DISCRIMINATION BY ANTIBODIES BETWEEN LOCAL DEFECTS IN DNA INDUCED BY 2-AMINOFLUORENE DERIVATIVES

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1. Introduction

It is well known that most if not all carcinogens react with DNA. In vitro studies of the reaction between the ultimate metabolite of a carcinogen and DNA have shown that the covalent linkage of these products to DNA modifies to various extents the conformation of the double helix (reviewed [1–3]). This conformational change may be of importance in the initiation step of the tumorogenic process and it appears thus to be interesting to characterize these modifications. Specific antibodies can be useful to this purpose [4].

Here we present some results on the interactions between purified antibodies and samples of DNA modified in vitro with *N*-acetoxy-*N*-2-acetylaminofluorene (*N*-AcO-AAF), *N*-acetoxy-*N*-2-acetylamino-7-iodofluorene (*N*-AcO-AAIF) and by *N*-hydroxy-*N*-2-aminofluorene (*N*-OH-AF), these compounds being considered as models of the ultimate metabolite of the strong carcinogen *N*-2-acetylaminofluorene. Extensive studies have led to the conclusion that the location of the fluorene ring as well as the size of the destabilized regions were different in DNA–AAF and DNA–AAIF, respectively [5–8]. However, recent experiments are in favor of a close interaction between AF residues and the bases in DNA–AF [9].

From the study of the reactivity of these modified DNAs with antibodies directed against adenosine, cytidine, guanosine–AAF and DNA–AAF, further knowledge on the nature of the destabilized regions is obtained.

2. Material and methods

The synthesis of *N*-AcO-AAF, *N*-AcO-AAIF and *N*-OH-AF, the reaction with DNA and the purifications of the samples have been reported [5,8,9] as well as the purification of the specific antibodies [10–13]. The percentage of modified bases in DNA will be indicated in brackets (for example DNA–AAF (4%)) and when necessary the symbols n and d will be used to indicate that DNA was native or denatured, respectively. The interactions between antibodies and DNAs have been studied by radioimmunoassays as in [9,13]. All experiments were done at 4°C in 0.2 M NaCl, 5 mM Tris–HCl (pH 7.5).

3. Results

The inhibition of precipitation (expressed in %) of [^{3}H]nDNA–AAF or [^{3}H]dDNA are plotted as a function of the logarithm of the concentration of the inhibitor expressed in mol carcinogen modified bases or in mol bases (adenine, cytosine), depending upon the inhibitors (modified DNAs, dDNA) and the antibodies (fig.1).

3.1. *Antibodies to nDNA–AAF (fig.1A)*

Two samples of nDNA–AAF have been used, having 4% and 8% of modified bases, respectively. Within experimental error, the same inhibition was found and only one set of points is given in fig.1A. The precipitation of [^{3}H]DNA–AAF is slightly less

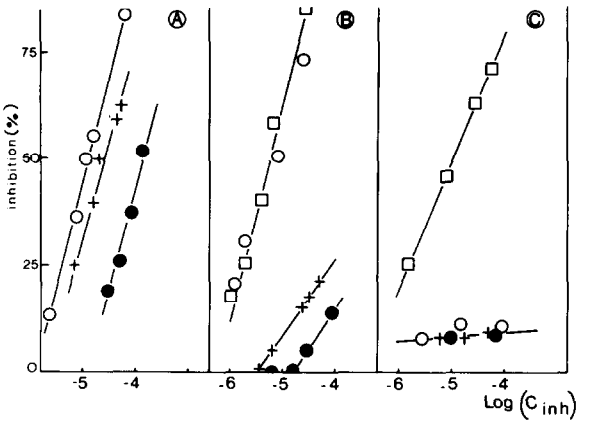

Fig.1. Radioimmunoassays. Percent of inhibition as a function of the logarithm of inhibitor concentration. (A) Competition between antibodies to DNA–AAF, [^{3}H]DNA–AAF and (o) nDNA–AAF (4%), (+) nDNA–AAIF (3.6%), (•) nDNA–AF (3.6%). Concentrations of inhibitor are expressed in mol fluorene-modified bases. (B) Competition between antibodies to cytidine, [^{3}H]DNA and (□) dDNA, (o) nDNA–AAF (5.5%), (+) nDNA–AAIF (3%), (•) nDNA–AF (5.5%). Concentrations of inhibitor are expressed in mol cytosine for dDNA and in mol fluorene-modified bases in all other cases. (C) Competition between antibodies to adenosine, [^{3}H]-dDNA and (□) dDNA, (o) nDNA–AAF (5.5%), (+) nDNA–AAIF (3%), (•) nDNA–AF (5.5%). Concentrations of inhibitor are expressed in mol adenosine for dDNA and in mol fluorene-modified bases in all other cases.

inhibited by nDNA–AAIF than by nDNA–AAF while nDNA–AF is less efficient. Similar results (not shown) were obtained with antibodies to Guo–AAF.

