Binding of pyrene to DNA, base sequence specificity and its implication

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ABSTRACT

Solubilization as well as spectral studies of pyrene in natural DNA and synthetic deoxypolynucleotide solutions at neutral pH reveal at least two binding modes. Sites I are predominant in native DNA and in poly(dA-dT):poly(dA-dT) whereas sites II are found with denatured DNA and other polynucleotides such as poly(dA):poly(dT) and three different types of guanine containing copolymers which solubilize pyrene to a lesser extent. Spectral comparison with the covalent adducts of trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene (anti-BPDE) and the physical complexes of its tetraols lead to the suggestion of a base sequence specific binding model for this carcinogenic metabolite to account for the puzzling fact that although its physical binding is predominantly intercalative, the covalent adducts appear not to be intercalated. It is speculated that in neutral solutions, intercalation may have little, if any, to do with the chemical lesion of this metabolite to the guanine base of the DNA and may, on the contrary, provide an efficient pathway for detoxification.

INTRODUCTION

Some polycyclic aromatic hydrocarbons (PAHs) have been known to be carcinogenic as well as mutagenic and their pathways consist of metabolic conversion to reactive diol epoxide intermediates which bind covalently to nucleic acids in living cells. The most widely studied PAH has been benzo(a)pyrene (BaP) and there is strong evidence to suggest that (+)trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene ((+)anti-BPDE) is the major ultimate carcinogenic metabolite (1-5). Anti-BPDE (racemate) has been shown to preferentially form covalent bonds to the exocyclic amino group of guanine (6-7). This covalent linkage is quite stereoselective and is generally believed to be the consequence of stereospecific physical binding such as intercalation (8). Consequently, physical binding studies are essential for a

detailed understanding of such chemical lesions.

Physical binding between anti-BPDE and DNA has been demonstrated (9,10) and appears to be predominantly intercalative in nature. This noncovalently intercalated complex is rapidly hydrolyzed to its tetraol (BPT). Due to its stability, this compound (BPT) was used to carry out more detailed binding studies with DNA (11) to mimic the physical interaction of its parent compound. It was found that BPT binds noncovalently to DNA and is characterized by two types of binding sites. Site I exhibits a 10-nm red shift in the absorption spectrum of the pyrene chromophore, a complete quenching of the fluorescence of BPT and a negative linear dichroism suggesting an intercalative mode. Site II shows no shift in the absorption spectrum with respect to that of free BPT molecules, an unchanged fluorescence yield and decay time, and susceptibility to oxygen quenching indicative of an external mode. The spectroscopic properties of the covalent adducts of anti-BPDE with DNA also exhibit outside binding characteristics (12-13). Since physical binding is dominated by the intercalative mode, it is puzzling that the covalent adduct should be non-intercalative. Although the obvious answer is that covalent linkage occurs only at the external binding site, the possibility of geometric rearrangement after covalent lesion at the intercalative site cannot be ruled out.

To help distinguish these two possibilities, we have carried out solubilization studies of pyrene in neutral solutions of synthetic deoxypolynucleotides. The relevance of pyrene to the question at hand stems from the fact that both anti-BPDE and BPT contain a pyrene moiety and the physical binding, especially the intercalative mode, is believed to be hydrophobic in nature (π -electron interaction). Indeed, binding studies of pyrene to natural DNA (14-15) reveal two binding modes very similar to those of BPT. We thus believe that pyrene can mimic the physical binding of BaP metabolites to DNA more closely than BaP itself. The limited availability and the reactive nature of these BaP metabolites make the binding studies with model systems essential.

