Circular dichroism of poly(dG-dC) modified by the carcinogens N-methyl-4-aminoazobenzene or 4-aminobiphenyl

Perlet A.Abuaf, Fred F.Kadlubar¹ and Dezider Grunberger

Institute of Cancer Research/Comprehensive Cancer Center, College of Physicians and Surgeons, Columbia University, New York, NY 10032 and ¹National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA

Received May 7, 1987; Revised and Accepted July 30, 1987

ABSTRACT

Poly(dG-dC) was modified to different extents by the carcinogens 4-aminobiphenyl (ABP) or N-methyl-4-aminoazobenzene (MAB). HPLC analysis of the enzymatically hydrolyzed modified polymers indicates that more than 90% of the ABP and 81% of the MAB modification occurs at the C8 position of guanine. The conformational changes of the unmodified and modified polymers were studied as a function of ethanol and magnesium ion concentrations by the use of circular dichroism (CD). The modified polymers show a CD inversion pattern similar to that of the salt-induced B to Z transition of poly(dG-dC). Both of the modified polymers require less salt or ethanol than the unmodified polymer for the inversion of the spectra. The amount of ethanol or magnesium needed to induce the inverted CD spectrum is inversely proportional to the percentage of bound ABP or MAB. These data indicate that ABP and MAB can enhance conversion from B to Z conformation in alternating purine-pyrimidine sequences.

INTRODUCTION

A critical event in the initiation stage of chemical carcinogenesis is the covalent binding of chemicals to DNA that affect its structure and function (1-3). Since it has been shown that DNA can adopt a variety of conformations depending on its sequence and physicochemical environment, it is important to determine how the binding of some carcinogens to DNA may affect its conformation and subsequently its function.

Deviation from the normal right-handed B-DNA is most evident in left-handed Z-DNA in which orientation of bases about their glycosidic bonds alternates between the anti and syn conformations (4-6). Because purines are more stable in the syn conformation than pyrimidines, Z-DNA is favored by alternating purine-pyrimidine sequences (7).

Transition of B to Z is facilitated by various conditions, such as negative supercoiling of DNA, ethanol, changes in ionic strength or methylation of C5 in cytosine residues (8-13). As a consequence of the syn conformation of purine residues in the Z-form, the N7 and C8 atoms are exposed on the convex outer surface of the

helix. Binding of bulky molecules to these positions in alternating purine-pyrimidine sequences generally stabilizes the Z-conformation (14-21). Indeed, modification of poly(dG-dC) at C8 of dG by N-acetyl-2-aminofluorene (AAF), 2-aminofluorene (AF), or bromine atoms induces the Z-form (15-19,21). Similarly, methylation of N7 of dG by alkylating carcinogens facilitates formation of Z-DNA (14). In the case of the antitumor drug cis-dichlorodiammineplatinum (II), the Z-conformation is stabilized only if the drug forms a monodentate adduct by binding to the N7 of dG residues (22,23). On the other hand, the potent hepatocarcinogen aflatoxin, which reacts only with N7 of dG stabilizes the B-conformation and moreover it prevents conversion of DNA into Z-form (24). Similarly, other substances such as adriamycin and daunomycin which are active mutagens and carcinogens form only an intercalated B-form (25,26).

Due to the diverse effects of the covalently bound carcinogens upon the DNA conformation, we have investigated whether or not modification of poly(dG-dC) by two chemical carcinogens, 4-aminobiphenyl (ABP) and N-methyl-4-aminoazobenzene (MAB), will affect the B to Z transition. ABP, which induces cancers of the liver, mammary gland, and urinary bladder, forms N-(deoxyguanosin-8-yl)-ABP as the major carcinogen-DNA adduct in vivo (27-29). In addition, this C8-substituted guanine derivative is relatively persistent in the DNA of the carcinogen-target tissue and does not appear to undergo DNA repair. MAB, which is a hepatocarcinogen, also forms a C8-substituted guanine derivative as the major DNA adduct in vivo (30,31). However, N-(deoxyguanosin-8-yl)-MAB is rapidly removed from the liver DNA and its presence does not correlate with target tissue specificity. Thus, it was of further interest to determine if conformational differences in these adducts might correlate with these biological properties.

