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DNA bending induced by covalently bound drugs

Gel electrophoresis and chemical probe studies

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Modification of nucleotide residues arising from the covalent binding of a drug or as a result of irradiation with ultraviolet light can induce distortion of the DNA double helix. The purpose of this review is to show that, from investigation of the electrophoretic mobility of the modified DNA fragments, one can deduce whether the distortions behave more as the centers of directed bends or as hinge joints. It is also demonstrated that chemical probes are a complementary tool for the analysis of distortions at the nucleotide level.

1. Introduction

Many mutagens, carcinogens and antitumor drugs bind covalently to DNA and this form of binding is generally assumed to be related to their mechanism of action. Structural information on the distortions induced in DNA by these drugs is of importance in the elucidation of their effects on the regulation of gene expression, the behavior of the DNA replication machinery and DNA repair enzymes. In solution and under normal conditions, natural double-stranded DNA adopts mainly the B-form and behaves as an elastically deformable rod. Several studies support the concept of conformational flexibility of DNA. Changes in experimental conditions and/or topological constraints can induce conformational transitions from the B-form to an unwound species or to complex conformations, such as slippage and cruciform structures, left-handed double helix, triple-stranded helix or to thus far unknown structures. The DNA base sequence is a key parameter for the relative stability of these conformations. Any alteration in the sequence due to physical or chemical modification of the nucleotide residues can affect the B-form, the unusual structures if present and the B-form–unusual structure transitions (for general reviews, see refs. 1–9). Consequently, both short- and long-range interactions between DNA and ions, small molecules or proteins can be greatly modified. In other words, in the cell, signals between DNA and proteins can be changed.

The purpose of this review is to show that, on the basis of studying the electrophoretic mobility of DNA modified by a chemical or physical agent, it can be deduced whether the damaged regions behave more as hinge joints or as the centers of directed bends. The present article is focussed on gel electrophoresis which has been a key method employed in studies of the sequence-directed bending of DNA fragments. Gel electrophoresis

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reveals a global distortion of DNA. In addition, this review describes the approach to the characterization of the distortion at the nucleotide level by means of chemical probes.

2. Electrophoresis

It is well known that on polyacrylamide gels, the electrophoretic mobility of DNA with certain repeated sequences is considerably lower than that of DNA with random sequences. DNA restriction fragments derived from trypanosome kinetoplast minicircles and synthetic DNA fragments, both of which contain short runs of (dA) · (dT), are typical examples. The anomalous electrophoretic behavior of these fragments is attributed to the presence of the short stretches of (dA) · (dT) which are able to confer intrinsic curvature to a DNA molecule in the absence of external constraints (for general reviews, see refs. 10–13). Electron microscopy studies [14,15], rotational diffusion measurements [16,17] and ligase-catalyzed DNA circle formation [18] provide a demonstration of the stable axial curvature of these DNA fragments. It should be borne in mind that, in these fragments, the (dA) · (dT) runs are approximately in phase with the helix screw. The distortions sum coherently which results in a macroscopic curvature and thus facilitates detection. When the (dA) · (dT) runs are separated by a half-integral number of turns, the DNA fragments display normal electrophoretic mobility. This in-phase and out-of-phase effect strongly supports the view that the distortion occurs predominantly in a fixed plane. More generally, a decrease in the DNA end-to-end distance, due to stable curvature of the helix axis or increased isotropic flexibility, leads to decreased gel electrophoretic mobility [19,20]. The phasing assay allows one to distinguish between these two possibilities, since isotropic flexibility is not expected to display a phase dependence [13,21].

Although the circular permutation test [22] has often been used for the study of natural DNA fragments, it appears preferable in the case of modified samples to study the multimers of the ligated duplexes. The experimental procedure can be summarized as follows [10–13]. After ^{32}P 5′

