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Effects of Ionizing Radiation (γ-Radiation) on Super Coiled Plasmid DNA Interactions with Zn (II), Ni (II), Co (II)

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ABSTRACT

M-DNA is a complex between DNA and cobalt (II), nickel (II) and 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, γ-radiation caused the very efficient formation of interstrand cross links that was not observed with B-DNA, nor with the Ni^{2+} or Zn^{2+} forms of M-DNA.

KEYWORDS: *DNA-metals interactions, 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. Metal cations interact extensively with DNA in solution [1] 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 [2, 3]. 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 [4]. 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.

MATERIALS AND METHODS

Sample Preparation

The samples for these experiments were prepared as described here unless otherwise stated. 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 M^{2+} chloride and 10 m M NaCl. For γ-irradiations, the solutions were prepared and 20 µ L aliquots for each time interval were transferred to 0.5 mL micro

centrifuge tubes before 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 ^{60}Co source with an approximate dose rate of 1440 rad min^{-1}. 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 [5]. Aliquots of 18 µ L, taken from the irradiated samples containing EDTA, were read in 2 mL of "pH 12 ethidium assay buffer" (0.5 µ g mL^{-1} ethidium bromide, 20mM 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. *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-HCl, 0.5 µ g 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 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.

RESULTS AND DISCUSSION

Effects of Ionizing Radiation on DNA with M^{2+}

To investigate the responses of B-DNA and M-DNA to ionizing radiation, DNA was exposed to y-radiation at pH 7.5 or 9.0 in the presence of Co^{2+}, Ni^{+2} or Zn^{2+}. At pH 9.0, these samples have the M-DNA conformation but at pH 7.5 they remain in the B -DNA conformation. Additionally, Mg^{2+} and Mn^{2+} were used as controls because neither promote the M-DNA conformation at either pH. An additional control was exposure without any M^{2+} present. Damage was estimated from changes in ethidium fluorescence assays [6]. Normally, the fluorescence of bound ethidium is proportional to the concentration of duplex DNA because ethidium does not bind to single-stranded regions. Similarly ethidium does not bind to pyrimidine dimers or hydrated bases so that a loss of fluorescence can be correlated with DNA damage [1]. Cross linking of DNA can also be included under this category of "general DNA damage" since if many cross links are present, the topology of the DNA might be restrained in such a way that ethidium binding is inhibited. However, cross linking of the DNA duplex can be measured after a heating and cooling step performed at pH 12. Upon heating, the duplex denatures and on cooling at this high pH no duplex reforms so that all of the ethidium fluorescence is lost. However, if a crosslink is present, it acts as a nucleation site to overcome the repulsion between strands and the duplex will re-anneal on cooling resulting in a complete return of fluorescence. Finally, DNA strand breaks can be assayed with covalently closed circular plasmid DNA. Upon heating at pH 12 the two strands normally cannot separate so that the fluorescence returns on cooling. However, if a strand break occurs two effects are observed. First, the fluorescence before heating increases by as much as 40% because an open circular DNA can bind more ethidium than a topologically constrained circular DNA. Second, the fluorescence after heating and cooling is eliminated since even one strand break is sufficient to allow strand separation. All of these assays can also be used to assess damage to M-DNA as long as the measurements are

performed after conversion to B-DNA by the addition of EDTA.

For plasmid DNA at pH 7.5 the fluorescence before heating increased with dose of ionizing radiation for all of the samples (**Figure 1**).

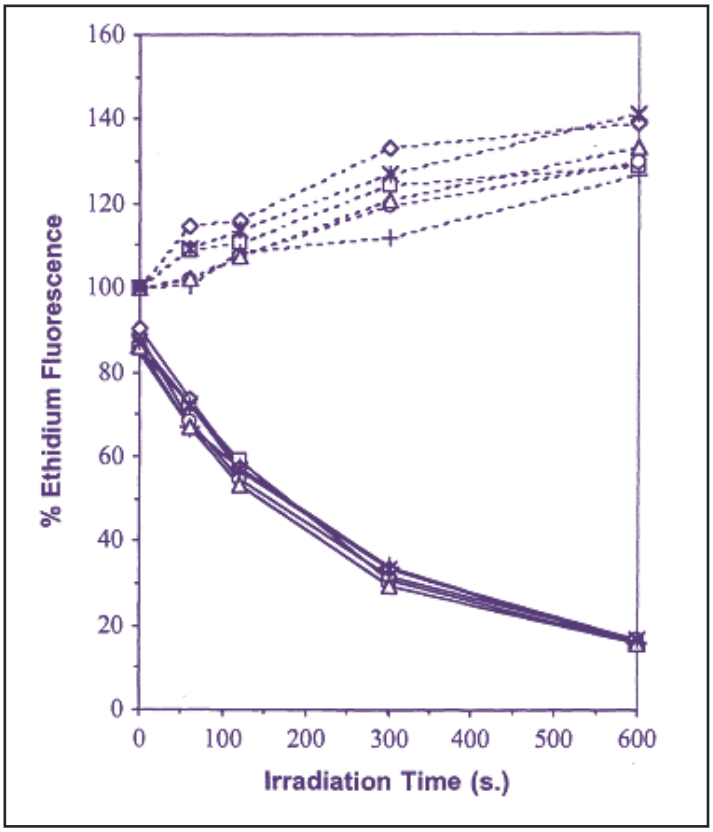

Figure 1: Gamma irradiation of plasmid DNA at pH 7.5 in the presence of M^{2+}. Samples contained Co^{2+} (diamonds), Ni^{2+} (circles), Zn^{2+}(triangles), Mg^{2+}(squares),Mn^{2+} (crosses) or no M^{2+} (stars). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

