Salt-Induced Conversion of B-DNA to Z-DNA Inhibited by Aflatoxin B1

Abstract. *The carcinogen aflatoxin B1 was reacted with a polymer of alternating deoxyguanine and deoxycytosine residues to determine the effect that adduct formation has on the conversion of this polymer from the right-handed B-DNA form found at low salt concentrations to the left-handed Z-DNA form found at high salt concentrations. Reaction with aflatoxin strongly inhibited the salt-induced conversion of this polymer from B-DNA to Z-DNA. This inhibition could be detected even at relatively low binding levels.*

Aflatoxin B1 (AFB_1) is a potent hepatocarcinogen produced by certain strains of *Aspergillus flavus* and related fungi. The growth of these fungi on human food supplies poses a serious public health hazard (*1*). Aflatoxin B1 reacts with a variety of cellular macromolecules, and its covalent binding to DNA is believed to have a role in its carcinogenicity (*2*) (see Fig. 1). DNA is normally found as a right-handed double helix (B-DNA), but under certain conditions it can adopt a left-handed double helical form (Z-DNA), which is favored by sequences with alternating purines and pyrimidines—especially alternating guanine and cytosine residues. Z-DNA was initially observed in crystallographic studies of DNA oligomers (*3*) but has been identified in the polytene chromosomes of *Drosophila* (*4*). Ionic conditions (5, 6) as well as chemical modifications of DNA play an important role in Z-DNA formation. The carcinogen 2-acetylaminofluorene, for example, reacting with the C-8 position of guanine, readily facilitates the conversion of poly(dG-dC) (dG, deoxyguanylate; dC, deoxycytidilate) from B-DNA to Z-DNA (*7, 8*). Likewise, methylation of cytosine at C-5 (*9*) and guanine at N-7 (*10*) in the same polymer facilitates its conversion to Z-DNA. Many methylating carcinogens react with guanine at N-7, as does AFB_1 (*11*). We have examined the effect of AFB_1 adduct formation on the salt-induced conversion of poly(dG-dC) from the B to the Z form and now report that, instead of facilitating the conversion to Z-DNA, AFB_1 has the property of stabilizing the polymer in the B conformation and preventing its conversion to Z-DNA in high-salt solutions.

