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RESEARCH ARTICLE

GAMMA IRRADIATION OFF MICROBIAL AND SYNTHETIC DNA SEQUENCES IN THE PRESENCE OF CO^{2+}

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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. For the Co^{2+} form of M-DNA, y-radiation caused the very efficient formation of interstrand crosslinks that was not observed with B-DNA and the cross links occurred in A-T & G-C base pairs.

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 in the G•C content. DNA that has a higher G•C content denatures at a higher temperature then does DNA with a higher A•T content when measured under similar solvent conditions. Thus, at pH 7 with NaCl concentration of 0.15 M, the bacterial DNA from *Clostridium perfringens* has a G•C content of 27% and a Tm of 80.5 °C while DNA from *Micrococcus lysodecticus* has a G•C content of 72% and a Tm of 99.5 °C. *Echerichia coli* has a G•C content of 50% and a Tm of 90.5 °C. Metal cations interact extensively with DNA in solution (Kazakov *et al*, 1996, Lee *et al*,2011, Vitthal *et al*,2011) 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 (Kazakov *et al*, 1996, Saenger *et al*, 1984). 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.

Radiation

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 (Breen *et al*, 1995). Absorption of ionizing radiation directly by the bases of DNA can occur. Alternatively (Vitthal, *et al*, 2011) there can

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be formation of free radical species generated from water molecules that surround the DNA. The water molecule is exited by ionizing radiation to give the exited species [H_2O*]. Which undergoes hemolysis to give •OH and H•.

$$H_2O \rightarrow H_2O^* \rightarrow OH^{\bullet} + H^{\bullet}$$

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.

METERIALS AND METHODS

Nucleic acid preparation: The DNA in this set of experiments included bacterial DNA from *E. coli*, *C. perfringens* and *M. lysodiecticus*, 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). DNA was first isolated from solution by ethanol precipitation (Sambrook *et al*, 1989) 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, 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 for γ-irradiations 40 mM HEPES buffer (pH 7.5) or boric acid buffer (pH 9.0), 40 μ M in base-pairs of DNA, 400 μM of $M2^+$ chloride and 10 mM NaCl. 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. The DNA concentration is 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 rad min^{-1}. In this 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 $M2^+$ from their interactions with DNA.

Ethidium Fluorescence Assay

An alkaline ethidium fluorescence assay is used to investigate the induction of nicks and interstrand crosslinks in DNA upon exposure to radiation. 5 Aliquots of 18 μL, taken from the irradiated samples containing EDTA, read in 2 mL of pH 12 ethidium assay buffer (0.5 μg mL^{-1} ethidium bromide, 20mM potassium phosphate buffer, pH 11.8, and 0.5 mM EDTA) at an excitation wavelength of 525 nm and 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 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 *E. coli* and *C. perfringens* DNA were analyzed at pH 11.8, while *M. lysodiecticus* DNA was analyzed at pH 12.2. The synthetic DNA sequences were analyzed at pH 11.9, except for poly(dG)-poly(dC) which was at pH 12.1 and poly(dA)-poly(dT) for which the assay buffer was pH 8 (5 mM TRIS-HCI, 0.5 μg mL-1ethidium 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 water bath for at least 2 minutes. Fluorescence readings of the resulting samples gave the "after-heat" results. All results reported the mean of two independent experiments.

RESULTS AND DISCUSSION

Effects of ionizing radiation on microbial DNA with CO^{2+}

The interstrand cross linking in the CO^{2+} form of M-DNA upon exposure to γ-radiation, it is interest to determine whether the observed reaction is dependent on the DNA composition. *C. perfringens*, *E. coli* and *M.lysodiecticus* bacterial DNAs were chosen because they contain G.C contents of 27%, 50% and 72%, respectively. All three samples followed a similar pattern to the presence of Co^{2+}.

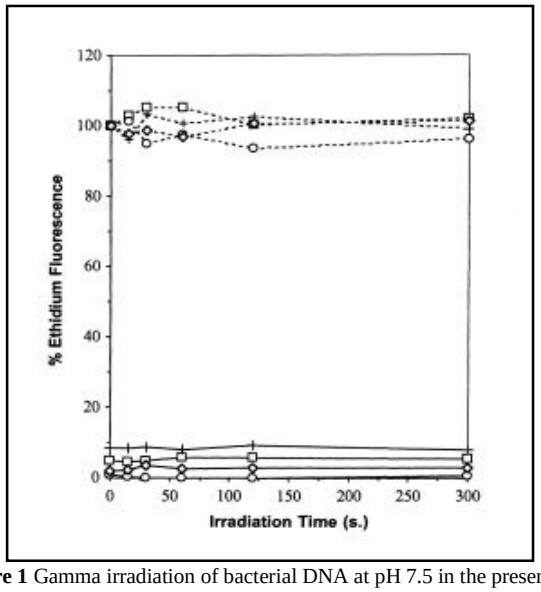

Figure 1 Gamma irradiation of bacterial DNA at pH 7.5 in the presence of Co^{2+}. DNA samples were from calf thymus (squares), *E. coli* (crosses), *C. perfringens* (circles) and *M. lysodiecticus* (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

At pH 7.5 Figure 1 there is no loss of fluorescence with increasing dose demonstrating that overall damage that would prevent ethidium binding to the DNA is minimal. Similarly there is no increase in fluorescence after heating and cooling showing that no cross links were being formed under any of the conditions tested.