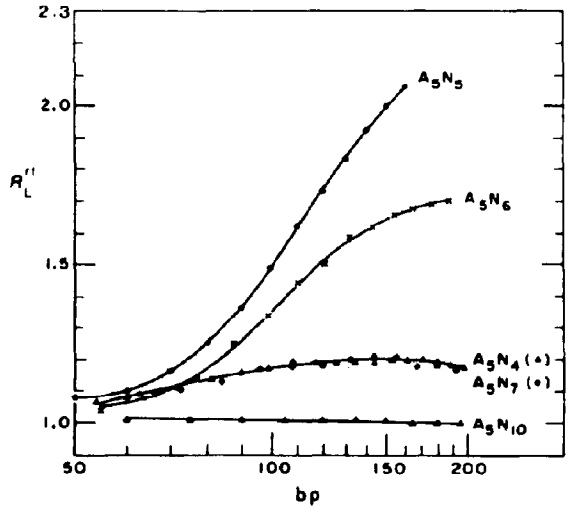

Fig. 1. Plots of the ratio R_L^{II} (at room temperature) of apparent multimer size, determined by comparison with the electrophoresis markers, as a function of the actual chain length. A_5N_5, A_5N_6, etc. denote the multimers of the duplexes in which the sequence phasing of the A_5 tract is 10 (A_5N_5), 11 (A_5N_6), etc. Sequence of monomer A_5N_5, d(GGCAAAAACG)·d(CCCGTTTTTG); sequence of monomer A_5N_6, d(GGCCAAAAACG)·(CCCGTTTTTGG). Both duplexes are constructed with 2 base-pair protruding 5′ ends. (Reproduced from *Nature* 320 (1986) 501–506; courtesy of Macmillan Magazines Ltd.)

end labelling, the complementary oligonucleotides are hybridized. The resulting duplexes must have cohesive ends which allows ligation in a unique polarization to form longer DNA molecules. After ligation of the duplexes, the multimers are run on a non-denaturing polyacrylamide gel. The electrophoretic migration of the multimers is compared to that of size standards. In general, one plots the ratio R of apparent multimer size as a function of actual chain length. An example, given in fig. 1, is relative to the migration of multimers of duplexes containing five A residues in a row [13]. Increasing values of the ratio R indicate an anomalous structure which is interpreted here as DNA bending. The R values are highest for the d $(A_5N_5)_n$ series in which the 10 base-pair phasing nearly matches the expected helix screw of 10.3 base-pairs per turn. R is equal to unity for the $(A_5N_{10})_n$ series in which the A_5 residues are separated by a half-integral number of turns.

It should be emphasized that the complete theory for the migration of DNA fragments through polyacrylamide gels remains to be developed [23] and that interpretation (in terms of base-step angular parameters) of the data relative to DNA fragments containing short runs of (dA)·(dT) is still in progress [23–26]. Nevertheless, all these results provide a useful guide for investigation of DNA fragments modified by a physical or chemical agent.

2.1. *cis-Diamminedichloroplatinum(II)*

In vivo and in vitro, the antitumor drug *cis*-diamminedichloroplatinum(II) reacts preferentially with the N7 of adjacent purine residues in DNA. The major adducts arise from intrastrand cross-links between two adjacent G residues and between an A residue adjacent to a G residue. Single and double-stranded oligonucleotides platinated at a d(GG) site have been studied in detail (for general reviews, see refs. 27–29)). It is worth mentioning that an X-ray study of platinated d(pGpG) crystals has demonstrated the dihedral angle between the G planes to lie within the range 76–87°, thereby completely disrupting base-base stacking interactions [30]. NMR studies [31–34] and molecular mechanics calculations [35] suggest that, in double-stranded oligonucleotides platinated at a d(GG) site, the double helix is distorted, however the platinated G residues are paired with the complementary C residues.

Rice et al. [36] have recently studied the electrophoretic migration of multimers of a 22 base-pair double-stranded oligonucleotide platinated at a single d(GG) site. The autoradiogram of the gel from polyacrylamide electrophoresis of the ligated fragments reveals that the ligation yields a mixture of circular and linear multimers. They all present a strongly anomalous electrophoretic mobility. Analysis of these results leads to the conclusion that the d(GG) adducts bend DNA and that the platinum-induced bend lies within the range 35–45°.

The direction of the platinum-induced bend was determined from the study of DNA fragments obtained by copolymerizing a duplex containing the platinated 22-mer strand with a series of synthetic decamers containing a tract of five A residues. Exploiting the fact that the A tracts bend DNA toward the minor groove, it has been deduced that the platinum residues (located in the major groove) bend DNA toward the major groove [36].

A few studies have been devoted to the distortions induced by the d(AG) adducts. NMR studies of platinated single-stranded oligonucleotides [37,38] and competition experiments with monoclonal antibodies [39] favor a close similarity between the structures of the d(AG) and d(GG) adducts.

The electrophoretic migration of multimers of a 20 base-pair duplex and of those of a 15 base-pair duplex, each duplex containing a single d(AG) adduct, suggests that the d(AG) adducts bend DNA [40]. For a given molecular weight, the multimers of the 20 base-pair duplex migrate more slowly than those of the 15 base-pair species. However, the R ratio for the latter multimers is greater than unity. It is assumed that this value is mainly due to the fact that the helical repeat of the platinated fragments is no longer 10–11 base-pairs/turn.

