Scientific Journal of Impact Factor (SJIF): 4.14

e-ISSN (O): 2348-4470
p-ISSN (P): 2348-6406

International Journal of Advance Engineering and Research Development

Volume 3, Issue 11, November -2016

U.V irradiation of synthetic DNA sequence in the presence of Co^{2+}

[1]Chandra Sekhar Rao, [2]Nagalakshmi.

[1]*Associate Professor H&S Department, Vardhaman College Of Engineering, Kacharam,Shamshabad, Hyderabad-501218.*

[2] *Assistant Professor H&S Department, BITS Engineering College, Hindupur, Ananthapuram-515001.*

Abstract: *M-DNA is a complex between DNA and cobalt (II), nickel (II) or zinc (II) that forms under alkaline conditions. It has been postulated that the imino proton of guanine or thymine is replaced by the metal cation in each base-pair. The complex is thought to maintain a double-helical structure similar to B-DNA but has unusual properties. M-DNA acts as an electron conductor making it a potential candidate for future nanotechnology applications. Crosslinks induced by UV radiation also formed at a faster rate for the Co^{2+}, forms of M-DNA compared to B-DNA. In this case crosslinking occurred in all DNA but was more prominent in A-T base-pairs*

Key words: *Synthetic DNA- co^{2+} Interaction, Non-ionizing radiation.*

INTRODUCTION

The primary structure of DNA consists of a polymer of deoxynucleotides. Each deoxynucleotide consists of a nitrogenous base attached to cyclic 2'-deoxy-D- ribose, which in turn is attached to a phosphate group. A major intrinsic property of the DNA that affects its stability is the G•C content. DNA that has a higher G•C content denatures at a higher temperature than does DNA with a higher A•T content. Although Tm is proportional to G•C content, it is evidently not the case that the number of hydrogen bonds between base pairs determines the stability of a helix. This is especially obvious in synthetic sequences having the same G•C content but differing in the sequence. In many cases, these have been shown to have different Tm values.

Metal cations interact extensively with DNA in solution and must be considered along with the DNA structure. In fact, with very low concentrations of metal cations, DNA denatures even at relatively low temperatures. Often, changes in the stability or conformation of the DNA result from interactions with various metal cations at different sites on the DNA. Group 1 and group 2 metal cations generally dissolve in water giving complete charge separation and their interactions are largely ionic. For example, NaCl dissolved in water gives the Na^+ and Cl^- cation and anion, each interacting ionically with water molecules. Transition metal compounds do not usually follow this trend. There is often a change in the stability of DNA when metal ions interact with it In general, the interaction of metal cations with negatively charged phosphate oxygen atoms is nonspecific and serves to stabilize the DNA duplex due to shielding of the negative charges of the phosphate backbone. Gamma radiation is a type of ionizing radiation and causes nicking and damage to bases in DNA. These types of damage are attributable to one of two general processes .Absorption of ionizing radiation directly by the bases of DNA can occur. Alternatively, there can be formation of free radical species generated from water molecules that surround the DNA.

The free radicals that are generated by these processes react with DNA to cause damage of the free radical species formed, $^{\bullet}OH$ is the most important and usually acts either by abstracting hydrogen atoms from the sugars of DNA or by addition to the double bonds of bases.

Materials and Methods:

Nucleic Acid Preparation:

The DNA in this set of experiments included the synthetic DNA sequences poly[d(TG)] poly[d(CA)]• poly[d(TC)] •poly[d(GA)], poly(dG) poly(dC) and poly(dA)•poly(dT) was studied.

DNA was first isolated from solution by ethanol precipitation using NaCI as the monovalent cation. All DNA was stored at -20 °C in 10 mM NaCI and 10 mM N-(2-hydroxyethyl) piperazine-N'-(2 ethanesulfonic acid) (HEPES), pH 7.5, TRIS (pH 7.5) or sodium borate (pH 9.0). Before use, the bacterial and synthetic DNA sequences (but not the plasmid DNA) were sheared by passing through one-half inch 30 gauge needles five times in order to obtain fragments of uniform length.During this procedure, maximum thumb pressure was used to pass the DNA solution through the needle from a syringe.

Sample Preparation:

The samples for these experiments were prepared as described here unless otherwise stated. For γ-irradiation experiments: 40 mM HEPES buffer (pH 7.5) or boric acid buffer (pH 9.0), 40 J.1M in base-pairs of DNA, 400 μM of M^{2+} chloride and 10 mM NaCl. For UV radiation experiments, conditions were the same except that TRIS buffer (pH 7.5) was used instead of HEPES. For γ-irradiations, the solutions were prepared and 20 μL aliquots for each time interval were transferred to 0.5 mL micro centrifuge tubes before exposure. In the case of UV irradiations the samples were prepared then transferred to 1mL quartz cuvettes covered with thin plastic wrap to prevent evaporation during exposure. The DNA concentration was estimated from the absorbance at 260 nm with an extinction coefficient of 6600 Cm^{-1} M^{-1}. Thus, 1.0 A_{260} is equivalent to 0.075 mM in base-pairs of DNA.

