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Peroxynitration of albumin creates binding site for autoantibodies in diabetes mellitus

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It has been suggested that stress can play a major role in the etiopathogenesis of numerous diseases including diabetes mellitus. Superoxide and nitric oxide are two such stressors produced during inflammation in high amount. They can combine to produce peroxynitrite anion ($ONOO^-$) which is potent oxidizing and nitrating agent. Thus, its interaction with biomolecules can cause oxidation as well as nitration. In the present study, albumin was modified by peroxynitrite and structural changes have been studied by UV, fluorescence, CD and Congo red binding. Analysis of modified-albumin showed increased level of carbonyl, nitrotyrosine and dityrosine. Thiol content was significantly reduced in modified-HSA. Reduction of plasma antioxidant power has been reported in diabetes mellitus and under such conditions peroxynitrite may modify albumin. This may modify the antigenic properties of albumin. Subsequent processing of modified-albumin by immune cells may generate autoantibodies. Thus, peroxynitrite-modified-HSA was used as antigen for detecting autoantibodies in diabetes mellitus sera by ELISA. Peroxynitrite-modified-HSA was bound by the diabetic autoantibodies. The study demonstrates that peroxynitration can generate immunologically active epitope on HSA.

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Physicochemical and immunological studies on hydroxylated IgG: Its possible role in the generation of autoantibodies in rheumatoid arthritis

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Immunoglobulin G (IgG) is the most abundant immunoglobulin of the total immunoglobulin pool in the serum and has been found susceptible to damage by reactive oxygen species (ROS). This study aims at exploring the alterations in the structural characteristics using various biophysical and biochemical methods. Modified IgG showed hyperchromicity in UV-vis spectroscopy, quenching in tyrosine fluorescence and cross linking in SDS PAGE. Changes in secondary structure were evident by Far UV-CD and FTIR. The modified IgG showed enhanced hydrophobicity, increase in carbonyl and reduction in the sulfydryl content. DLS studies point towards increase in the hydrodynamic radii, while DSC analysis showed enhanced thermodynamic stability of the modified IgG. Hydroxyl radical induced aggregation was confirmed by enhanced ThT specific fluorescence intensity and a red shift in the Congo red specific fluorescence intensity in the modified IgG and by the transmission electron microscopy. The immunogenic potential of native and $OH^{\bullet}$ treated IgG was probed in experimental animal. The modified IgG was highly immunogenic inducing high titer antibodies. Furthermore, antibodies against native and $OH^{\bullet}$ modified IgG in RA patients were detected by direct binding and inhibition ELISA. The data showed preferential binding of RA autoantibodies to hydroxylated IgG in comparison to the native counterpart. Thus it can be concluded that structural changes generated neoepitopes on IgG causing enhanced antibody production. Also, $OH^{\bullet}$ modified IgG can serve as a novel antigen with a possible role in etiopathogenesis of RA.

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