Before any irradiation, the after-heat fluorescence was less than the before-heat fluorescence. This means that some of the plasmid DNA was already in the open circular form. The increase in before-heat fluorescence can be attributed to nicking of the DNA since the open circular form of the plasmid binds more ethidium. This interpretation was confirmed by the after-heat measurements since in this case nicking leads to loss of fluorescence. As can be seen on (Figure 1), the results were almost identical in the presence of all of the M^{2+} and these in turn were virtually identical to the results with no M^{2+} present.

The results at pH 9.0 were slightly more complex (**Figure 2**). For all of the samples, the rate of nicking was more rapid than at pH 7.5 because the before-heat fluorescence increased more rapidly. Also, except in the case of Co^{2+}, there was a corresponding faster decrease in the fluorescence after heat at pH 9.0. After the initial rapid increase in the before-heat fluorescence there was generally a slow decrease with increasing exposure time. This may be attributed to general DNA damage. In the presence of Co^{2+} at pH 9.0 under M-DNA conditions, the after-heat fluorescence returned to near maximal values throughout the experiment, indicating crosslink formation even at short exposure times.

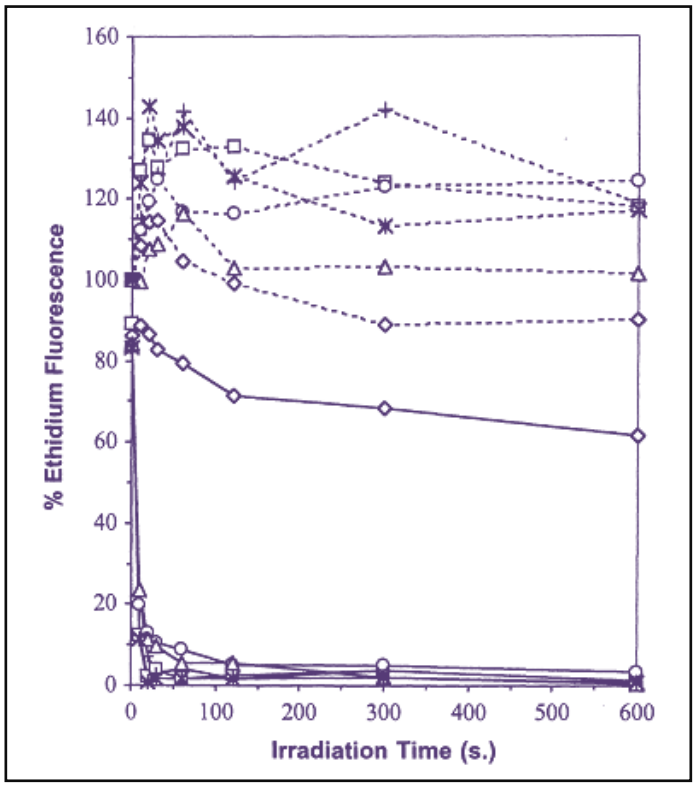

Figure 2: Gamma irradiation of plasmid DNA at pH 9.0 in the presence of M^{2+}. Samples contained Co^{2+} (diamonds), Ni^{2+} (circles), Zn^{2+} (triangles), Mg^{2+} (squares), Mn2+ (crosses) or no M^{2+} (stars). Dashed lines represent before-heat readings and solid lines represent after-heat readings.

CONCLUSIONS

For the Co^{2+} form of M-DNA, γ-radiation caused the very efficient formation of interstrand cross links that was not observed with B-DNA, nor with the Ni^{2+}or Zn^{2+} forms of M-DNA. The cross links occurred in both A-T and G-C base-pairs but did not form in the presence of a free radical scavenger. Cross links induced by UV radiation also formed at a faster rate for the Co^{2+}, Ni^{2+} or Zn^{2+} forms of M-DNA compared to B-DNA. In this case cross linking occurred in all DNA but was more prominent in A-T base-pairs and was not inhibited by a free radical scavenger.

REFERENCE

1. Protz, W. A. Measurement ofCopper-Dependent Oxidative DNA Damage by HOCl and H_20_2 with the Ethidium-Binding Assay. J. Biochem. Bioph. Methods 32: 125-135 (1996)
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. Saenger, W. (1984) Principles ofNucleic Acid Structure. Springer Verlag, New York.
4. Breen, A. P. and Murphy, J. A. Reactions of Oxyl Radicals with DNA. Free Radical Bioi. Med. 18: 1033-1077, (1995).
5. Morgan, A. R., Evans, D. H., Lee, J. S. and Pulleyblank, D. p. Review: Ethidium Fluorescence Assays. Part II. Enzymatic Studies and DNA-Protein Interactions. Nucleic Acids Res. 7: 571-594, (1979)
6. Morgan, A. R., Lee, J. S., Pulleyblank, D. E., Murray, N. L. and Evans, D. H. Review: Ethidium Fluorescence Assays. Part 1. Physiochemical Studies. Nucleic Acids Res. 7: 547-569, (1979b).

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