Radiation Exposure:

Samples were γ-irradiated using a 60Co source with an approximate dose rate of 1440 rate min^{-1}. A UV Stratalinker was used for UV exposure and contained five 8 W germicidal bulbs having a peak irradiance of 254 nm. In this case, a 20 μL sample was removed from the quartz cuvettes after each time interval. Following exposure, 2 μL of 200 mM EDTA, pH 8.0 was added to each 20 μL sample to remove the M^{2+} from their interactions with DNA.

Ethidium Fluorescence Assay:

An alkaline ethidium fluorescence assay was used to investigate the induction of nicks and interstrand crosslinks in DNA upon exposure to radiation (Morgan et al., 1979b). Aliquots of 18 μL, taken from the irradiated samples containing EDTA, were read in 2 mL of "pH 12 ethidium assay buffer" (0.5 μg mL^{-1} ethidium bromide, 20 mM potassium phosphate buffer, pH 11.8, and 0.5 mM EDTA) at an excitation wavelength of 525 nm and an emission wavelength of 600 nm in a fluorescence spectrophotometer. The pH of the ethidium assay buffer was adjusted with small amounts of alkali for different types of DNA since sequences with higher G•C content required more alkaline conditions for strand

separation to occur on heating. Appropriate pH values were found by trial and error such that linear DNA gave a good fluorescence reading but denatured DNA would not. The synthetic DNA sequences were analyzed at pH 7.5, except for poly(dG)•poly(dC) which was at pH 9.0 and poly(dA)•poly(dT) for which the assay buffer was pH 8 (5 mM TRIS-HCI, 0.5 μg mL^{-1}ethidium bromide and 0.5 mM EDTA).

The fluorescence values of the "before-heat" samples were recorded and the samples heated in a boiling water bath for 2 minutes followed by immersion in a room temperature in water bath for at least 2 minutes. Fluorescence readings of the resulting samples gave the "after-heat" results. All results reported represent the mean of two independent experiments.

Result and Discussion: Effects of non ionizing radiation on synthetic DNA sequence with CO^{2+}.

The synthetic sequences showed only a small increase in after-heat fluorescence accompanied by a small decrease in before-heat fluorescence at pH 7.5 in the presence of Co^{2+}

