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Detection of intercalation-induced changes in DNA structure by reaction with diethyl pyrocarbonate or potassium permanganate

Evidence against the induction of Hoogsteen base pairing by echinomycin

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Binding of the bis-intercalators echinomycin and *N,N'*-di(9-acridinyl)spermidine or the mono-intercalators 9-aminoacridine and ethidium to DNA induces hypersensitivity of adenines towards reaction with diethyl pyrocarbonate. It is proposed that this hyperreactivity is due to the DNA helix unwinding and extension induced by intercalation, thereby exposing N7 in the major groove, and not as previously suggested to the formation of Hoogsteen base pairing. Hypersensitivity of thymines towards oxidation with permanganate is also induced upon binding of these drugs (especially the bis-intercalators) to DNA. This thymine hyperreactivity is both sequence- and intercalator-dependent, thereby indicating the potential of $KMnO_4$ as a useful probe for analyzing the structure of intercalator-DNA complexes in solution.

Intercalation; DNA conformation; Chemical probing; Hoogsteen base pair; Echinomycin

1. INTRODUCTION

The principal features of the intercalative binding of polycyclic aromatic molecules to double-stranded helical B-DNA are now well described on the basis of X-ray crystallographic and fiber diffraction, as well as numerous physicochemical investigations (reviews [1,2]). Evidence has steadily been accumulating, however, that the conformation of the intercalation complex is strongly dependent on both the intercalator and the DNA sequence constituting and surrounding the intercalation site. In particular, footprinting experiments using DNase I have indicated that changes in the DNA conformation occur outside the reagent intercalation site (e.g. [3–5]). Furthermore, hypersensitivity to reaction with diethyl pyrocarbonate (DEPC) of specific adenine residues of DNA-echinomycin complexes were interpreted in favour of the formation of Hoogsteen base pairing proximal to echinomycin intercalation sites [6]. This feature was originally observed by X-ray crystallographic analyses [7,8].

In the present study we have used the adenine (guanine) specific reagent, DEPC, and the thymine specific reagent, $KMnO_4$, to detect changes in DNA structure/conformation induced in B-DNA by intercalative binding of ethidium, 9-aminoacridine (9-AA), *N,N*-di(9-acridinyl)spermidine (diAcr) and echinomycin (fig.1).

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Abbreviations: 9-AA, 9-aminoacridine; DEPC, diethyl pyrocarbonate; diAcr, *N,N'*-di(9-acridinyl)spermidine

2. MATERIALS AND METHODS

The 232 base pair *Eco*RI-*Pvu*II fragment from the plasmid pUC19 was 3'-end labeled (at the *Eco*RI site) and purified using standard techniques. The reactions were carried out in 100 μl buffer (10 mM sodium cacodylate (pH 7.2), 1 mM EDTA and 0.25 μg calf thymus DNA) containing 1–2 nmol ^{32}P-labeled

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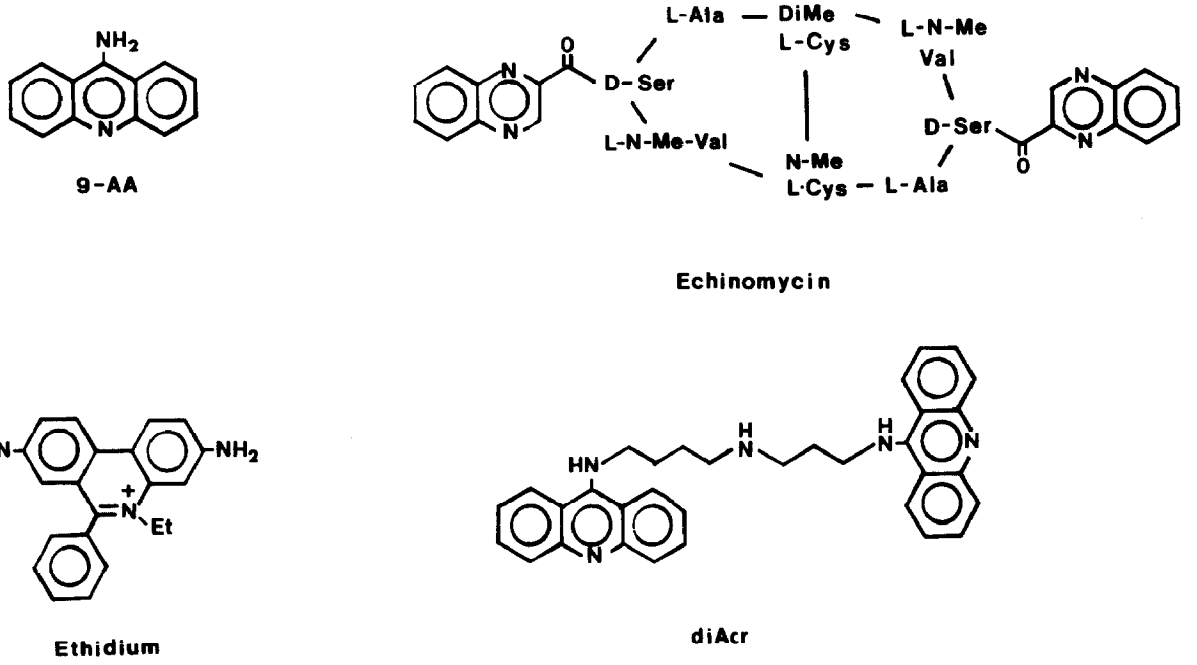