MATERIALS AND METHODS

Modification of poly(dG-dC) by ABP and MAB

Poly(dG-dC) samples containing ABP-nucleoside adducts were prepared by incubation with N-acetoxy-N-trifluoroacetyl-4-aminobiphenyl (32), a synthetic electrophilic ester that is known to react with DNA to give the same nonacetylated arylamine-nucleoside adducts that are formed in vivo from ABP and in vitro from N-hydroxy-ABP (27, and unpublished studies). The reaction mixture (1 ml), which was saturated with argon, contained 1 mg poly(dG-dC) (Pharmacia P-L Biochemicals, Piscataway, NJ) in 5 mM Bis Tris-HCl buffer (pH 7.1), to which was added 0.003 to 0.025 ml of 30 mM [³H]acetoxy-N-trifluoroacetyl-4-aminobiphenyl (12 mCi/mMol) in absolute ethanol. After incubation at 37° for 30 min under argon, the addition/incubation was repeated four times.

Poly(dG-dC) samples containing MAB-nucleoside adducts were prepared by reaction with N-benzoyloxy-N-methyl-4-aminoazobenzene (N-BzO-MAB), a synthetic analogue of the ultimate carcinogenic metabolite, N-sulfonyloxy-N-methyl-4-aminoazobenzene (33, 34). The reaction mixture (1 ml) contained 1 mg poly(dG-dC) in 10 mM potassium citrate buffer (pH 7.0), to which was added 0.02 or 0.05 ml of 50 mM [^3H]N-BzO-MAB (50.5 mCi/mMol; 34) in absolute ethanol. After incubation at 37° for 30 min, the MAB-modified poly(dG-dC) was purified by multiple solvent extractions and precipitations as described for MAB-modified DNA (35, 36). To prepare poly(dG-dC) samples with higher levels of MAB modification, either a second addition/incubation of [^3H]N-BzO-MAB was made to the above reaction medium or the purified MAB-poly(dG-dC) samples prepared above were again reacted with [^3H]N-BzO-MAB. The level of modification of the polymers were determined from the radioactivity measured by scintillation counting.

Characterization of the modified polymers

To determine the sites of modification, the polymers were enzymatically hydrolyzed according to published procedure in the presence of DNase I, nuclease P_1 , alkaline and acid phosphatases (37). HPLC analysis of the enzymatically hydrolyzed [^3H]ABP-modified poly(dG-dC) (28) was carried out on a Waters $\mu\text{Bondapak C}_{18}$ analytical reversed-phase column (0.39 x 30 cm) using a water-methanol gradient and a flow rate of 2 ml/min. HPLC analysis of the hydrolyzed [^3H]MAB-modified poly(dG-dC) (30) was carried out on a Waters $\mu\text{Bondapak C}_{18}$ Semi-prep column (0.79 x 30 cm) using a water-methanol gradient and a flow rate of 4 ml/min. The identity of the ^3H -labeled carcinogen-deoxyribonucleoside adducts was based on comparisons of their HPLC retention times with those of synthetic standards as previously described for ABP (27-29) and MAB (30).

Circular dichroism (CD) spectra were recorded on an AVIV spectropolarimeter, Model 60DS. Spectra were recorded in a 0.1-cm path-length cell at ambient temperature. Spectra used for computations represent an average of three spectra. Corresponding blanks for all samples were prepared by substituting the buffer for the polynucleotide.

RESULTS

It has been shown that covalent modification at the C8 position of guanine with bulky substituents as AAF, AF, and bromine induces the Z-conformation in poly(dG-dC) (15-19,21). Because ABP and MAB bind preferentially to the C8 atom of guanine in DNA, we were interested in whether or not modification of poly(dG-dC) by these bulky molecules would similarly induce the Z-conformation. We have used CD

Fig. 1. HPLC profile of ABP-modified poly(dG-dC). The histogram shows the radioactivity eluted from the column and the dashed line shows the water-methanol gradient elution program. The peaks designated C8-dG and r.o. C8-dG represent N-(deoxyguanosin-8-yl)-ABP and its purine ring-opened decomposition product, respectively.

spectroscopy to detect the conformational changes in ABP- and MAB-modified poly(dG-dC) in the presence of agents like ethanol and magnesium salt, and compare these changes to those in the unmodified polymer.