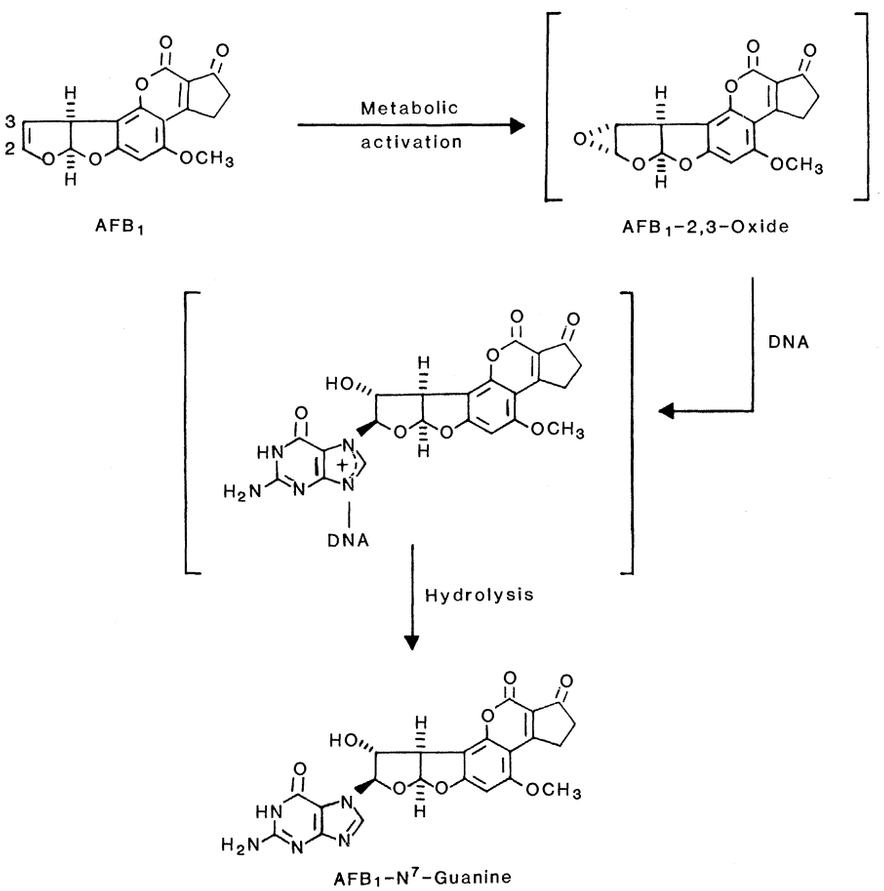

Fig. 1. The metabolic activation of aflatoxin B1 leading to the DNA adduct. The product isolated upon hydrolysis is also shown.

Poly(dG-dC) DNA was reacted with AFB_1 to varying binding levels by a modification of the method of Garner *et al.* (*12*). The poly(dG-dC)–AFB_1 adducts (Fig. 1) were analyzed by acid hydrolysis and an established high-performance liquid chromatography procedure (*13*). Approximately 64 percent of bound ^3H-labeled AFB_1 was present as the AFB_1–N^7-guanine adduct (Fig. 1), and the rest was distributed among a series of minor derivatives. The profile was qualitatively equivalent to that of calf thymus DNA modified under similar conditions or by enzymatic activation of AFB_1 (*11, 13*). Figure 2 shows the circular dichroism of unmodified and AFB_1-reacted poly(dG-dC) at various molarities of NaCl. The binding level is defined as the number of nucleotides required for the binding of one aflatoxin molecule. With unmodified polymer (BL ∞), raising the salt concentration produces gradual inversion of the circular dichroism (*5*). At a binding level of one aflatoxin molecule per 42 nucleotides, however, the form of the circular dichroism remains virtually unchanged for NaCl concentrations ranging from 0.1*M* to 4*M*. The positive band of the adducted polymer between 270 and 290 nm remains positive for all concentrations of NaCl, in marked contrast to the strong negative values found in the unmodified, aflatoxin-free polymer. The negative band at 250 nm is virtually unaffected by the salt concentration. Although NaCl has an effect on the circular dichroism spectrum of the aflatoxin-reacted polymer, the effect is a minor one compared to the drastic changes found in the unmodified salt-induced polymer. In 0.1*M* salt, the positive value of the Cotton effect in the region 270 to 290 nm in the aflatoxin-treated polymer is slightly higher than the value of the untreated polymer—undoubtedly a result of aflatoxin absorbance. Raising the salt concentration to 4*M* NaCl produced only a small lowering of the band to a level roughly comparable to that seen in 1*M* NaCl in the unmodified polymer. These data indicate that the salt-induced conversion of B-DNA to Z-DNA has been blocked by the presence of aflatoxin.

We explored the effect of varying levels of aflatoxin binding on the salt-induced B to Z conversion (Fig. 2). The degree of transition, θ_{290}, as used by Pohl and Jovin (*5*) was plotted as a function of NaCl concentration for different aflatoxin binding levels. The de-

gree of transition was obtained by measuring the change in the circular dichroism at 290 nm (legend to Fig. 3). The unmodified polymer (BL ∞) shows a strong sigmoidal transition curve with a midpoint near 2.7*M* NaCl, indicating the cooperativity of the transition. At binding levels of one aflatoxin for every 42, 175, or 300 nucleotides, the transition is only partial as measured by the change in the circular dichroism. The curves do not show the sigmoidal character found in the unmodified polymer, but appear to be linear. The change in the circular dichroism of the aflatoxin-treated polymer (BL 42) is visible even in 0.1*M* NaCl; this suggests that at the highest aflatoxin binding level, the changes shown in Fig. 2 do not represent an actual conversion of B-DNA to Z-DNA. The changes may be perturbations in the circular dichroism spectrum associated with aflatoxin binding rather than DNA conformational changes. A plot of the degree of transition at 250 nm as a function of NaCl shows very little change in the degree of transition for a binding level of 42. Examination of the effect of salt on the circular dichroism spectrum at all four binding levels shows that there is significant alteration even at a binding level of one aflatoxin residue per 300 nucleotides. Upon the addition of salt, the spectrum moves in the same direction as the unmodified polymer, but only partially. This suggests that the DNA double helix is stabilized in a right-handed conformation around the positions where aflatoxin B1 has reacted, and although some of the segments can begin to convert to left-handed Z-DNA in the unmodified stretches between the aflatoxin residues, this conversion is only partial. Aflatoxin may act to prevent the propagation of the Z-DNA conformation along the entire molecule.

