proteins, and 3-nitrotyrosine is a relatively specific marker of peroxynitrite mediated damage to proteins. Human serum albumin (HSA), the most abundant protein in circulatory system, is a monomeric multi-domain protein of 585 amino acid residues responsible for many physiological functions. In the present study, HSA modified with varying doses of peroxynitrite in presence of sodium bicarbonate. The incurred damage on HSA by peroxynitrite was evaluated by various physico-chemical techniques. The results revealed structural changes, increased carbonyl, nitrotyrosine, nitrotyrptophan and dityrosine content in peroxynitrite-modified-HSA. The aggregate formation in peroxynitrite-modified-HSA was perceptible from melting temperature (Tm) and transmission electron microscopy (TEM). Direct binding and inhibition studies performed on serum samples/IgG of type 1 diabetic patients revealed that autoantibodies in these patients were recognizing peroxynitrite-modified-HSA. Normal human serum (NHS) showed negligible binding with the antigen. The results suggest role of nitroxidized proteins in the initiation/ progression of type 1 DM.

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