Reactivity of B and Z-DNA towards N-acetoxy-2-acetylaminofluorene

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ABSTRACT

Poly d(G-C).poly d(G-C) in B-form, on one hand, and poly d(G-br5C).poly d(G-br5C) and poly d(G-m5C).poly d(G-m5C) in Z-form, on another hand, were treated with N-AcO-[3H]AAF and the kinetics of these reactions were followed by radioactivity. Covalent binding of carcinogen to the polymers was evaluated after separation of the reacted polymers from non-reacted carcinogen by thin-layer chromatography. We found that B-form polymer reacts twice faster than the Z-form polymers. Proportions of main adducts in the three polymers are almost the same. Results are discussed in relation to the calculated electrostatic potential minima and steric accessibility at the reactive site (1, 2).

A quite general mechanism of action of chemical carcinogens towards DNA is the reaction between an electrophilic derivative of the carcinogen and nucleophilic sites of bases in DNA (3,4). Such reactions are obviously dependent at least on the electrostatic potential minima at the nucleophilic site and on the steric accessibility of the site (2,5). Recent calculations of these parameters for different sites of guanine and cytosine in right-handed B-DNA and in left-handed Z-DNA show important differences of values between the two conformations (1,2). These differences may lead to different reactivities of B and Z-DNA towards carcinogens.

Several factors such as methylation (6,7), bromination (8) and carcinogen binding (9,10) were found to induce a Z-conformation of poly d(G-C). poly d(G-C). Recently, the natural occurrence of Z-DNA regions was revealed in Drosophila polytene chromosomes (11,12). Thus, an eventual difference of reactivity of B- and Z-forms towards carcinogens, might lead to a differential susceptibility of certain zones of DNA to the attack of carcinogens.

In the present work we investigate the in vitro reaction of N-acetoxy-2-acetylaminofluorene (N-AcO-AAF) an active derivative of the carcinogen 2-acetylaminofluorene (2-AAF) with poly d(G-C).poly d(G-C) on one hand, and with poly d(G-br5C).poly d(G-br5C) and poly d(G-m5C).poly d(G-m5C) on another
hand. In the conditions of reaction employed (see below), poly d(G-C).poly d(G-C) adopts a B-conformation (13,14) and poly d(G-br\textsuperscript{5}C).poly d(br\textsuperscript{5}C) (8) and poly d(G-m\textsuperscript{5}C).poly d(G-m\textsuperscript{5}C) a Z-conformation (7). The level of binding of AAF residues to poly d(G-C).poly d(G-C) during the reaction is sufficiently low not to induce the B → Z transition of an important part of the polymer (less than 2 % of the polymer is in Z-form) (10).

We followed the kinetics of the covalent binding of AAF residues to guanosine in the three polymers, as well as in native calf thymus DNA, and in dGMP. The proportion of main adducts in these reactions were determined as well.

EXPERIMENTAL

Reactivity of the polymers towards N-AcO-AAF

N-AcO-[\textsuperscript{3}H]AAF (specific activity 878 mCi/mMol) in ethanol was incubated at 37°C with native DNA (Calf thymus, Sigma), with poly d(G-C).poly d(G-C) (P.L. Biochemicals), and with poly d(G-br\textsuperscript{5}C).poly d(G-br\textsuperscript{5}C) (prepared as previously described (8)) in 2×10\textsuperscript{-3} M Na citrate, 10\textsuperscript{-2} M NaCl\textsubscript{2} (pH 7.5) buffer (ethanol:buffer, v/v : 1:2). The ratio of the carcinogen concentration (3.6×10\textsuperscript{-4} M) and the concentration in guanosine residues in all three compared polymers (0.9×10\textsuperscript{-4} M) was the same in the three experiments (r = 0.4). Reactions were performed in the dark.

Thin-layer chromatography was used to separate the reacted polymers from non-reacted carcinogen (and its degradation products). Aliquots of 10 \lambda of the reaction mixture were sampled in function of time and applied on TLC plates of Silica gel F\textsubscript{254} (5 cm x 10 cm, Merck). The solvent system was phenol-chloroform-isoamylic alcohol 25:24:1, v/v/v. In this solvent system N-AcO-AAF and its degradation products are extracted and migrate; the polymer bearing covalently bound AAF residues does not migrate. The radioactivity of the non-migrating material on Silica gel was measured with an LKB 1216-8 counter. The complete extraction of non-reacted carcinogen by this solvent system was verified by the equality of radioactivity of the non-migrating material and that of the same quantity of polymer on which a usual phenol extraction in solution was performed.