Fig.1. Structures of the intercalators.

fragment and the drug at the desired concentrations (diluted from 10 mg/ml DMSO stock solutions). The samples were left at 20°C for 30 min for equilibration, and were subsequently treated with 2.5 μl DEPC at 20°C for 15 min (occasional shaking) or with 5 μl 50 mM $KMnO_4$ for 90 s at 20°C. The reactions were stopped by addition of 50 μl ice-cold stop-buffer (1.5 M NaAc, pH 7.0, 1 M 2-mercaptoethanol) and immediately precipitated with 400 μl 96% ethanol (precooled to −20°C). The pellets were resuspended in 250 μl 0.3 M NaAc (4°C) and reprecipitated. Following lyophilization the samples were treated with 1 M piperidine at 90°C for 30 min, precipitated with *n*-butanol, dried and finally taken up in 98% formamide loading buffer. The samples were analyzed on 8% polyacrylamide, 50% urea gels run in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.3) followed by autoradiography.

9-AA and ethidium bromide were of commercial grade. DiAcr was prepared as described [9] and echinomycin was a generous gift from Dr Michael Waring.

3. RESULTS

Adenines and guanines in double-stranded B-DNA are quite unreactive towards DEPC. In Z-DNA [10,11] and crusiform loops [12,13], however, the N7-atoms of purines are exposed, thereby accounting for the hyperreactivity towards DEPC of DNA in these conformations. Recently it was shown that the bis-intercalator, echinomycin, induces sequence-dependent DEPC hyperreactivity [6].

The results shown in fig.2A are in accordance with this finding: B-DNA has low DEPC reactivity (lane 2) while the presence of echinomycin induces DEPC reactive sites at some adenines (lanes 12–14). For example, a hypersensitive site is found at the sequence: TCGA*, which is known to be a preferred binding site for echinomycin [5,6], in agreement with the DNase I footprinting results presented in fig.3.

Most interestingly, however, we find that DEPC reactivity of adenines is also induced by a diacridine (fig.2A, lanes 9–11). It should be kept in mind that the effect of echinomycin is localized to specific sites due to the pronounced sequence preference exhibited by this drug when binding to DNA [4,5], whereas the effect of the diacridine is 'spread out' over the entire DNA sequence due to very low sequence specificity. Thus the effect of the diacridine is only detected at higher total concentrations.

Even mono-intercalators such as 9-amino-acridine or ethidium induce DEPC reactivity of the adenines in DNA, although the effect of these drugs is seen at considerably higher concentrations when compared to the bis-intercalators (fig.2A, lanes 3–8).