Fig. 2. CD spectra of unmodified (—), 1.56% ABP-modified (....), and 7.03% ABP-modified poly(dG-dC) (----) in 37% ethanol/5 mM Bis Tris-HCl (pH 7.02) mixture.

Fig. 3. CD spectra of unmodified (—), 7.03% ABP-modified (----), and 1.56% ABP-modified poly(dG-dC) (....) in 5 mM Bis Tris-HCl (pH 7.02)/10 mM NaCl/350 mM MgCl_2 mixture.

Analysis of ABP-modified poly(dG-dC)

For determining the sites of modification, we have enzymatically hydrolyzed the modified polymer and analyzed it by HPLC. Figure 1 shows the HPLC profile of the hydrolysis products of [^3H]ABP-modified poly(dG-dC). N-(Deoxyguanosin-8-yl)-ABP

Fig. 4. Percent ABP modification level as a function of ethanol concentration needed for a complete CD inversion.

Fig. 5. HPLC profile of MAB-modified poly(dG-dC). The histogram shows the radioactivity eluted from the column and the dashed line shows the water-methanol gradient elution program. The peaks designated N^2 -dG, C8-dG (cis) and C8-dG (trans), and C8-G represent 3-(deoxyguanosin- N^2 -yl)-MAB, the cis and trans isomers of N-(deoxyguanosin-8-yl)-MAB, and their decomposition product, N-(guan-8-yl)-MAB, respectively.

accounted for 81% of the total radioactivity and its purine ring-opened derivatives, which form spontaneously during the hydrolysis procedure, accounted for another 10%. Thus, at least 90% of the ABP modification occurred at the C8 position of guanine.

For the detection of conformational changes induced in poly(dG-dC) by ABP modification in the presence of ethanol and magnesium ion, we have used CD analyses. Figure 2 shows the CD spectra of unmodified, 1.56% and 7.03% ABP-modified poly(dG-dC) in 37% ethanol/5 mM Bis Tris-HCl (pH 7.02) mixture. The unmodified polymer at this ethanol concentration has a spectrum typical of a B-like form with a positive band centered at 285 nm followed by a negative band at 253 nm. However, in the presence of 37% ethanol, the spectra are markedly changed upon modification of the polymers with ABP. In the 1.56%-modified polymer, the CD spectrum has a negative band at 294 nm, a positive band at 271 nm, and a negative band at 250 nm. At a modification level of 7.03%, the negative band at 294 nm becomes more intense, whereas the band at 250 nm becomes positive.

Similar CD changes were observed in the presence of magnesium salts. Figure 3 shows the CD spectra of unmodified, 1.56% and 7.03% ABP-modified poly(dG-dC) in 5 mM Bis Tris-HCl buffer (pH 7.02) /10 mM NaCl and 0.35 M $MgCl_2$. Upon modification, the positive band at 285 nm changes into a negative band at 294 nm, and the intensity of the negative band at 252 nm decreases. The relative intensities of the bands depend on the percentage of bound ABP.

Fig. 6. CD spectra of unmodified (—), 0.43% MAB-modified (....), and 1.97% MAB-modified poly(dG-dC) (---) in 45% ethanol/5 mM Bis Tris-HCl (pH 7.02) mixture.

Figure 4 shows the amount of ethanol required to produce a complete CD inversion at different ABP modification levels. Extrapolation of the data suggests that 17% ABP-modified poly(dG-dC) would be in the Z-form in the absence of ethanol.

Fig. 7. Percent ellipticity change at 293 nm as a function of Mg^{++} concentration. O, 2.27% MAB-modified poly(dG-dC); O, 0.43% MAB-modified poly(dG-dC); X, poly(dG-dC) in 5 mM Bis Tris-HCl (pH 7.02)/10 mM NaCl mixtures.

Fig. 8. CD spectra of 2.92% ABP-modified (—), and 2.27% MAB-modified poly(dG-dC) (....) in 40% ethanol/5 mM Bis Tris-HCl (pH 7.02) mixture.