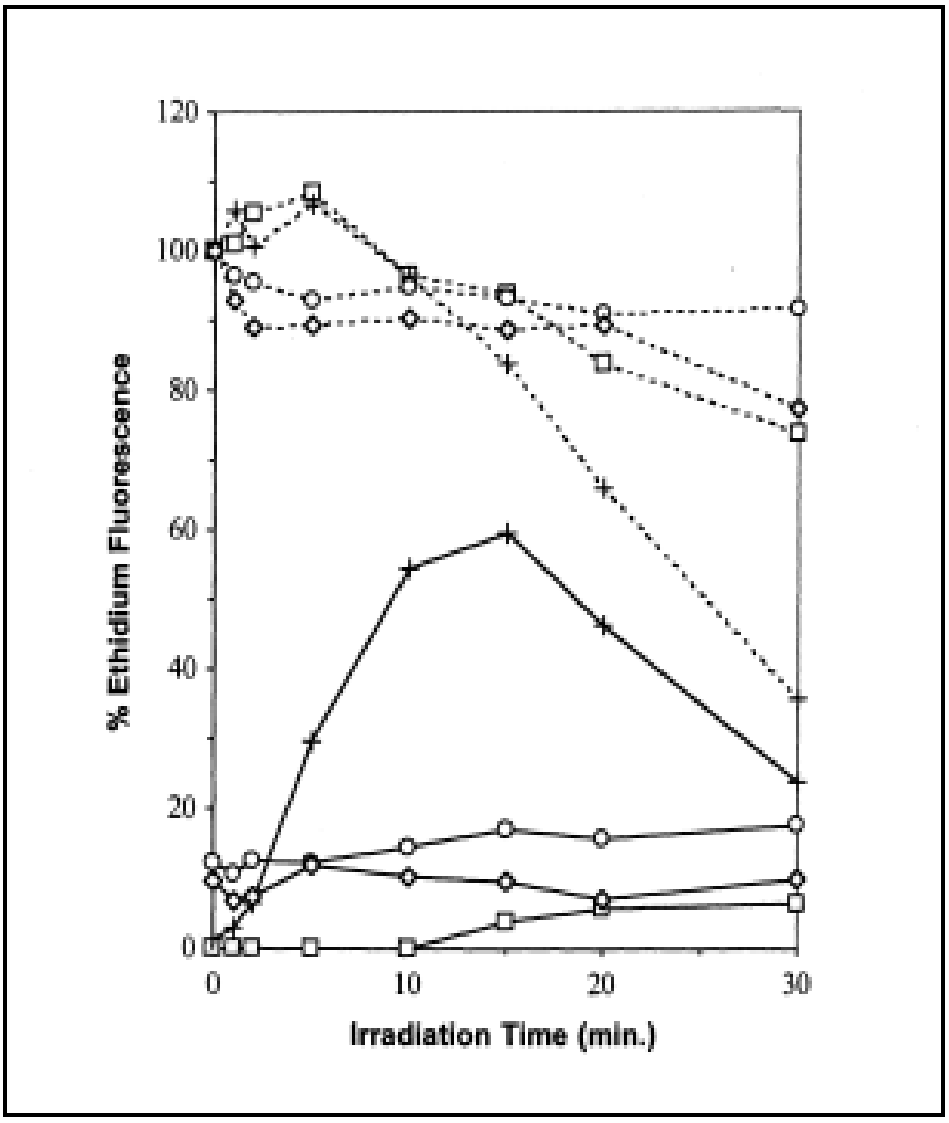

Figure1. UV irradiation of synthetic DNA sequences at pH 7.5 in the presence of Co^{2+}. Samples contained poly[d(TC)] • poly[d(GA)] (squares), poly(dA) • poly(dT) (crosses), poly[d(TG)] • poly[d(CA)] (circles) and poly(dG) • poly(dC) (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

Poly(dA)•poly(dT) showed a strong decrease in before-heat fluorescence (less than 40% after 30 minutes of exposure) that can at least partially be explained by the formation of cyclobutyl pyrimidine dimmers. Surprisingly, there was also a strong increase in after-heat fluoroscence (about 60% after 15 minutes of exposure). This was completely different from exposure with ionizing radiation and indicated a different mechanism of crosslink formation.

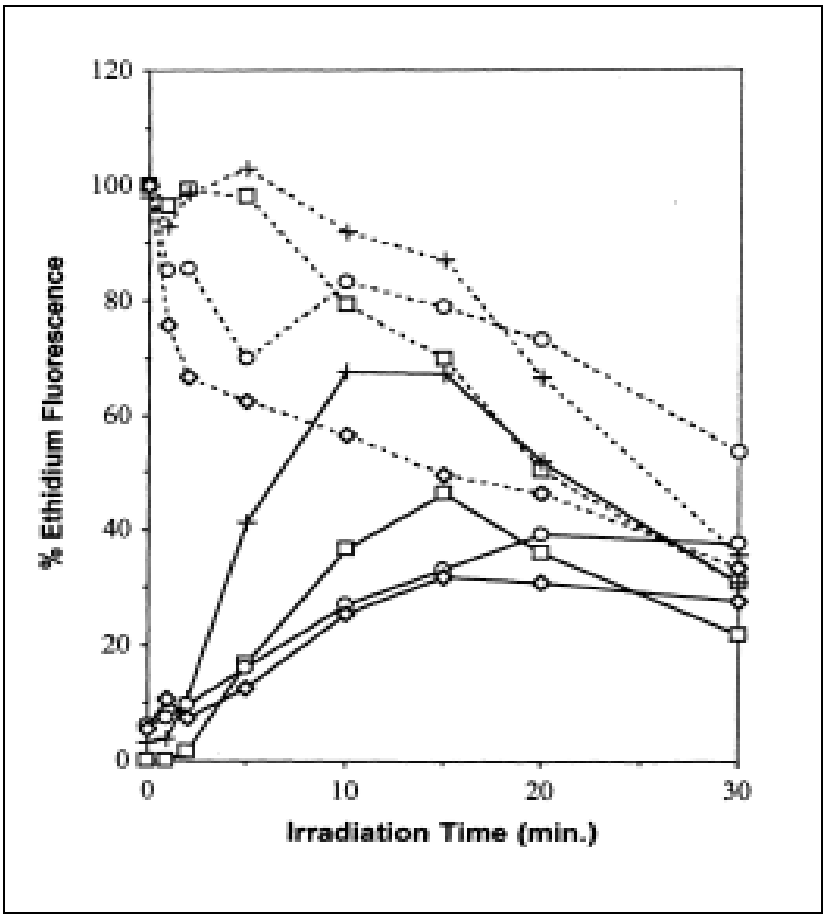

Figure 2. UV irradiation of synthetic DNA sequences at pH 9.0 in the presence of Co^{2+}. Samples contained poly[d(TC)] • poly[d(GA)] (squares), poly(dA) • poly(dT) (crosses), poly[d(TG)] • poly[d(CA)] (circles) and poly(dG) • poly (dC) (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat readings. UV radiation caused increased cross linking with all of the synthetic DNA sequences assayed in the presence of $C0^{2+}$ at pH 9.0 (Figure 2). Compared to pH 7.5, poly[d(TC)] •poly[d(GA)] showed the greatest increase followed by poly[d(TG)] •poly[d(CA)] and poly(dG) • poly(dC). Poly (dA)• poly(dT) showed only a small increase compared to pH 7.5, the return in after-heat fluorescence being unexpectedly high in both cases.