3.2. *Antibodies to cytidine (fig.1B)*

The inhibition of precipitation of [^{3}H]dDNA by dDNA and nDNA–AAF are identical. On the other hand, the inhibition is much smaller with nDNA–AAIF and even smaller with nDNA–AF.

3.3. *Antibodies to adenosine (fig.1C)*

The same low level of inhibition was obtained with the 3 carcinogen-modified DNAs. There is some inhibition which increases very slowly with the concentration of inhibitor. It was not possible to work at higher concentration of inhibitors since the percentages of modified bases are relatively small. At higher concentrations, some non-specific interactions (probably electrostatic interactions) occur between DNA and antibodies.

4. Discussion

In the reaction of *N*-AcO-AAF or *N*-AcO-AAIF with DNA, the major target is the C8 of guanine residues [14,15,21]. The studies of DNA–AAF led to the proposal of the insertion–denaturation model [3,16] or the base displacement model [2,17] in which the fluorene ring is located inside the double helix and the guanine residue outside, conclusion in agreement with studies of modified oligonucleotides [14,18].

Probably due to steric hindrance of the iodine atom, it was also proposed an outside binding model [5,7,8] in which the iodofluorene ring is not inserted between the bases and therefore the fluorene ring in DNA–AAIF is believed to be outside the double helix. Recent results on DNA–AF are in favour of an insertion of the fluorene ring between the bases [9]. However, the sites of guanine involved in the linkage with AF residues are not yet firmly established.

Antibodies to adenosine and to cytidine react with dDNA but not with nDNA [19]. The formation of a complex between antibodies and bases in a polynucleotide is possible only if the bases are accessible [10,19]. On the other hand, antibodies to DNA–AAF or to Guo–AFF have the same affinity for nDNA–AAF and dDNA–AAF [12,13].

Here, various antibodies were used as probes to study the conformation of DNAs which were modified by the reaction with *N*-hydroxy-*N*-2-amino-fluorene and some of its derivatives. The anti-cytidine antibodies bind to dDNA and nDNA–AAF with the same affinity. (We recall that the concentration of dDNA was expressed in cytosine residues and the concentration of nDNA–AAF in AAF residues.) The simplest explanation of this result is that the accessibility of cytosine residues in both DNAs is the same. Thus the covalent linkage of AAF residue to the C8 of guanine residue induces a local denaturation of the double helix. This is in agreement with the insertion–denaturation model [2,3,16].

The affinity of the anti-cytidine antibodies to nDNA–AAIF and to nDNA–AF is much smaller, showing that the cytosine residues are less accessible in these DNAs than in nDNA–AAF. Therefore the covalent binding of AF or AAIF residues is less denaturing for DNA than the covalent linkage of AAF residues. This fits the outside binding model proposed

for DNA–AAIF [5,7,8]. Whether or not the conformational change introduced by AF is similar to that introduced by AAIF is not yet clear. However as far as the kinetics of hydrolysis by endonuclease S_1 from *Aspergillus oryzae* are concerned, DNA–AAIF [7] and DNA–AF [20] behave similarly, both DNAs being much less susceptible to degradation by this endonuclease than DNA–AAF.

The 3 modified DNAs interact very weakly with the anti-adenosine antibodies as compared to dDNA. The binding of the carcinogen even in DNA–AAF does not destabilize a large enough region to expose adenine residues to the antibodies. Formaldehyde unwinding experiments have shown that at 44°C in 42 mM sodium borate buffer (pH 9), 1.05 M formaldehyde, ~ 7 basepairs are disrupted around each carcinogen in DNA–AAF [6]. In our conditions, 4°C and 0.2 M NaCl (pH 7.5) the destabilized region is much smaller.

The antibodies to DNA–AAF (or to Guo–AAF) recognize the dGMP–AAF residues but not dGMP or the fluorene ring alone [12,13]. The antibodies bind to DNA–AAF and to DNA–AAIF with about the same affinity. This can be understood assuming that the antigenic determinant (which is probably a part of the Guo–AAF molecule) is equally well accessible in both DNAs, the fluorene ring being inside the helix and the guanine residue outside (insertion–denaturation model) or vice versa (outside-binding model). This is possible at least with space filling models (CPK). This would also mean that AAF residues are not completely buried in the DNA helix even in the insertion–denaturation model. One could also postulate a dynamic structure in which the modified residues rotate easily around the glycosidic bond and thus the antibodies cannot differentiate between the two models. Finally the inhibition by DNA–AF is less efficient. This probably reflects the smaller affinity of these antibodies to GMP–AF as compared to GMP–AAF [13] and might indicate a different accessibility of Guo–AF residues.

In conclusion, this paper shows that specific antibodies are able to recognize differences in conformational changes induced by the covalent binding of carcinogen to native DNA. It is therefore a useful tool to test for defects induced by a variety of carcinogens.

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