Analysis of MAB-modified poly(dG-dC)

Figure 5 shows the HPLC profile of the enzymatic hydrolysates of the [^3H] MAB-modified poly(dG-dC). As can be seen from the HPLC profile, 81% of the MAB modification occurs at the C8 position position of guanine: *cis* N-(deoxyguanosin-8-yl)-MAB, *trans* N-(deoxyguanosin-8-yl)-MAB, and N-(guanine-8-yl)-MAB account for 37%, 40% and 4% of the total carcinogen-nucleoside adducts, respectively; 15% is accounted for by 3-(deoxyguanosin- N^2 -yl)-MAB.

Figure 6 shows the CD spectra of the unmodified, 0.43% and 1.97% MAB-modified poly(dG-dC) in 45% ethanol/5 mM Bis Tris-HCl (pH 7.02) mixture. Like ABP modification, binding of MAB to poly(dG-dC) also alters the CD spectrum markedly. Whereas the unmodified polymer has a B-like spectrum under the given conditions, the spectrum of the 0.43% MAB-modified polymer shows a negative band at 293 nm, the intensity of which increases when modification level increases to 1.97%. The negative band at 252 nm decreases drastically upon modifying the polymer with 0.43% MAB and becomes positive at 1.97% modification level.

Figure 7 shows the percent molar ellipticity change at 293 nm of the unmodified, 0.43% and 2.27% MAB-modified poly(dG-dC) in 5 mM Bis Tris-HCl (pH 7.02)/10 mM NaCl mixture, as a function of magnesium concentration. The transition midpoints are 0.6 M, 0.4 M, and 0.3 M for the unmodified, 0.43%, and 2.27% MAB-modified polymers, respectively. These results indicate that at higher modification levels, less

amount of cations are needed to induce a conformational change in poly(dG-dC).

To compare the effects of ABP and MAB modifications on their ability to invert the CD spectrum of poly(dG-dC), we measured the CD spectra of polymers modified to similar extents under identical conditions. Figure 8 shows that poly(dG-dC) modified with 2.92% ABP at 40% ethanol has already a completely inverted spectrum with a negative band at 292 nm, and a positive band at 270 nm. On the other hand, the spectrum of poly(dG-dC) modified with 2.27% MAB in the presence of 40% ethanol is only partially in Z conformation, retaining still a negative band at 252 nm.

DISCUSSION

Since modification of guanine residues by bulky substituents may induce different conformations in the DNA, we studied by CD measurements the conformations of poly(dG-dC), and poly(dG-dC) modified by ABP and MAB. Our results show that both of these substituents promote B to Z transition in poly(dG-dC). This has been shown by an inversion of CD spectra similar to that seen in the unmodified polymer in high salt or ethanol solution (10-12). Due to steric factors, the substituents used in our study probably induced syn conformation in the deoxyguanosine residues by facilitating the B to Z transition. Theoretical calculations are in agreement with these assumptions (38). Using minimized potential energy calculations, it was shown that in ABP-modified d(CpG), the global minimum energy conformation has syn deoxyguanosine and carcinogen-base stacking instead of base-base stacking seen in the unmodified dinucleotide (39).

All of our CD data indicate that at higher modification levels, less drastic extrinsic factors are needed to promote the CD inversion. In unmodified poly(dG-dC) inversion of CD spectra starts at 0.75 to 1.0 M MgCl_2 , and is complete at 1.5 to 1.7 M MgCl_2 solution. However, already at 0.35 M MgCl_2 solution both 1.56% and 7.03% ABP-modified poly(dG-dC) show partially inverted CD spectra (Fig. 3). Furthermore, unmodified poly(dG-dC) requires 55 to 60% ethanol for a complete inversion of CD spectrum. However, at 37% ethanol solution, 1.56% ABP-modified poly(dG-dC) already has a partially inverted CD spectrum (Fig. 2). Furthermore, at 37% ethanol solution, 7.03% ABP-modified poly(dG-dC) shows a completely inverted CD spectrum (Fig. 2), as observed when poly(dG-dC) is in Z-form in the presence of 60% ethanol (18). Similarly, the 0.43% MAB-modified poly(dG-dC) has a partially inverted CD spectrum in 45% ethanol, whereas the 1.97% MAB-modified poly(dG-dC) shows a completely inverted CD spectrum (Fig. 6).