Since pyrene is only sparingly soluble in water ($\sim 0.3\mu\text{M}$) the conventional methods used for binding studies, such as the use of Scatchard plot, are thus not applicable. However, one can take

advantage of the fact that in the presence of DNA the solubility of pyrene in the aqueous solution is greatly enhanced as a result of binding to this macromolecule. Thus, the extent of solubilization of pyrene in a particular DNA solution offers some quantitative measure of the DNA binding ability of this compound. By using sodium dodecyl sulfate (SDS) to dissociate pyrene from DNA, we have recently developed a simple method which not only gives more reliable results on the pyrene solubility in DNA solutions, but also makes possible the direct comparison of pyrene spectra before and after dissociation. In fact, the most telling results of our polynucleotide studies are obtained by just such spectral observations. The two binding modes which were characterized by a 10-nm versus no shift in pyrene absorption spectra on binding (type I and II, respectively) can now further be characterized by hyperchromicities of 150% versus 30% upon dissociation (15). The absorbance at the characteristic maximum of 336.5 nm after dissociation will be used to quantify pyrene in a DNA solution.

Since pyrene is a planar molecule, it is optically inactive when free in the solution. However, upon binding to a dissymmetric environment such as DNA, strong Cotton effects can be induced in the pyrene spectral region. The circular dichroic (CD) measurements as well as some melting data will be presented to complement the absorption spectral observations. The results of these pyrene studies coupled with spectral comparison with the covalent adducts of anti-BPDE and the physical complexes of its tetraols will then be used to propose a binding model for the anti-BPDE+DNA system.

MATERIALS AND METHODS

The polynucleotides and the extinction coefficients used for their concentration determination are summarized as follows:

Polynucleotide	Source	Lot No.	ϵ (nm)
poly(dA-dT):poly(dA-dT)	P-L Biochem.	658/92	6,600(262)
poly(dG-dC):poly(dG-dC)	P-L Biochem.	692/62	8,400(255)
poly(dA-dC):poly(dG-dT)	Boehringer	1320405	6,500(260)
poly(dA):poly(dT)	P-L Biochem.	648/60	6,000(260)
poly(dG):poly(dC)	Sigma	71f-0134-1	7,400(253)

To ensure double helical formation at room temperature at which all the measurements were made, the polynucleotides were dissolved in 0.01 M NaCl and the pHs were determined to be between 6 and 8.

Lyophilized calf-thymus DNA was purchased from Sigma Chemical Co. and deproteinized according to the procedure described in (16). The stock solution was then dialyzed against double distilled water for three 18-hr periods. The concentration of the stock solution was determined by using $\epsilon(260\text{nm})=6,550 \text{ M}^{-1} \text{ cm}^{-1}$ per phosphate and other DNA concentrations were obtained by dilution and appropriate amount of NaCl added to achieve the desired salt concentration. Denatured DNA solutions were prepared by heating in a boiling water bath for thirty minutes and rapidly cooling with an ice-water mixture.

Pyrene was purchased from Eastman Kodak and used without further purification. Anti-BPDE was provided by the NCI Chemical Repository and the solutions of desired concentrations were prepared using tetrahydrofuran (THF) as the solvent. SDS was obtained from the Pierce Chemical Co. An extinction coefficient of $45,000 \text{ M}^{-1} \text{ cm}^{-1}$ at 336.5 nm in 1% SDS solution was used for all pyrene concentration determinations.

DNA solutions saturated with pyrene were prepared by placing solid pyrene in a vial containing 3-5 mL of desired solution. The mixture was then stirred with a micro-spinbar in a refrigerator for at least two days. The solution was then centrifuged for clarity and the supernatant transferred to an ultracentrifuge tube. The solution was further centrifuged at 25,000 rpm for 30 minutes in a Beckman L5-50B preparatory ultracentrifuge to separate out any residual solid. A 1.25-mL portion of this transparent supernatant was then placed in one compartment of a tandem cuvette while the other compartment was filled with an equal volume of 2% SDS solution. An absorption spectrum was then measured to show the characteristic spectral features of pyrene bound to DNA. The solutions were then mixed by shaking and a spectrum was again taken to show the features of the dissociated pyrene. The characteristic absorption maximum at 336.5 nm was then used to calculate the total pyrene concentration in the solution. The validity of Beer's law for this peak as well as a

more detailed procedure for solubilization studies have been described earlier (15).

The anti-BPDE+DNA covalent adducts were prepared by adding 3 μ L of 20 mM anti-BPDE in THF to 3 mL of DNA solutions. Five extractions with 20 mL each of ether were made in order to remove the physically bound BPT after the mixtures were incubated at room temperature for 30 minutes.