Permanganate oxidation of thymines in DNA

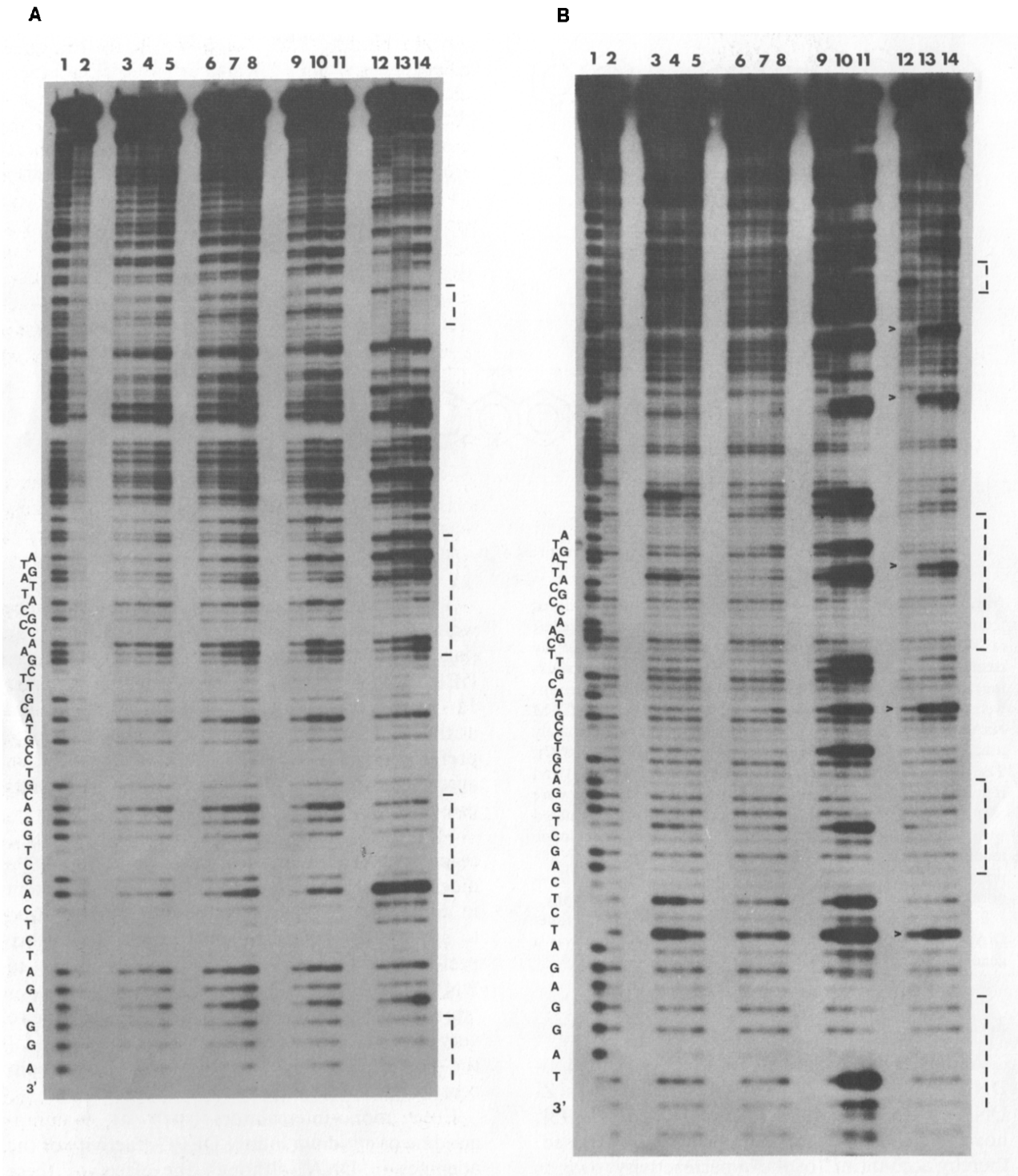

Fig.2. DEPC (A) or $KMnO_4$ (B) probing of DNA-intercalator complexes. Lane 1, A+G sequence reaction; lane 2, control without intercalator added; lanes 3–5, ethidium bromide (0.2, 1 or 5 μg/ml, respectively); lanes 6–8, 9-AA (0.2, 1 or 5 μg/ml); lanes 9–11, diAcr (0.05, 0.25 or 1.25 μg/ml); lanes 12–14, echinomycin (0.05, 0.25 or 1.25 μg/ml). Strong binding sites for echinomycin are indicated by boxes (from the results presented in fig.3).