Two duplexes (20-mer) containing either a single d(AG) single d(GC) adduct were studied [41]. Apart from a single exception, all of the base-pairs are identical (the 5′ G·C base-pair adjacent to the central G·C base-pair is replaced by an A·T base-pair). The multimers of these two platinated duplexes exhibit the same gel migration anomaly. The conclusion is that, from a global point of view, the d(AG) and d(GG) adducts induce the same distortion in the DNA double helix.

2.2. *Thymine photodimers*

Numerous studies have shown that adjacent thymine residues in a DNA molecule form cyclobutane-type photodimers upon irradiation with ultraviolet light and that the pyrimidine dimer is a frequent damage inflicted on DNA by the environment (for general reviews, see refs. 42 and 43). To date, no crystalline structures of oligonucleotides containing dimers have been resolved. An NMR investigation of a duplex containing a

thymine photodimer shows that all the base-pairs are paired although the hydrogen bonds between the A residues and the dimer are weakened [44]. The duplex is in the B-form with some distortion at the level of the thymine dimer, in agreement with the results from molecular mechanics calculations [45,46]. Solution studies on the distribution of topological isomers indicate that the double helix is underwound by 14.3° upon ultraviolet irradiation [47].

Recently, Husain et al. [48] have proposed that thymine dimers bend DNA. The multimers of a 32 base-pair DNA fragment containing a thymine dimer were studied by gel electrophoresis and electron microscopy. The multimers in which the thymine dimers are approximately in phase with the helix screw display an anomalously slow rate of migration in polyacrylamide gels. Repair of the dimers by DNA photolyase abolishes the anomaly. The thymine dimers facilitate the formation of small covalent circles as shown by two-dimensional gel electrophoresis and electron microscopy. The angle of the thymine dimer-induced bend is estimated to be about 30°.

2.3. *Psoralens*

Psoralens are largely used medically in the treatment of vitilago and psoriasis and for the investigation of nucleic acid structure and function. Psoralens are bifunctional photoreagents which form covalent bonds with the pyrimidine bases of nucleic acids. They intercalate into double-stranded nucleic acids and upon irradiation with ultraviolet light (320–400 nm), the binding of both ends of psoralens to opposite strands of nucleic acids results in the formation of covalent interstrand cross-links (for general reviews, see refs. 49 and 50). X-ray crystallographic analysis of a thymine-psoralen mono-adduct [51,52] and NMR investigation of a double-stranded psoralen cross-linked oligonucleotide [53] suggest that psoralen cross-links bend DNA.

Studies employing the electrophoresis of DNA restriction fragments photoreacted with 4,5′,8-trimethylpsoralen [54] and of multimers of duplexes photoreacted with 4,5′8-trimethylpsoralen or 4′-(hydroxymethyl)-4,5′8-trimethylpsoralen [55,56], respectively show migration to be close to that of the unmodified DNA fragments. The conclusions drawn are that DNA is not significantly bent, that it is unwound at each cross-link by about one base-pair and that it becomes stiffer. As pointed out by Haran and Crothers [56], psoralens behave as classical intercalators.

2.4. *N-Acetylaminofluorene*

In vivo, the hepatocarcinogen *N*-acetylaminofluorene binds covalently to DNA. It reacts mainly with the G residues and one type of adduct results from the covalent binding of an acetylaminofluorene (AAF) residue at the C(8) position. In vitro, this type of adduct is formed during the reaction between *N*-acetoxy-*N*-acetylaminofluorene and DNA. Generally, the AAF residues induce local denaturation of B-DNA, the AAF residues being inserted between the base-pairs and the G residues being rotated outside the double helix (insertion-denaturation model or base-displacement model) (for general reviews, see refs. 42 and 57–59). Experiments based on light scattering [60] and linear dichroism [61] using AAF-modified calf thymus DNA suggest that the AAF residues induce hinge points. Solution studies of AAF-modified circular DNA indicate that the unwinding angle of one AAF residue ranges between 20 and 30° [62–64].