All absorption spectra were measured by a Cary 210-plus spectrophotometric system. Melting experiments were performed in stoppered quartz cuvettes by using the "chart" utility of the temperature readout accessory. A 0.2°C per minute heating rate was maintained by a Neslab RTE-8 refrigerated circulating bath and a EPT-4RC temperature programmer. Circular dichroism (CD) spectra were measured with a JASCO-500A recording spectropolarimeter at room temperature.

RESULTS

Spectral Characteristics of Pyrene in DNA Solutions

As stated in the introduction earlier studies with natural DNA have indicated the existence of two binding modes for pyrene, type I and II, with the former being characterized by a 10-nm red shift upon binding while little or no shift is observed for the latter. This is illustrated in Fig. 1 for pyrene solubilized in aqueous 1.2 mM native and denatured DNA solutions. It is apparent that the duplex DNA solubilizes more pyrene than the denatured form and the type I pyrene exhibits vibronic maxima at 344 and 328 nm, in contrast to those of 334 and 318 nm of the type II. Upon mixing with an equal volume of 2% SDS, the bound pyrenes are dissociated from the macromolecules and indeed both solutions now exhibit the characteristic 336.5 nm bands for pyrene in 1% SDS whose intensities were used to obtain the pyrene solubilities of 11 and 3 μ M in 1.2 mM native and denatured DNA solutions, respectively.

No salts were added in order to minimize residual duplex formation in the denatured solution. Since the pyrene solubility is strongly dependent on the ionic strength (14-15), only the native DNA solution without salt is shown for comparison. The predominance of the duplex form at room temperature for this aqueous DNA solution is indicated by a 37°C melting

Fig. 1 . Absorption spectra for pyrene solubilized in aqueous 1.2 mM native and denatured DNA solutions using a tandem cuvette containing two 4-mm path length compartments. One side is filled with 1.25 mL of 2% SDS solution and the other with an equal volume of pyrene-saturated polynucleotide solution. Native DNA : before(---) and after(....) mixing. Denatured DNA : before(—) and after(— · —) mixing.

temperature and a hyperchromicity of 30%, in addition to having spectral characteristics similar to the other DNA solutions containing NaCl.

Aside from the indicated spectral shift, one other distinguishing feature between these two binding modes is the difference in the hyperchromicity resulting from such SDS dissociation (the percent amplitude increase of this 336.5 nm peak compared to the longwavelength maximum (344 or 334 nm) of the bound spectrum). A striking 160% hyperchromicity for the duplex versus that of 50% for the denatured DNA is clearly evident from the spectra. The broadness of the bound spectrum in the denatured solution, however, indicates that a substantial contribution from the residual duplex still exists in this solution and may account for the 50% hyperchromicity as compared to a mere 30% in the more dilute denatured DNA solutions (15). This suggests that the solubility determined may have overestimated the binding ability of the single stranded form.

Solubilization Studies of Pyrene in Polynucleotide Solutions

The spectra for pyrene solubilized in various synthetic polynucleotide solutions are shown in Fig. 2. It is readily apparent that aside from having the largest amplitude, the pyrene spectrum in poly(dA-dT):poly(dA-dT) exhibits the spectral characteristics of the native DNA, i.e. the 344 and 328 nm maxima,

Fig. 2 . Spectral characteristics for pyrene solubilized in various deoxypolynucleotide solutions with 0.01 M NaCl. Measured with a tandem cuvette prior to mixing with SDS.