Further experiments were carried out with the polymer that had reacted at a binding level of one aflatoxin per 300 residues. Lowering the salt concentration from 4*M* to 0.1*M* showed that the circular dichroism spectrum readily reverted to the spectrum for the low-salt solution. This is consistent with the idea that aflatoxin has a local influence in maintaining a right-handed conformation but does not prevent partial Z formation in the intervening segments from being reversible when the salt concentration is lowered. The effect of the aflatoxin adducts on the inhibition of the B to Z conversion was quite stable. Examination of the circular dichroism spectra after the polymers had been stored for 4 days at 4°C in either 0.1*M* or 4*M* NaCl (*p*H 6.6) showed the behavior of the polymers to be identical to that described above.

Treatment of the aflatoxin adduct at *p*H 12.5 induces a cleavage in the guanine imidazole ring to generate a modified adduct (*14*). Which of the adducts is the biologically active carcinogen is not known. The *p*H of the polymer was raised to 12.5 by adding 0.1*N* NaOH; after 30 minutes at 37°C, the DNA was precipitated with ethanol and resuspended in a phosphate buffer at *p*H 6.6. Measurements of the effect of NaCl on the circular dichroism suggested that the ring-cleaved form of the adduct behaves like the uncleaved adduct.

We tried to determine whether the Z and B forms of the polymer were equally good DNA substrates for aflatoxin binding; however, no conditions could be found that allowed stabilization of poly(dG-dC) in Z-DNA without inhibiting the aflatoxin binding reaction. DNA melting experiments were carried out to show that the polymer was still in a double helix after the aflatoxin reaction. The absorbance at 260 nm as a function