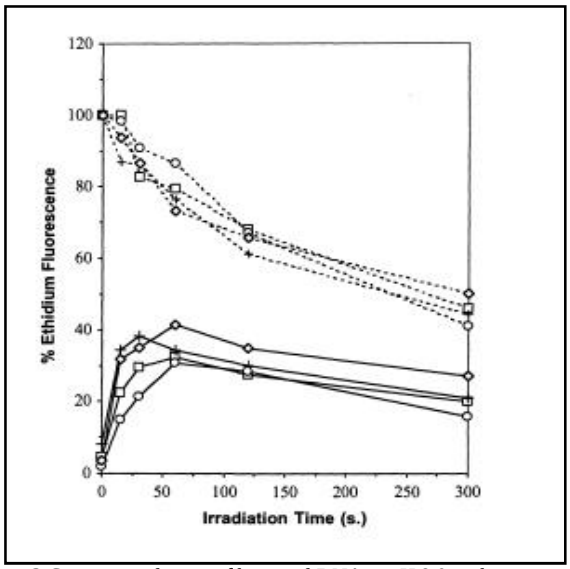

Figure 2 Gamma irradiation of bacterial DNA at pH 9.0 in the presence of Co^{2+}. DNA samples were from calf thymus (squares), *E. coli* (crosses), *C. perfringens* (circles) and *M. lysodiecticus* (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

At pH 9.0 Figure 2 pattern again became more complex. The general trend is decrease in the before-heat fluorescence with increasing dose so that damage is being inflicted on the DNA that prevented ethidium from binding. In the presence of Co^{2+} the fluorescence after heating increased to nearly 50% at short exposure times so that considerable cross linking must have been occurring in the M-DNA. After 30 seconds of exposure, the return in fluorescence decreased, indicating that general DNA damage is starting to dominate. Without any radiation exposure, all of the samples showed similar before-heat ethidium fluorescence, indicating that the DNA had not become single stranded in the presence of M^{2+} at pH 9.0.

Effects of ionizing radiation on synthetic DNA sequence with co^{2+}

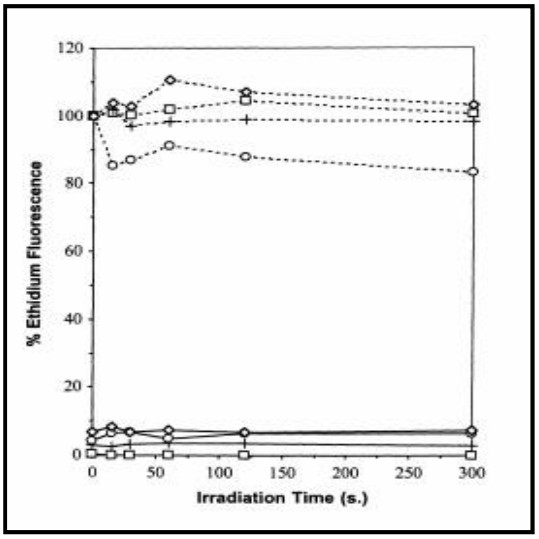

Figure 3 Gamma irradiation of synthetic DNA sequences at pH 7.5 in the presence of Co^{2+}. Samples were poly[d(TC)]•poly[d(GA)] (squares), poly(dA)•poly(dT) (crosses), poly[d(TG)]•poly[d(CA)] (circles) and poly(dG)•poly(dC) (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

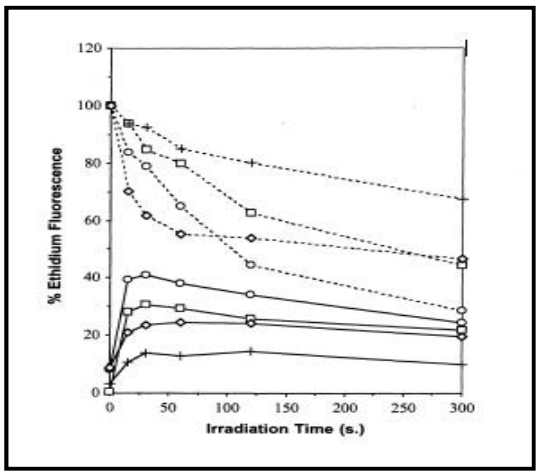

Figure 4 Gamma irradiation of synthetic DNA sequences at pH 9.0 in the presence of Co^{2+}. Samples were poly[d(TC)] •poly[d(GA)] (squares), poly(dA) •poly(dT) (crosses), poly[d(TG)] •poly[d(CA)] (circles) and poly(dG) •poly(dC) (diamonds). Dashed lines represent before-heat readings and solid lines represent after-heat Readings