On polyacrylamide gels, the electrophoretic migration of multimers of a 20 base-pair or 15 base-pair duplex, both the duplexes being modified at a single G residue, has been investigated [65]. All the modified multimers exhibit diminished mobility. The values of the ratio R are low and moreover are slightly smaller for the multimers of the AAF-modified 20-mer as compared to the 15-mer. The above results together with the fact that the AAF-modified G residues are no longer paired with the complementary C demonstrate that the behavior of AAF-modified G residues is closer to that of hinge joints in comparison with the centers of directed bends. It is worth noting that similar experiments performed with DNA fragments modified by aminofluorene residues lead to the electrophoretic

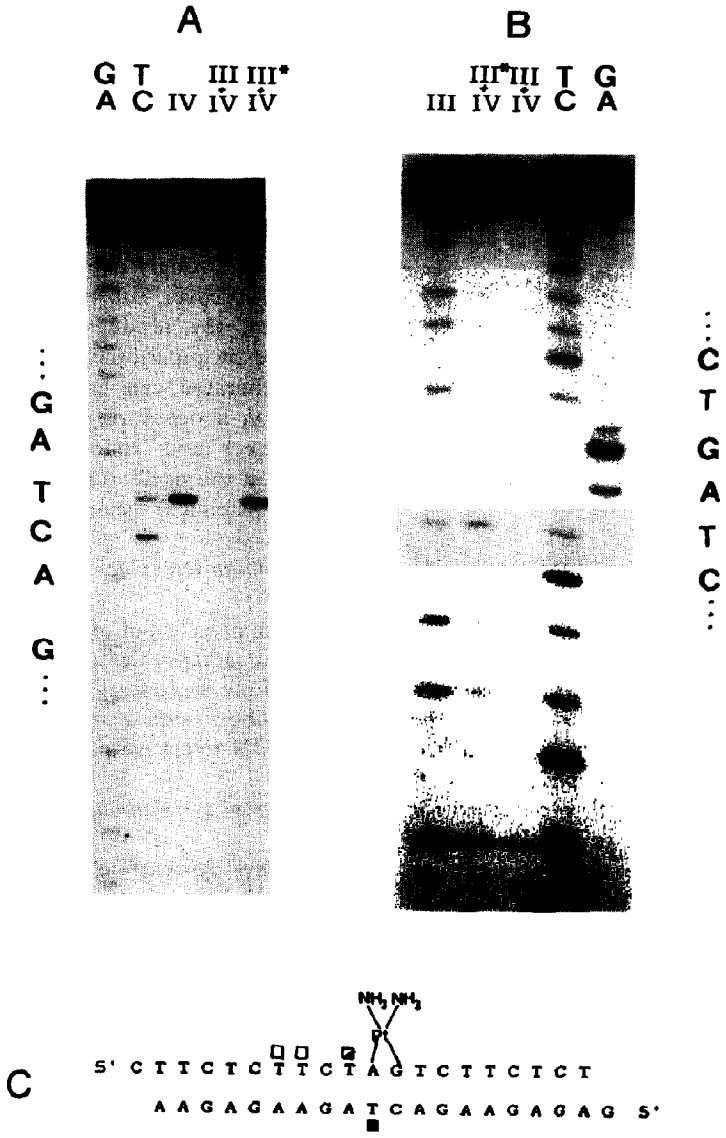

Fig. 2. Piperidine-induced specific strand cleavage at OsO_4-modified bases in the platinated (or unplatinated) duplex. (c) Formula of the duplex. (III) Unplatinated and (III*) platinated upper strands, (IV) lower strand. (A) Reaction of probe with IV, with IV in III + IV and with IV in III* + IV, respectively. (B) Reaction of probe with III, with III* in III* + IV and with III in III + IV. A summary of the changes in chemical reactivity is given in panel C. Filled, half-filled and open symbols indicate strong, intermediate and low hyperreactivity, respectively. (Reproduced from *Biochemistry* 28 (1989) 1454–1461; Courtesy of the American Chemical Society.)

migration being observed to be close to that of the unmodified DNA fragments [65].

The binding of AAF residues to DNA increases the flexibility of the double helix. Local denaturation due to AAF residues reduces the rigidity of DNA. This effect might in part be counterbalanced by the stacking of AAF residues with adjacent base-pairs. The importance of the local

Table 1

Conformational changes induced in DNA by modified nucleotide residues

	Effect	Adduct-induced bend angle (°)
cis-Platinum d(AG) and d(GG) adducts	bending	35–45
Thymine photodimer	bending	30
Psoralen interstrand cross-link	weak or no bending	<10
Acetylaminofluorene	hinge point	
Aminofluorene	no effect	
Apurinic site	hinge point	

denaturation can be estimated qualitatively by the study of duplexes in which one guanine has been removed (apurinic site).