(—) 0.27 mM poly(dA):poly(dT); (---) 0.36 mM poly(dG):poly(dC); (···) 0.27 mM poly(dA-dC):poly(dG-dT); (-·-·-) 0.24 mM poly(dG-dC):poly(dG-dC); (- - - - -) 0.39 mM poly(dA-dT):poly(dA-dT).

in distinct contrast to those of the other polynucleotides which exhibit features of the denatured DNA, i.e. the 334 and 318 nm maxima. These points are more dramatically illustrated in Fig. 3 with the inclusion of spectra after dissociation by the SDS. The spectral similarity between Fig. 1 and Fig.3 with poly(dA-dT):poly(dA-dT) and the native DNA on the one hand and the other deoxypolynucleotides typified by poly(dG-dC):poly(dG-dC) and the denatured DNA on the other is rather striking. A

Fig. 3 . Spectral comparison for pyrene bound to and dissociated from synthetic deoxypolynucleotides. 0.39 mM poly(dA-dT):poly(dA-dT) before(---) and after(-·-·-) mixing with SDS. 0.24 mM poly(dG-dC):poly(dG-dC) before(—) and after(···) dissociation. A tandem cuvette used.

TABLE I

Solubility of Pyrene in Polynucleotide Solutions with 0.01M NaCl

Polynucleotide	[DNA] ^a (M _p , mM)	[Pyrene] ^b (M _h , μM)	M _h /M _p (10 ⁻³)	Hyperchromicity ^c (%)
poly(dA-dT): poly(dA-dT)	0.39	11.7	30	160(344nm)
poly(dG-dC): poly(dG-dC)	0.24	3.5	15	60(336nm)
poly(dA-dC): poly(dG-dT)	0.27	2.1	7.7	70(336nm)
poly(dG): poly(dC)	0.36	1.0	2.9	30(336nm)
poly(dA): poly(dT)	0.27	0.8	3.0	20(334nm)

a. Concentration expressed per phosphate.

b. Concentration determined with $\epsilon(336.5\text{nm}) = 45,000 \text{ M}^{-1} \text{ cm}^{-1}$ in 1% SDS solution.

c. Percentage increase in intensity at 336.5nm maximum after SDS dissociation as compared to the peak intensity indicated in the parenthesis when bound.

hyperchromicity of 150% upon dissociation for pyrene in poly(dA-dT):poly(dA-dT) and 60% for that of poly(dG-dC):poly(dG-dC) are almost identical to the corresponding native and denatured DNA solutions. It is thus clear that pyrene binds to poly(dA-dT):poly(dA-dT) predominantly with type I mode but to other polynucleotides with type II characteristics.

The quantitative pyrene solubility results of these deoxypolynucleotide solutions are extracted from the 336.5 nm intensities and summarized in Table I along with the hyperchromicity after dissociation. It is seen that poly(dA-dT):poly(dA-dT) solubilizes pyrene an order of magnitude more than its sequence isomer poly(dA):poly(dT) as well as poly(dG):poly(dC) and about 2-4 times higher than poly(dG-dC):poly(dG-dC) and poly(dA-dC):poly(dG-dT). Since all the alternating deoxypolynucleotides used in this study are purine-pyrimidine copolymer in each strand, it appears that deoxypolynucleotides with alternating purine-pyrimidine bases bind more pyrene than homo-deoxypolynucleotides. With the total pyrene concentration determined by this method and assuming the measured

pyrene solubility in water of $0.3 \mu\text{M}$ to be that of the free pyrene concentration, one can obtain very rough estimates of the binding constants with the formula $K = [\text{bound pyrene}] / [\text{free pyrene}][\text{DNA}]$, where the unbound DNA concentration is in base-pair. The estimated values are: 2, 0.8, and $0.4 \times 10^5 \text{ M}^{-1}$ for poly(dA-dT):poly(dA-dT), poly(dG-dC):poly(dG-dC), and poly(dA-dC):poly(dG-dT), respectively and $0.1 \times 10^5 \text{ M}^{-1}$ for both poly(dG):poly(dC) and poly(dA):poly(dT). These are to be compared with $1 \times 10^5 \text{ M}^{-1}$ obtained for the natural duplex DNA (15).