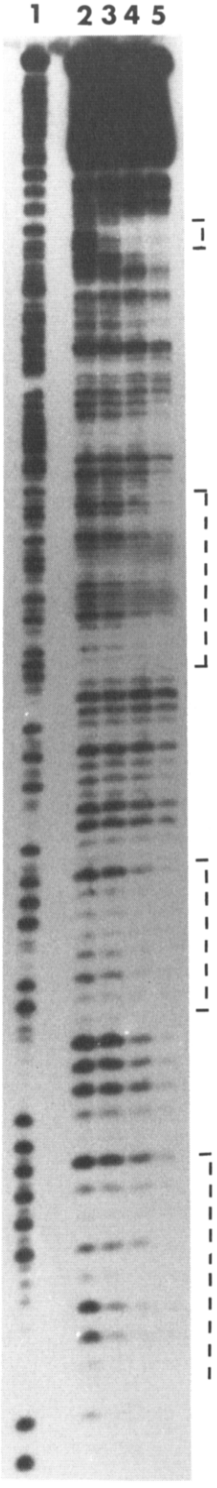

Fig.3. DNase I footprinting of the binding sites of echinomycin on the DNA fragments also used for DEPC and $KMnO_4$ probing. Lane 1, A + G sequence reaction; lanes 2–5, DNase I digestion (1 ng/ml, 5 min at 20°C) in the presence of 0, 0.05, 0.25 or 1.25 μg/ml of echinomycin. Identified strong binding sites are indicated by boxes.

has been used to study DNA conformation upon protein binding [14]. As shown in fig.2B, intercalators, especially bis-intercalators, also induce changes in the DNA conformation which make it reactive towards permanganate oxidation. In the case of diAcr some sequence dependent thymine-reactivity is observed at low drug/DNA ratios (lane 9), but at higher ratios all thymines show comparable reactivity (lanes 10,11). For echinomycin the thymine-hyperreactivity is confined to specific sites which are changing as a function of the drug/DNA ratio (lanes 12–14). 9-AA induces practically no thymine reactivity, whereas ethidium does so but only at non-saturating drug/DNA ratios (lanes 3,4).

4. DISCUSSION

The present results show that the structural changes induced in B-DNA by intercalative drug binding make the adenines susceptible to reaction with DEPC and the thymines reactive towards oxidation with permanganate (most pronounced in case of bis-intercalation). It has been proposed [6] that the purine hypersensitivity towards DEPC induced by echinomycin is due to the formation of Hoogsteen base pairing by exposure of N1 and N3. We find this explanation very unlikely. To form a Hoogsteen base pair from a normal Watson-Crick base pair, the purine has to 'flip' 180°, i.e., the hydrogen bonds have to be broken and the DNA has to breathe ~6 Å in order to accommodate the turning of the base. This change is comparable to that occurring in the B-Z transition of a base pair, which is a slow process [15].

Recent NMR studies [16] have likewise indicated that echinomycin-oligonucleotide complexes only exist in the Hoogsteen conformation in some cases (ACGT but not TCGA). Thus, it is unlikely that the complex of large DNA fragments and the simple intercalators, 9-AA, ethidium or diAcr should acquire the Hoogsteen conformation at all A-T base pairs. Rather we suggest that the increased reactivity of adenines towards DEPC is due to the helix extension and unwinding of the DNA induced by intercalation, which significantly expands the major groove thereby exposing N7 to attack by DEPC, especially since both echinomycin and diAcr intercalates from the minor groove.

A similar argument is valid in the case of permanganate. A widening of the major groove exposes the 5–6 double bond of thymine which is the target of permanganate oxidation [17,18]. It is noteworthy that the increased thymine reactivity is confined to specific intercalators, and to certain sequences, indicating that permanganate is a sensitive probe for sequence and intercalator dependent changes in DNA conformation. In particular it is interesting that most of the permanganate reactive thymines are found at positions (fig.2B, arrows) in between the echinomycin binding sites. This may indicate a 'long-range' effect on the DNA structure induced by echinomycin binding.

It is not clear at present what determines the sequence-dependent thymine hyperreactivity. The width of the major groove could be a decisive factor. If this is the case permanganate could be a useful probe for analyzing sequence-dependent conformations of intercalator-DNA complexes.

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