Similar results were obtained with 28% AAF-modified poly(dG-dC) which was in the Z-form even in the absence of ethanol (18). However, with ABP and MAB we

were not able to achieve that high level of modification. Even after repeated modifications, the highest level of ABP-modified poly(dG-dC) was only 7.03%.

Therefore, some extrinsic factors were needed to achieve inversion of CD spectra. We assume that if higher modification levels were possible, we would be able to observe inverted CD spectra in the absence of ethanol. In fact, extrapolation of the data indicates that about 17% ABP-modified poly(dG-dC) can exist in the Z-form in the absence of ethanol (Fig.4). The attempt to perform modification of poly(dG-dC) in the presence of 60% ethanol, when the polymer is in the Z-form, failed because the high level of ethanol affected the reaction and lowered the level of modification.

Comparison of ABP and MAB modifications shows ABP to be better than MAB at inducing B to Z transition in poly(dG-dC). (Fig.8). This suggests that ABP induces syn conformation of guanine residues in DNA more readily than MAB, causing a larger distortion of DNA conformation. Although it is generally assumed that the repair of modified bases is better in more distorted DNA (40), the results with the repair of ABP- and MAB-adducts in vivo are not in accord with this assumption. In fact, ABP-C8-guanine adducts are much more persistent and less readily repaired in vivo than the MAB-C8-guanine adducts (29). Thus, it is possible that conversion to Z-DNA may result in inhibition of DNA adduct repair. In this regard, enzymatic repair of O⁶-methylguanine in DNA has been reported to be impaired by conversion to the Z-form (41).

Taken together, based on the inverted CD spectra, we assume that the conformational changes occurring in poly(dG-dC) modified by ABP and MAB are due to B to Z transition. However, we are aware that CD studies, unlike NMR, do not provide definitive conformational information. For instance, it has been shown that inverted CD spectra can result also from factors other than the presence of Z-DNA (42). Therefore, although the changes in the CD spectra induced in ABP- and MAB-modified poly(dG-dC) are similar to those induced by Z-DNA, additional conformational information will need to be obtained by using NMR and/or specific Z-DNA antibodies.

ACKNOWLEDGEMENTS

The authors are thankful to Drs. C. Teeters and B. A. Wallace of Columbia University for providing programs for the CD data analysis, and Dr. D. Norman for his valuable suggestions and discussions. This investigation was supported by NIH Grants CA 21111 and CA 31696.

REFERENCES

1. Miller, E.C. (1978), *Cancer Res.* **38**, 1479-1496.
2. Miller, E.C., and Miller, J.A. (1981), *Cancer* **47**, 2327-2345.