On polyacrylamide gels, the multimers of 15 base pair and 20 base-pair oligonucleotides with an apurinic site migrate more slowly than the corresponding multimers of the unmodified oligonucleotides [65]. The same variation in R ratio as a function of DNA length is observed for the 15-mer and 20-mer ligated to multimers. The conclusion is drawn that the apurinic sites increase the flexibility of DNA. This is in good agreement with two different theoretical approaches showing that, once a single base-pair has been disrupted, DNA becomes very flexible [66,67].

To summarize, several chemically or physically modified DNA fragments have been studied by gel electrophoresis on polyacrylamide gels under non-denaturing conditions. As collected in table 1, the consequences of the modification are very different.

In all such experiments, one obtains a global view of the distortion. In order to provide a better description of the distortion, one requires information at the nucleotide level. For example, does the distortion induced by *cis*-platinum spread over several base-pairs (smooth bend) or merely a few (kink)? How many base-pairs are unpaired after the covalent binding of acetylaminofluorene residues to DNA? Chemical probes can, in part, help in attempting to resolve these questions.

3. Chemical probes

It has been found that the rates of reactivity of several chemical compounds with nucleotide residues are strongly dependent on the structure of DNA. Moreover, subsequent to the specific modifications, the residues are directly sensitive to cleavage by piperidine, and hence the fragments thus generated can be resolved as a ladder of bands on a denaturing gel. In other words, one can detect and characterize a conformational change at the nucleotide level.

Four chemical reagents have been mainly used.

3.1. Osmium tetroxide

In the presence of pyridine, OsO_4 binds through addition across the 5,6 double bond of the pyrimidine residues. It is hyperreactive with pyrimidine residues in single-stranded and distorted DNA relative to B-DNA [68–70].

3.2. Diethyl pyrocarbonate

Diethyl pyrocarbonate carbethoxylates purines at the N7 position. It is hyperreactive with purine residues in single-stranded and Z-DNA as compared to B-DNA [68,71–73].

3.3. Bromoacetaldehyde and chloroacetaldehyde

These compounds which react with N1 and N^6 of adenosine and N3 and N^4 of cytidine are hyperreactive with these residues in single-stranded DNA, in the junctions between B- and Z-DNA and with A residues in Z-DNA as compared to B-DNA [74–77].

3.4. Hydroxylamine

Hydroxylamine reacts with cytosine at the C4 and C6 positions. Hyperreactivity of hydroxylamine with C residues in single-stranded and distorted DNA has been observed [68].

Experimentally, uniquely end-labelled DNA fragments are reacted with the chemical probe. Treatment with piperidine and sample preparation are identical to those for sequencing reactions [78]. The resulting fragments are resolved on a sequencing gel along with the Maxam-Gilbert sequencing ladders [78] of the corresponding unmodified DNA fragments. As an example, the reactivity of OsO_4 with a double stranded oligonucleotide platinated at a d(AG) site is represented in fig. 2. A striking result is that several T residues on the 5'side of the adduct but not those on the 3'side are sensitive to OsO_4. Chloroacetaldehyde does not react with the A residues complementary to these reactive T residues [40] and thus the double helix is distorted to a greater extent, but not locally denatured, on the 5'side of the adduct than on its 3'side. The T residue complementary to the platinated A residue reacts with OsO_4. This demonstrates greater accessibility of the T residue to the probe due to either distortion of the double helix or unpairing of the bases.

Chemical probes have been widely used for studying unusual structures in negatively supercoiled DNAs [68–77]. Recent papers indicate the interest currently being attracted by the use of chemical probes for characterization of duplexes respectively modified by *cis*-platinum [40], acetylaminofluorene [65], aminofluorene [65] and apurinic site [65].

Emphasis should be placed on the qualitative aspects of the chemical probes. OsO_4 reacts with thymine residues in distorted B-DNA. The minimum rotation necessary to render these residues accessible to OsO_4 remains to be determined. Another consideration to be made is that DNA can facilitate some reactions by its surface properties [79–84]. Moreover, DNA is known to be subject to thermal fluctuations resulting in transient conformations with opened base-pairs [85–87]. Both of the aforementioned factors might explain why, in Z-DNA the A residues which are paired with the T residues react with chloroacetaldehyde [77]. Therefore in studying any nucleic acid structure by means of chemical probes, some of the pitfalls can be avoided by using several chemical probes.

4. Conclusion

Experiments based on the electrophoretic mobility of DNA fragments on polyacrylamide gels are undoubtedly convenient and sufficiently sensitive in order to reveal and to characterize conformational changes induced in the double helix by the covalent binding of a drug. Such procedures provide a global view of distortions. Finally, chemical probes represent useful tools for the analysis of distortion at the nucleotide level.

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