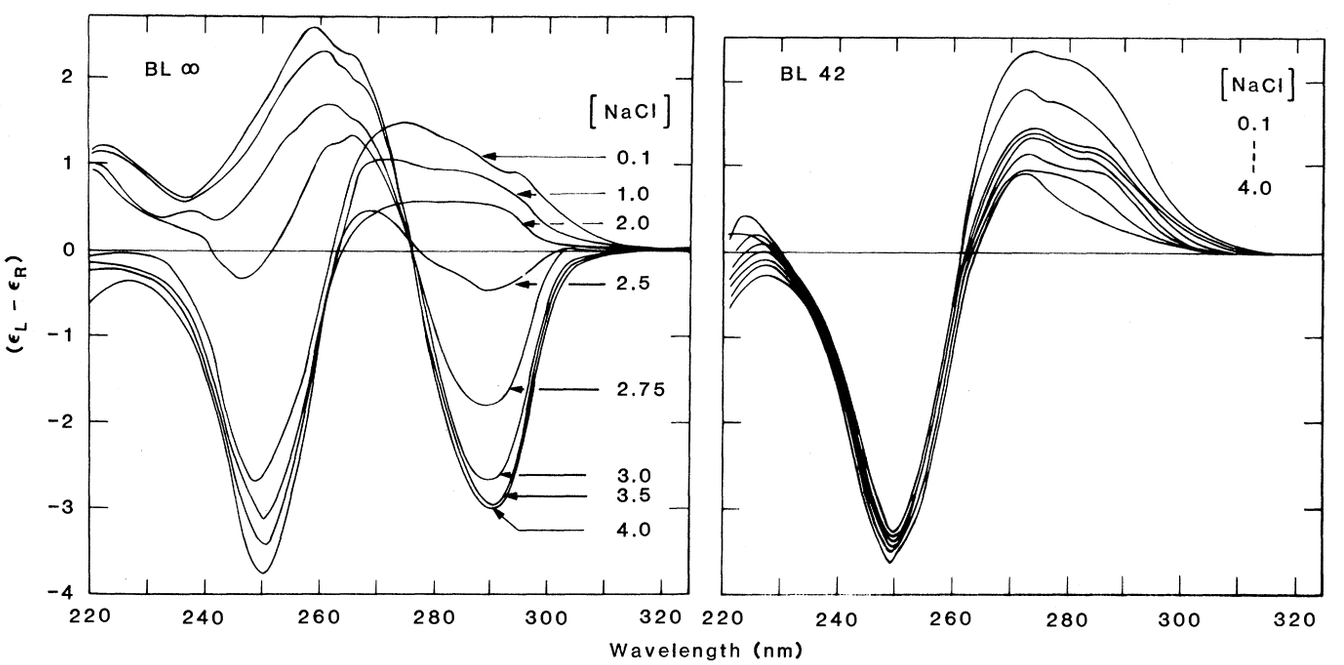

Fig. 2. Circular dichroism spectra of poly(dG-dC) in solution with different concentrations of NaCl. The polymers were buffered in 20 m*M* $NaHPO_4$ (*p*H 6.6). They were reacted with aflatoxin B1 to the extent indicated by the binding level BL (moles of nucleotide per mole of aflatoxin). (Left) Unmodified DNA polymer (BL ∞); (right) DNA polymer highly adducted with aflatoxin B1 (BL 42). The adduct was prepared as follows (*13*): 1.4 nmole of [^{3}H]aflatoxin B1 (3.03 nCi/nmole, Moravek Biochemicals) were added to excess 3-chloroperbenzoic acid, dissolved in 0.2 ml CH_2Cl_2, and mixed vigorously for 4 hours at room temperature with 0.3 ml DNA solution [poly(dG-dC) (P-L Biochemicals), 1 mg/ml in 20 m*M* $NaHPO_4$, *p*H = 6.6]. Phases were separated by centrifugation and the DNA was precipitated out of the aqueous phase with ethanol. The DNA pellet was washed sequentially with ethanol (once), chloroform (twice), benzene (once), and ether (twice) to remove unbound carcinogen, and subsequently dried and resuspended in 1 ml of 20 m*M* $NaHPO_4$ buffer (*p*H = 6.6). Portions were removed for spectroscopic determination of DNA recovery and for quantitation of bound [^{3}H]aflatoxin B1 by liquid scintillation counting. The unmodified sample (BL ∞) was prepared in an identical manner except for omission of aflatoxin. Solid NaCl was added stepwise to the redissolved DNA adducts to obtain the indicated salt concentrations. After incubation for 45 minutes at room temperature, circular dichroic spectra were monitored at each step in a Cary 16 spectrophotometer.

of temperature was measured for the unreacted and adducted polymers. Although all of the polymers exhibited a similar rise in hypochromicity at an elevated temperature, the polymers that had reacted with aflatoxin showed a decrease in the melting temperature, suggesting that even in a low-salt solution the aflatoxin reaction had introduced some modifications into the double helix. The greatest decrease in melting temperature corresponded to the highest level of aflatoxin reactivity.

The effect of aflatoxin in inhibiting the B to Z transition seems paradoxical. Segments of $(dG\text{-}dC)_n$ shorter than 42 base pairs inserted into bacterial plasmids can convert to the Z conformation when the salt concentration is raised (*15, 16*). Presumably the DNA at the ends of the insert is in the B conformation. The segments between the aflatoxin residues appear not to convert to Z-DNA; yet conversion to the Z conformation occurs readily in the plasmids with the cloned inserts. There are several possible explanations. The aflatoxin may bind to the DNA and hold it in a conformation that inhibits Z formation at further distances along the molecule than that found with normal B-DNA. Alternatively, it is possible that some conversion to Z-DNA in the regions between aflatoxin molecules is not detected by the circular dichroism.

It may be useful to classify substances that react with DNA according to their effect on the interconversion of B-DNA and Z-DNA. The carcinogen 2-acetylaminofluorene (*7, 8*) and N-7 methylating agents (*10*) both induce Z-DNA formation in poly(dG-dC). The bifunctional platinum reagents, *cis*-dichlorodiamino platinum(II) and *trans*-dichlorodiamino platinum(II), inhibit the salt-induced conversion of B-DNA to Z-DNA (*17, 18*), whereas the monofunctional platinum complex chlorodiethylene triamino platinum(II) chloride facilitates the conversion of B-DNA to Z-DNA. Aflatoxin B1 is considerably more effective than the platinum compounds described above in inhibiting the conversion of B-DNA to Z-DNA.