The reaction of dGMP (Sigma) with N-AcO-[\textsuperscript{3}H]AAF was performed in exactly the same conditions as for the polymers. The kinetics of formation of dGMP-C8-AAF was followed by measuring the radioactivity of the material chromatographing with a non-radioactive concentrated solution of dGMP-C8-AAF. Solvent system for chromatography was isopropanol-ammonia 25 %-water, 6:3:1 (v/v/v).
In this system, N-AcO-AAF and its degradation products are migrating with the front of the solvent and dGMP-C8-AAF with an $R_f = 0.72$.

The results of these kinetics are presented in figure 1. The parameters characterizing these kinetics, i.e., slope at origin and maximum level of carcinogen binding are presented in table I.

Another set of experiments was performed to test the reactivity of poly d(G-$m^5$C).poly d(G-$m^5$C) in Z-form. N-AcO-$[^3H]$AAF was incubated with poly d(G-C).poly d(G-C), with poly d(G-$br^5$C).poly d(G-$br^5$C) and with poly d(G-$m^5$C).poly d(G-$m^5$C) (prepared as previously described (7)) in 50 mM NaCl, 2 mM MgCl$_2$, 5 mM Tris-HCl (pH 8.0) buffer (Fig. 2). Same kinetics is observed in the case of binding to poly d(G-$br^5$C).poly d(G-$br^5$C) and poly d(G-$m^5$C).poly d(G-$m^5$C), both in Z-form. The value of the slope at origin, as well as the value of the maximum level of binding are half of those for poly d(G-C).poly d(G-C) in B-form. The same result was obtained when comparing poly d(G-$br^5$C).poly d(G-$br^5$C) in Z-form and poly d(G-C).poly d(G-C) in B-form in the first experiment.

![Figure 1 - Kinetics of covalent binding of $[^3H]$AAF residues to: dGMP (-•-), native DNA (-•-), poly d(G-C).poly d(G-C) (-△-) and poly d(G-$br^5$C).poly d(G-$br^5$C) (-○-) in 2x10$^{-3}$ M Na citrate, 10$^{-2}$ M NaCl, pH 7.5, 1% ethanol, at 37°C. Concentration in guanosine residues 0.9x10$^{-4}$ M, concentration in N-AcO-AAF 3.6x10$^{-5}$ M. In these conditions, poly d(G-C).poly d(G-C) is in B-form, and poly d(G-$br^5$C).poly d(G-$br^5$C), in Z-form.](https://academic.oup.com/nar/article-abstract/10/14/4423/2378999/Reactivity-of-B-and-Z-DNA-towards-N-acetoxy-2-acetylaminofluorene)
Table I - Parameters of kinetics experiments: $s_n$, slope at origin, and $p_n$, final degree of covalent binding of carcinogen to the studied polymers as evaluated from Figs 1 and 2.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$s_n$ $(10^3\text{cpm/min})$</th>
<th>$s_n/s_1$ (%) carcinogen/Guo</th>
<th>$p_n$</th>
<th>$p_n/p_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly d(G-C).poly d(G-C) (1)</td>
<td>0.8</td>
<td>1</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Poly d(G-br$^5$C).poly d(G-br$^5$C) (2)</td>
<td>0.45</td>
<td>0.56</td>
<td>1.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Native DNA (3)</td>
<td>1.1</td>
<td>1.37</td>
<td>3.6</td>
<td>1.40</td>
</tr>
</tbody>
</table>

A : Poly d(G-C).poly d(G-C) is in B-form and poly d(G-br$^5$C).poly d(G-br$^5$C) is in Z-form in the reaction conditions (2x10^{-3} M Na Citrate, 10^{-2} M NaCl, pH 7.5, 1 % ethanol, 37°C).

B : Poly d(G-C).poly d(G-C) is in B-form, while poly d(G-br$^5$C).poly d(G-br$^5$C) and poly d(G-m$^5$C).poly d(G-m$^5$C) are in Z-form in the reaction conditions (5x10^{-2} M NaCl, 2x10^{-3} M MgCl$_2$, 5x10^{-3} M Tris-HCl, pH 8.0, 1 % ethanol, 37°C).