CONCLUSIONS: In contrast to the effects of γ-radiation, the results show less nicking with UV radiation. As well, there is no pH-dependent effect on nicking in the case of UV radiation, indicating that a mechanism involving free radical formation outside of the DNA helix is unlikely. Compared to γ-radiation, UV radiation caused less general damage to the DNA and fewer cross links at pH 9.0 compared to pH 7.5. In general, however, there was some cross linking in the presence of all of the M^{2+} assayed even at pH 7.5. The major difference observed upon formation of M-DNA with either Co^{2+} is that while general damage, including nicking, increases slightly there is a large increase in the level of crosslinking

Interstrand cross linking of DNA exposed to UV radiation has also been observed by another group. It was proposed that the mechanism involves the photochemical dimerization of pyrimidine moieties on opposite strands requiring changes in strand conformation. The structure of this type of crosslink remains unknown but ispresumed to involve formation of a cyclobutyl dimer between double bonds in the bases on opposite strands. A mechanism involving •OH formation from the aqueous solution is unlikely since the results presented in the present work show that the presence of a radical scavenger does not prevent crosslink formation. As in the case with γ-irradiation, UV radiation caused some cross linking with all of the synthetic DNAs assayed in the presence of Co^{2+} at high pH. Interstrand cross linking was observed even between strands containing only purines and strands containing only pyrimidines as well as with mixed strands. Of considerable interest is the observation that poly (dA)•poly(dT) showed a high rate of cross linking even at pH 7.5

References:

1. Aich, P., Kraatz, H.-B. and Lee, J. S. (2000) M-DNA: pH Stability, Nuclease Resistance and Signal Transmission. 1. Biomol. Struct. Dyn. Conversation 11: 297-301.

2. Ciccarelli, R. B. and Wetterhahn, K. E. (1982) Nickel Distribution and DNA Lesions induced in Rat Tissues by the Carcinogen Nickel Carbonate. Cancer Res. 42:3544-3549.

3. Hery, M., Nazaret, S., Jaffre, T., Normand, P. and Navarro, E. (2003) Adaptation to Nickel Spiking of Bacterial Communities in Neocaledonian Soils. Environ. Microbiol. 5: 3-12.

4. Lindsay, L. and Clark, W. Jr. (1992) Preloading of Micromolar Intracellular Ca^{2+} During Capacitation of Sicyonia ingentis Sperm, and the Role of the pH Decrease During the Acrosome Reaction. The Journal of Experimental Zoology 262: 219-229.

5. Manuur, J. and Doty, P. (1962) Determinatin of the Base Composition of DeoxyribonucleicAcid from its Thermal Denaturation Temperature. J. Mol. Biol.5: 109-118.

6. Lee, J. S., Burkholder, G. D, Latimer, L. J. P., Haug, B. L. and Braun, R. P. (1987) A Monoclonal Antibody to Triplex DNA Binds to Eucaryotic Chromosomes. Nucleic Acids Res. 15: 1047-1061.

7. Breen, A. P. and Murphy, J. A. (1995) Reactions of Oxyl Radicals with DNA. Free Radical Biol. Med. 18: 1033-1077.

8. Pyeritz, R E., Schlegel, R A and Thomas C. A. Jr. (1972) Hydrodynamic Shear Breakage of DNA May Produce Single-Chained Tenninals. Biochim. Biophys. Acta 272: 504-509.

9. Douki, T. and Cadet, J. (1995) UV and Nucleic Acids. In Interface Between Chemistry and Biochemistry, (Edited by JoBes, P. and JomvaB, H.), Birkhauser, Basel.

10. Glisin, V. R. and Doty, P. (1967) The Cross-Linking of DNA by Ultraviolet Radiation. Biochim. Biophys. Acta 142: 314-322.Grady, J. K., Chasteen, N. D. and Harris, D. C. (1988) Radicals from "Good's" Buffers. Anal. Biochem. 173: 111-115.

11. Chandrasekaran, R., Radha, A. and Park, H.-S. (1995) Sodium Ions and Water Molecules in the Structure of Poly(dA)ePoly(dT). Acta Crystallogr. D51: 1025-1035.