The effect of the 2-acetylaminofluorene on the conversion of B to Z is readily understood in view of the fact that this bulky molecule reacts at the C-8 position of guanine where there is no room for this substituent in B-DNA. In Z-DNA, the guanosine is in the *syn* conformation and the C-8 atom is on the outside of the molecule where there is adequate room for a bulky substituent. Methylation at N-7 facilitates Z formation in poly(dG-dC) in part through the positive charge that it puts on the guanosine residue. This may facilitate the conversion of the polymer to Z-DNA by shielding the phosphate repulsions between the two polynucleotide chains (*10*). The effect of the bifunctional platinum reagents may be associated with the fact that they cross-link the DNA and stabilize it in a right-handed conformation, in contrast to the monofunctional one which is unable to cross-link. Aflatoxin B1 reacts at the N-7 position of guanine and puts a positive charge on the imidazole ring; in view of the results with N-7 methylation (*10*), this might be expected to facilitate Z-DNA formation. However, aflatoxin is a bulky molecule and it is possible that it may stabilize a right-handed conformation through its interaction with other components of the B-DNA double helix in the major groove. For example, its binding to the sugar-phosphate backbone through hydrogen bonding could stabilize the right-handed helical conformation, or it may simply block the conversion by its bulk.

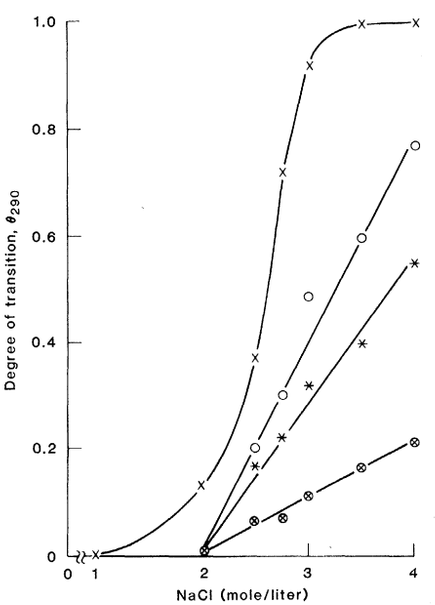

Fig. 3. Degree of the salt-induced conversion of poly(dG-dC) from the B to the Z form as a function of different levels of aflatoxin B1 adduct. The DNA–aflatoxin B1 adducts were formed as described in Fig. 2 with [^{3}H]aflatoxin B1 in amounts of 0, 0.14, 0.35, and 1.4 nmole. These yielded binding levels of (X) BL ∞, (○) BL 300, (*) BL 175, and (⊗) BL 42, respectively. For preparation of the BL 300 adduct, a higher specific activity was used (360.4 nCi/nmole). Salt titrations and circular dichroism measurements were performed as described in Fig. 2. The degree of the B to Z transition was calculated from the individual circular dichroism spectrum using the formula $\theta_{290} = (\alpha - \alpha_B)/(\alpha_Z - \alpha_B)$, where α is sample absorbance at 290 nm, α_B is absorbance at 290 nm of DNA in the B conformation, and α_Z is absorbance at 290 nm when the DNA is fully in the Z conformation (*5*).

Chemicals that modify DNA are often cytotoxic and some have carcinogenic activity. All of the chemicals discussed above have in common the property of interfering with the equilibrium between the right- and left-handed forms of DNA. This change in DNA conformation may be associated with regulation of gene function (*4*). The site of action of some carcinogens may be a regulatory region where the carcinogen-induced imbalance in the equilibrium between B and Z forms of DNA could interfere with normal transcriptional activity [for example, see (*19*)]. How such changes in gene expression could result in the inheritable defects found in the transformed state is, of course, still unknown.

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20. We thank J. Essigman for helpful discussions. This research was supported by grants from the National Institutes of Health, the American Cancer Society, and the National Aeronautics and Space Administration. A.N. was supported by a postdoctoral fellowship from the Charles A. King Trust of Boston, Mass.

26 May 1982; revised 29 November 1982