Figure 2 - Kinetics of covalent binding of [3H]$\alpha$AF residues to: poly d(G-C).poly d(G-C) (•••), poly d(G-br$^5$C).poly d(G-br$^5$C) (●●) and poly d(G-m$^5$C).poly d(G-m$^5$C) (○○) in 50 mM NaCl, 2 mM MgCl$_2$, 5 mM Tris-HCl, pH 8.0, 1 % ethanol at 47°C. Same concentrations as in Fig. 1. In these conditions, poly d(G-C).poly d(G-C) is in B-form, while poly d(G-br$^5$C).poly d(G-br$^5$C) and poly d(G-m$^5$C).poly d(G-m$^5$C) are in Z-form.
Degradation of N-AcO-AAF

Degradation of N-AcO-[\(^3\)H]AAF at 37°C in the 1% ethanol-buffer mixture was followed also by chromatography, as a function of time. The solvent system used was chloroform-acetone 95:5 (v/v). We measured the radioactivity of the product migrating with a control of pure non-radioactive (but concentrated) N-AcO-AAF. In this system \( R_f \) (N-AcO-AAF) = 0.55. A very rapid and important degradation of the metabolite was observed (Fig. 3). The half-life time was 3 minutes.

Nature and proportion of adducts

After 2 hours of incubation of N-AcO-[\(^3\)H]AAF with poly d(G-C).poly d(G-C), with poly d(G-br\(^5\)C).poly d(G-br\(^5\)C) and poly d(G-m\(^5\)C).poly d(G-m\(^5\)C), reaction mixtures were treated with water saturated phenol to extract non-reacted carcinogen. Aqueous phase was dialyzed for poly d(G-C).poly d(G-C) against 10 mM MgCl\(_2\), 5 mM bis Tris, 0.1 mM EDTA, pH 7.1 and for poly d(G-br\(^5\)C).poly d(G-br\(^5\)C) and poly d(G-m\(^5\)C).poly d(G-m\(^5\)C) against 50 mM Tris pH 8, 0.25 mM CaCl\(_2\).

Poly d(G-C).poly d(G-C) was heat-denatured and treated with DNase I (Boehringer, Manheim, 1 mg/1 mg polymer) and endonuclease from \( N.\ Crassa \) (Sigma, 6 units/mg polymer) overnight at 37°C. After adjustment to pH 8.0, phosphodiesterase from \( Crotalus\ durissus \) (Boehringer, Manheim, 0.25 units/mg

![Figure 3 - Degradation of N-AcO-[\(^3\)H]AAF during incubation at 37°C in 2x10\(^{-3}\) M Na citrate, 10\(^{-2}\) M NaCl, pH 7.5 buffer containing 1% ethanol.](image)

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polymer) and alkaline phosphatase from *E. coli* III-S (Sigma, 10 units/mg polymer) were added and incubation was continued overnight (15).

Poly d(G-brpentamer 5C).poly d(G-brpentamer 5C) and poly d(G-mpentamer 5C).poly d(G-mpentamer 5C) were hydrolyzed with Microccocal nuclease (Worthington, 25 units/μg polymer) overnight at 37°C and treated afterwards with phosphodiesterase and alkaline phosphatase as poly d(G-C).poly d(G-C) (8).

Aliquots of the three hydrolysates were applied on TLC Silica gel plates. Solvent system was n-butanol-acetic acid-water 100:22:50 (v/v/v). Radioactivity was measured for thin (2 mm) successive zones of the plates. Peaks of radioactivity were obtained at R, values corresponding to the main guanosine adducts in DNA: R, (dGuo-C8-AAF) = 0.67 (16), R, (dGuo-N2-AAF) = 0.54 (17), R, (unidentified adducts) = 0.3 (18). Their relative amounts were evaluated for the three polymers with an accuracy of 5 % (Table II).

**DISCUSSION**

The results presented here above show kinetics of reaction of N-AcO-AAF with dCMP, native DNA, poly d(G-C).poly d(G-C), poly d(G-brpentamer 5C).poly d(G-brpentamer 5C) and poly d(G-mpentamer 5C).poly d(G-mpentamer 5C) which are different in their rate and in the maximum degree of carcinogen binding. In all cases binding levels off quite fast (at around 30 minutes) because of the observed fast degradation of N-AcO-AAF in the reaction conditions. Therefore the maximum degree of binding becomes, in fact, the direct consequence of the reaction rate in the first minutes of reaction. In the following comparison of kinetics of reaction for the different products we shall, thus, limit ourselves to the very beginning of the kinetics, characterized by the slope at the origin of the curve.

We shall discuss our results in terms of steric accessibility and electrostatic potential minima at the reactive sites, the two parameters considered as determinant for the reactivity towards carcinogens (1,2,5).

