Abstract
Systemic lupus erythematosus (SLE) is an autoimmune disease associated with the presence of a wide variety of autoantibodies, including antibodies to DNA, RNA, ribonucleoproteins, phospholipids, cytoskeletal proteins and histones. The autoimmune phenomena found in SLE are mysterious, both in their induction and pathogenic mechanisms. Immune complex formation and their deposition in the glomeruli of the kidney is particularly harmful. The existence of autoantibodies in SLE specifically reactive with sequences and structures in RNA have also been demonstrated.

Reactive oxygen species (ROS) are formed continuously in living cells as a consequence of normal metabolic and biochemical reactions, as well as in certain pathological conditions. ROS are formed from oxygen in one electron reduction reactions and include superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH). Among the ROS, OH is the most damaging and can virtually react with any macromolecule it encounters. In vivo, hydroxyl radicals are formed by ionizing radiations or through the Haber-Weiss or Fenton reactions. Thus, causing DNA damage leading to strand breaks, base damage and conformational changes.

Native DNA is known to be a weak immunogen. It has been shown that modification of DNA renders it highly immunogenic. The hypothesis that ROS-modification of DNA is involved in the development of autoantibodies in SLE, has been supported by the enhanced reactivity of SLE anti-DNA autoantibodies to ROS-DNA. In view of these, the possible association of the ROS-modified and native polyribohomopolymer, polyinosinic acid [poly(I)] in the SLE disease activity has been speculated.

In the present study, polyinosinic acid [poly(I)] was modified with hydroxyl radical, generated by UV light in the presence of hydrogen peroxide. UV-absorption and difference spectroscopy was used to analyse the modifications/perturbations incurred on poly(I). Modified poly(I) sample showed a marked hypochromicity at 248 nm over the unmodified poly(I), reflecting the modification of hypoxanthine base in ROS-poly(I). The elimination of the expected negative peak at the $\lambda_{\text{max}}$ in between 200-280 nm in UV difference spectrum of the
modified poly(I) suggests the loss of chromophoric group, thus showing the ROS induced damage of the hypoxanthine base in poly(I). On formation of complex with poly(C), a change in absorbance ratio was observed with poly(I).poly(C) showing a ratio of 1.13 and ROS-modified poly(I).poly(C) of 0.98. Thus, reflects the occurrence of modification in poly(I) due to ROS.

Analysis and computation of thermodynamic parameters suggest the introduction of partial compactness in ROS-poly(I) at the site of ROS-modification, in contrast to native poly(I). Thermodynamically, ROS-poly(I) was found to be more stable than native poly(I).

Sephadex G-200 gel chromatography, agarose gel electrophoresis, densitometric scanning and gel diffusion were the techniques employed to detect the formation of strand breaks and base modification. Sephadex G-200 gel chromatographic pattern shows the modification of poly(I) to an extent of 57.8%. Enhanced mobility of ROS-poly(I) with decreased intensity is attributed to single strand breaks formation and base modification in poly(I) on exposure to 'OH radical. Further, densitometric scanning and gel diffusion pattern ascertain the enhanced mobility of ROS-poly(I) due to strand breaks formation and generation of low molecular weight species.

Photochemical changes are found to occur in purine and pyrimidine bases on attack of 'OH radical. The release of hypoxanthine base from poly(I) was detected by UV absorption spectra of acid hydrolysed poly(I), showing a λmax at 250 nm, typical of hypoxanthine base. Broadened absorption maxima at 250 nm in case of acid hydrolysed ROS-poly(I) showed base modification. These results were substantiated by UV difference spectroscopy. Ion exchange chromatography of the acid hydrolysed native and ROS-poly(I) suggest a modification of upto 71.8% in hypoxanthine base by 'OH and hence confirms the modifying effect of ROS.

To raise antibodies, rabbits were immunized intramuscularly with native and ROS-modified poly(I) complexed with methylated bovine serum albumin. Both the polymers were found to be potent immunogens, inducing high titer antibodies. The specificity of the induced antibodies were ascertained by competition
immunoassay, using poly(I) and ROS-poly(I) as inhibitors and were found to be highly specific towards the respective immunogen.

Experimentally induced antibodies exhibited polyreactivity, a property commonly associated with SLE anti-DNA autoantibodies. Anti-native poly(I) antibodies showed preferential binding to native forms of DNA, RNA, ribohomopolymers and guanine base (as inhibitors) in comparison to their ROS-modified counterparts. Anti-ROS-poly(I) antibodies showed specificity towards ROS-modified conformers as inhibitors.

A group of SLE sera were investigated for the evaluation of anti-DNA autoantibody titer. SLE sera from 14 patients showing high titer anti-DNA autoantibodies were analysed for their specificity towards native DNA by inhibition ELISA. The binding specificity of SLE anti-DNA autoantibodies with ROS modified and native poly(I) was analysed by ELISA, gel retardation assay and quantitative precipitin titration. Direct binding ELISA results show preferential binding of SLE autoantibodies to ROS-poly(I) in comparison to native poly(I). Further, inhibition ELISA reiterates the direct binding results. Gel retardation assay further substantiated the binding of native and modified poly(I) with anti-DNA autoantibodies. Protein A-Sepharose 4B purified SLE IgG showed appreciable binding to native and modified poly(I). The binding affinity of modified and unmodified poly(I) with SLE IgG was calculated using Langmuir plot. The association constant (Ka) for ROS-poly(I) was found to be highest, followed by native DNA and native poly(I), respectively. The binding patterns of SLE autoantibodies obtained from different patients were remarkably similar, indicating the recognition of the modified polymer by naturally occurring SLE anti-DNA autoantibodies. The results suggest that the photochemical modification of poly(I) cause perturbations, resulting in the generation of neo-epitopes making it a potential immunogen.

Deposition of the immune complexes between DNA and autoantibodies to DNA in kidney has been considered to elucidate the inflammatory reaction in lupus nephritis. Rats immunized with native and modified poly(I) induced high titer antibodies. Immunofluorescence of the kidney sections of rats showed the