To determine whether formations of the interstrand crosslinks are dependent on sequence considerations, the experiments were repeated using various synthetic DNA sequences. These assays showed no increase in after-heat fluorescence and before- heat fluorescence showed little or no decrease at pH 7.5 in the presence of Co^{2+} (Figure 3). At pH 9.0 (Figure 4) there was an increase in after-heat fluorescence with all samples tested, poly[d(TG)]•poly[d(CA)] being the highest followed by poly[d(TC)]• poly [d(GA)], poly(dG)•poly(dC) and poly(dA)•poly(dT). At pH 9.0, this is also accompanied by a decrease in before-heat fluorescence with radiation dose, which again demonstrated general DNA damage. This effect became dominant after 30 to 60 seconds resulting in a decrease in after-heat fluorescence after these times.

CONCLUSIONS

The mechanism of Co^{2+} induced crosslinking involves a free radical mechanism since the addition of the free radical scavenger TRIS nearly eliminates the effect completely. Since M-DNA can efficiently transport electrons up and down the helix, it is possible that free radicals produced near a particular base-pair could travel via the metal ions of M-DNA to a different base-pair where crosslink formation is more favorable. The degree of cross linking was similar in three bacterial DNAs ranging in G.C content from 27% to 72%. As well, the synthetic DNAs poly(dA)•poly(dT), poly(dG)•poly(dC) and poly[d(TC)]•poly[d(GA)] all showed crosslinking although the effect is most pronounced with poly[d(TG)•poly[d(CA)]. Therefore it can be concluded that the sequence or structure of the DNA can affect the rate of cross linking but that both A-T and G-C base-pairs can participate. The structure of the cross linked base-pair is unclear but Co cations are known to promote homolytic bond cleavage and formation in reactions catalyzed by co-enzyme B_{12}. One possibility is that the Co shuttles the electron from the •OH to a base by switching between +2 and +3 oxidation states. It is clear that the ionizing radiation-induced cross links are dependent on the M-DNA conformation. First, crosslink formation is not observed at pH 7.5 even in the presence of

Co^{2+}. Second crosslink formation is completely inhibited in the presence of EDTA. Both of these conditions cause reversion of M-DNA back to the B-DNA conformation. Third the degree of crosslink formation is dependent on the concentration of Co^{2+} present in solution at pH 9.0, reaching a maximum when the ratio of [DNA]: [Co^{2+}] is about 1:5. This is consists with the original studies on M-DNA that generally used an approximate 10-fold excess of M^{2+} in order to demonstrate the new conformation. The results obtained here demonstrate that such an excess of M^{2+} may not be necessary for formation of the M-DNA conformation. In fact, this assay provides a sensitive test for the M-DNA conformation although its usefulness is limited by the fact that the crosslinking reaction only occurs with Co^{2+}.

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References

1. Breen, A. P. and Murphy, J. A. (1995) Reactions of Oxyl Radicals with DNA. Free Radical Biol. Med. 18: 1033-1077.
2. Kazakov, S. A. (1996) Nucleic Acid Binding and Catalysis by Metal Ions. In Bioorganic Chemistry: Nucleic Acids (Edited by Hecht, S. M.), Oxford University Press, New York, pp. 244-260.
3. Lee Fang Chin, Siew Ming Kong, Hoi Ling Seng, Kong Soo Khoo, jamurthy Vikneswaran, Siang Guan Teoh, Munirah Ahmad, Soo Beng Alan hoo, Mohd Jamil Maah, Chew Hee Synthesis, (2011), characterization and biological properties of cobalt (II) complexes of 1, 10-phenanthroline and maltol, *Journal of inorganic biochemistry* 105, 339-347.
4. Saenger, w. (1984) Principles of nucleic acid structure, Springer-Verlag, pp 556. Springer-Verlag, New York.
5. Sambrook.J, Fritsch, E.F, and Maniatis.T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
6. Vitthal A. Kawade, Anupa A. Kumbhar, Avinash S. Kumbhar, Christian Näther, Andrea Erxleben, Uddhavesh B. Sonawaneand Rajendra R. Joshi, (2011) Mixed ligand cobalt(II) picolinate complexes: synthesis, characterization, DNA binding and photocleavage Dalton Trans., 40, 639-650.

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