3. Singer, B., and Kusmierek, J.T. (1982). *Annu. Rev. Biochem.* 52, 655-693.
4. Wang, A.H., Quigley, G.J., Kolpak, F.J., Cranford, J.L., Van Boom, J.A., Van der Macel, G., and Rich, A. (1979). *Nature* 282, 680-686.
5. Wang, A.H., Quigley, G.J., Kolpak, F.J., Van der Macel, G., Van Boom, J.A., and Rich, A. (1980). *Science* 211, 171-176.
6. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson, R.E. (1980). *Nature* 286, 567-573.
7. Rich, A., Nordheim, A., and Wang, A.H.J. (1984). *Ann. Rev. Biochem.* 53, 791-846.
8. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982). *Nature* 299, 312-316.
9. Nordheim, A., and Rich, A. (1983). *Proc. Natl. Acad. Sci. U.S.A.* 80, 1821-1825.
10. Pohl, F.M. (1976). *Nature* 260, 365-366.
11. Pohl, F.M., and Jovin, T.M. (1972). *J. Mol. Biol.* 67, 375-396.
12. Behe, M., and Felsenfeld, G. (1981). *Proc. Natl. Acad. Sci. U.S.A.* 78, 1619-1623.
13. Fujii, S., Wang, A.H.J., Van der Macel, G., Van Boom, J.H., and Rich, A. (1982). *Nucleic Acids Res.* 10, 7879-7892.
14. Moller, A., Nordheim, A., Nichols, S.R., and Rich, A. (1981). *Proc. Natl. Acad. Sci. U.S.A.* 78, 4774-4781.
15. Moller, A., Nordheim, A., Kozlowski, S.A., Patel, D., and Rich, A. (1984). *Biochemistry* 23, 54-62.
16. Sage, E., and Leng, M. (1980). *Proc. Natl. Acad. Sci. U.S.A.* 77, 4597-4601.
17. Santella, R.M., Grunberger, D., Broyde, S., and Hingerty, B.E. (1981). *Nucleic Acids Res.* 9, 5459-5467.
18. Santella, R.M., Grunberger, D., Weinstein, I.B., and Rich, A. (1981). *Proc. Natl. Acad. Sci. U.S.A.* 78, 1451-1455.
19. Grunberger, D., Santella, R.M., Hanau, L.H., and Erlanger, B.F. (1985). *Carcinogenesis* 10, 465-480.
20. Santella, R.M., Grunberger, D., Nordheim, A., and Rich, A. (1982). *Biochem. Biophys. Res. Comm.* 106, 1226-1232.
21. Kriek, E., Miller, J.A., Juhl, U., and Miller, E.C. (1967). *Biochemistry* 6, 177-182.
22. Malinge, J.M., and Leng, M. (1984). *EMBO J.* 3, 1273-1279.
23. Ushay, H.M., Santella, R.M., Coradonna, J.P., Grunberger, D., and Loippard, S.J. (1982). *Nucleic Acids Res.* 10, 3573-3588.
24. Nordheim, A., Hao, W.M., Wogan, G.N., and Rich, A. (1983). *Science* 219, 1434-1436.
25. Van Helden, P.D. (1983). *Nucleic Acids Res.* 11, 8415-8420.
26. Chairs, J.B. (1984). *Nucleic Acids Res.* 11, 8485-8494.
27. Beland, F.A., Beranek, D.T., Dooley, K.L., Heflich, R.H., and Kadlubar, F.F. (1983). *Environ Health Perspect.* 49, 125-134.
28. Kadlubar, F.F., Beland, F.A., Beranek, D.T., Dooley, K.L., Heflich, R.H., and Evans, F.E. (1982) in Sugimura, T., Kondo, S., and Takebe, H. (eds), *Environmental Mutagens and Carcinogens*, Alan R. Liss, New York, pp. 385-396.
29. Kriek, E. (1971). *Chem.-Biol. Interactions* 3, 19-28.
30. Tullis, D.L., Dooley, K.L., Miller, D.W., Baetcke, K.P., and Kadlubar, F.F. (1987). *Carcinogenesis* 8, 577-583.
31. Lin, J.K., Miller, J.A., and Miller, E.C. (1975). *Cancer Res.* 35, 844-850.
32. Lee, M.S., and King, C.M. (1981). *Chem. -Biol. Interactions* 34, 239-248.
33. Poirier, L.A., Miller, J.A., Miller, E.C., and Sato, K. (1967). *Cancer Res.* 27, 1600-1613.
34. Kadlubar, F.F., Miller, J.A., and Miller, E.C. (1976). *Cancer Res.* 36, 2350-2359.
35. Kadlubar, F.F., Miller, J.A., and Miller, E.C. (1977). *Cancer Res.* 37, 805-814.
36. Tullis, D.L., Straub, K.M., and Kadlubar, F.F. (1981). *Chem-Biol. Interactions* 38, 15-27.
37. Martin, C.N., Beland, F.A., Roth, R.W., and Kadlubar, F.F. (1982). *Cancer Res.* 42, 2678-2696.

38. Broyde, S., Hingerty, B.E., and Srinivasan, A.R. (1985). *Carcinogenesis* 6, 719-725.
39. Shapiro, R., Underwood, G.R., Zawadzka, H., Broyde, S., and Hingerty, B.E. (1986). *Biochemistry* 25, 2198-2205.
40. Beland, F.A., and Kadlubar, F.F. (1985). *Environ Health Perspect.* 62, 19-30.
41. Boiteux, S., Costa de Oliveira, R., and Laval, J. (1985). *J. Biol. Chem.* 260, 8711-8715.
42. Tomasz, M., Barton, J.K., Magliozzo, C.C., Tucker, D., Lafer, E.M., and Stollar, B.D. (1983). *Proc. Natl. Acad. Sci. U.S.A.* 80, 2874-2878.