**Table II - Relative proportions of main adducts in the studied polymers as evaluated from radioactivity of hydrolysates chromatograms (accuracy 5 %).**

<table>
<thead>
<tr>
<th>Adduct</th>
<th>DNA (from (18))</th>
<th>Poly d(G-C).poly d(G-C) in B-form</th>
<th>Poly d(G-brpentamer 5C).poly d(G-brpentamer 5C) in Z-form</th>
<th>Poly d(G-mpentamer 5C).poly d(G-mpentamer 5C) in Z-form</th>
</tr>
</thead>
<tbody>
<tr>
<td>dGuo-C8-AAF (Rf=0.67)</td>
<td>74 %</td>
<td>76 %</td>
<td>82 %</td>
<td>73 %</td>
</tr>
<tr>
<td>dGuo-N2-AAF (Rf=0.54)</td>
<td>10 %</td>
<td>12 %</td>
<td>4 %</td>
<td>12 %</td>
</tr>
<tr>
<td>Unidentified (Rf=0.3)</td>
<td>16 %</td>
<td>12 %</td>
<td>14 %</td>
<td>15 %</td>
</tr>
</tbody>
</table>
The highest reactivity of dGMP can be explained by the total accessibility of C8-position of guanine to the carcinogen. The lower reactivity of guanine in the polymers is certainly related to the much lower steric accessibility of the reactive sites. However, important electrostatic potential minima at the reactive sites, due to interaction of dGMP in the polyelectrolyte allow the reaction.

Our results show a higher reactivity for DNA than for poly d(G-C).poly d(G-C) (ε_DNA = 1.37 ε_G,C). However, both polymers present in the given conditions the B conformation, thus both accessibility and potentials at reactive sites are supposed to be the same. The difference may be due to the different composition in bases. Since A-T pairs are less stable than G-C pairs open regions may transiently appear in the given conditions. This "breathing" may improve the accessibility of guanine to the carcinogen. On another hand, the concentration in phosphates in the DNA experiment is 2.4 higher than in poly d(G-C).poly d(G-C) (for the same concentration in guanosine) which may influence the reaction.

But the principal aim of the present work is the comparison of reactivity of right-handed B-DNA and that of left-handed Z-DNA towards the carcinogen. Our results show twofold faster kinetics (and in consequence a twofold higher final degree of carcinogen binding) for poly d(G-C).poly d(G-C) in B-form than for poly d(G-br^5C).poly d(G-br^5C) or poly d(G-m^5C).poly d(G-m^5C) both in Z-form. The lower reactivity of Z conformation may be explained by the much lower absolute values of electrostatic potential minima at reactive sites of guanine. The calculated values of potentials are -110 Kcal/mol for C8-position and -116 Kcal/mol for N2 position of guanine in screened B-DNA and only -30 Kcal/mol and -40 Kcal/mol respectively for the same positions in screened Z-DNA (1). The better steric accessibility of C8-position (accessible area of 2 Å^2 instead of 1 Å^2) and N2-position (0.3 Å^2 instead of 0.0) seems not sufficient to counterpoise the effect of diminished (in absolute values) electrostatic potential minima at the reactive positions of guanine in Z-DNA as compared to B-DNA.

Certainly, a doubt exists concerning the possible role of bromin atom or methyl group present at position 5 of cytosine on the reactivity of guanosine in the Z-polymers. However, the identity of results for the two polymers bearing substituents very different in electronegativity disfavors the idea of a major role of cytosine substituents on the reactivity of guanosine in poly d(G-br^5C).poly d(G-br^5C) or poly d(G-m^5C).poly d(G-m^5C).

The results concerning the adducts show the presence of the same adducts.
and almost the same proportion of them in poly d(G-C).poly d(G-C)-AAF as in DNA-AAF. The nature and proportions of adducts in poly d(G-br\textsuperscript{5}C).poly d(G-br\textsuperscript{5}C)-AAF and of poly d(G-m\textsuperscript{5}C).poly d(G-m\textsuperscript{5}C)-AAF are the same too.

In conclusion, if we would extrapolate from poly d(C-C).poly d(C-C) and poly d(G-br\textsuperscript{5}C).poly d(G-br\textsuperscript{5}C) or poly d(G-m\textsuperscript{5}C).poly d(G-m\textsuperscript{5}C) to B- and Z-regions eventually coexisting in natural DNA our results would predict a lower susceptibility to carcinogen (N-AcO-AAF) attack of Z-DNA regions. The nature and proportions of adducts would, however, be the same in B- and Z-DNA regions.

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Abbreviations

N-AcO-AAF : N-acetoxy-2-acetylaminofluorene
dGuo-C\textsuperscript{8}-AAF : N\text-(deoxyguanosin-8-yl)-N-acetyl-2-aminofluorene
dGuo-N\textsuperscript{2}-AAF : 3-(deoxyguanosin-N\textsuperscript{2}-yl)-2-acetylaminofluorene.

REFERENCES