ABSTRACT

DNA double helix has considerable conformational flexibility. It is a dynamic structure in which different conformations are in equilibrium with each other. The concept of structural flexibility in DNA was sharply illustrated by the discovery of left handed Z-DNA. Both right handed B-DNA and left handed Z-DNA are double helical conformations with antiparallel chains that are held together by Watson-Crick hydrogen bonding between the bases. All the nucleotides along the B-DNA have the same conformation. However, the nucleotides along the left handed double helix alternate in syn and anti conformations. An equilibrium exists between B-DNA and Z-DNA.

Double stranded calf thymus DNA was brominated in high salt concentration. Conformational change in DNA was detected by various techniques like UV absorption spectra, thermal denaturation studies and S1 nuclease sensitivity. Characteristic property of Z-DNA viz. red shift in UV spectra and hyperchromacity at 295 nm was observed in brominated DNA indicates that the polymer had adopted Z- or Z-like conformation.

Thermal denaturation data as well indicates that brominated DNA is thermodynamically less stable than its correspondingly unmodified polymer. The electrophoretic mobility of brominated DNA (Br-DNA) was increased as compared to native DNA (nDNA) indicates the generation of some single stranded portions reiterating that the molecule undergo B -> Z or Z-like conformation as a result of bromination in high salt concentration. After nuclease S1 treatment of Br-DNA, the band shifted its position to lower side which indicates that these portions are digested on the treatment with S1 nuclease.
In brominated nDNA, cytosine and guanine were modified to the extent of 20.8% and 39.2% respectively, which suggests that guanine is a better substrate for bromination as compared to cytosine. Similar results have also been reported in the case of typical Z-DNA (i.e. brominated poly(dG-dC).poly(dG-dC)) where after bromination 35% of the guanine residues had reacted in the C8 position and 17% of the cytosine residues at C5 position.

Antibodies were generated in experimental animals against Br-DNA. The immunogenecity and specificity of induced antibodies was probed against various nucleic acids antigens by direct binding ELISA and competition ELISA. On the basis of competition experiments the antibodies recognized only the modified form of the polymer i.e. Br-DNA and not the unmodified form. Anti-Br-DNA antibodies also recognized poly(dG-dm5C).poly(dG-dm5C) and Br-poly(dG-dC).poly(dG-dC). These polymers are known to exist in Z-conformation in solutions under physiological condition. No binding of anti-Br-DNA antibodies was observed with bases. The induced antibodies are thus conformational specific reacting only with the modified form of native DNA.

Quantitative evaluation of antibody binding was ascertained by precipitin titration. Immune complex formation was also detected by gel retardation assay in which retarded mobility of Br-DNA-anti-Br DNA antibody complex was seen as a further indication of antibody interaction.

The importance of lysine residues on the binding characteristics of immune IgG was ascertained by trinitrophenylation of lysine residues. Results demonstrated a direct relationship between the degree of lysine modification and antigen binding.
Antibodies specific for Z-DNA arise in certain autoimmune states and are found in both murine and human systemic lupus erythematosus. In the present study, various normal human sera and sera of patients with SLE were checked for the presence of antibodies against nDNA and Z-DNA. Anti-DNA autoantibodies were found to be specific for native double stranded DNA with poor reactivity with single stranded polymer. SLE anti-native antibodies also showed appreciable binding with brominated DNA.

In conclusion, bromination of native calf thymus DNA in high salt concentration converts some portions of DNA from right handed conformations to left handed Z-conformations. Modified polymer is highly immunogenic. The antibodies induced against brominated DNA appears to be highly specific for the modified polymer recognizing the changed conformations of the modified polymer. The altered polymer showed appreciable binding with human anti-DNA autoantibodies. The possibility of an altered polymer acting as antigen for the production of human autoantibodies could be one of the factors for the pathogenesis of SLE.