The extent of the type I binding in each polynucleotide is indicated by the absorbance at 344 nm in the bound spectrum. This can be seen more clearly from the difference spectrum between the bound and the SDS dissociated spectra. The results are presented in Fig. 4. Again the overwhelming importance of the type I mode in poly(dA-dT):poly(dA-dT) is obvious as seen from its disproportionately large 344 nm difference peak. Minor but significant contribution from this mode in poly(dG-dC):poly(dG-dC) and poly(dA-dC):poly(dG-dT) (represented in the figure by the latter) is seen by a small difference band at 344 nm in contrast to none seen in poly(dA):poly(dT) and poly(dG):poly(dC) (represented in the figure by the former). This minor type I contribution for these two alternating polynucleotides may account for the observed 60-70% hyperchromicity upon dissociation as compared to the 20-30% for the two homopolynucleotides.

The relative binding abilities of type I versus type II modes for these deoxypolynucleotides can also be demonstrated by the spectral comparison between pyrene solubilized in polynucleotide solutions with and without salt added. In the absence of salt, the polynucleotides at these concentrations are presumably existing mainly in the single stranded form and indeed the 334 nm band predominates even in the poly(dA-dT):poly(dA-dT) solution (although the 344 nm shoulder is clearly visible in this solution indicating some residual duplex contribution). Upon addition of NaCl ($\sim 0.1\text{M}$) to ensure duplex formation, however, a spectral shift to 344 nm occurs only for poly(dA-dT):poly(dA-dT) (results not shown) indicating strong preference for the type I mode in this polynucleotide but not the others.

Fig. 4 . Difference spectra for pyrene in deoxypolynucleotides before and after dissociation. Spectrum after mixing with SDS subtracted from that of before mixing. (---) 0.39 mM poly(dA-dT):poly(dA-dT); (···) 0.27 mM poly(dA-dC):poly(dG-dT); (—) 0.27 mM poly(dA):poly(dT). All spectra are normalized to 0.3 mM.

The relative unimportance of the type I mode in the deoxypolynucleotides other than poly(dA-dT):poly(dA-dT), as indicated by the pyrene-saturated solutions, raises the question as to whether this is the consequence of lower binding constants for type I in these polynucleotides or that the type II binding sites are simply more numerous. Binding experiments using a tandem cuvette with one compartment filled with water saturated with pyrene and the other filled with a polynucleotide solution were carried out to answer this question. A spectrum for the free pyrene was measured before the mixing and a pyrene bound spectrum was again taken after the solutions were mixed. The difference spectrum obtained from after and before mixing for each polynucleotide solution is given in Fig. 5. The rationale for this experiment being that since the pyrene concentration in water is extremely low, there are presumably more than enough sites in either mode to accommodate all the pyrene molecules and consequently the relative binding constants dictate the extent of binding to these modes. For poly(dA):poly(dT) and poly(dG):poly(dC), no 344 nm difference band is observed, indicating that the binding constant for type I is indeed small in these polynucleotides but for poly(dG-dC):poly(dG-dC) and poly(dA-dC):poly(dG-dT), sizable 344 nm maxima are observed, albeit smaller but comparable to that of poly(dA-dT):poly(dA-dT). This indicates that the binding constants for the type I mode in

Fig. 5 . Difference spectra for pyrene in water before and after binding to deoxypolynucleotides. A tandem cuvette was used with one compartment filled with 1.25 mL of water saturated with pyrene and the other with an equal volume of polynucleotide solution. Spectrum before mixing was subtracted from that of after mixing. Smoothed out curves were drawn. (----) 0.39 mM poly(dA-dT):poly(dA-dT); (···) 0.27 mM poly(dA-dC):poly(dG-dT); (—) 0.24 mM poly(dG-dC):poly(dG-dC); (- - -) 0.27 mM poly(dA):poly(dT).

these polynucleotides are comparable or even larger than those of the type II mode. Thus, to reconcile with the results of the solubilization studies, it is suggested that the type I sites in poly(dG-dC):poly(dG-dC) and poly(dA-dC):poly(dG-dT) are rather limited and are easily outnumbered or blocked by the type II sites. This assertion is reasonable if one assumes that the type II binding is the nonspecific trapping of pyrene in the minor groove of the DNA and the type I is that of intercalation.

In addition to a 10-nm red shift on the pyrene spectrum upon binding to poly(dA-dT):poly(dA-dT), this strong binding can also be demonstrated by the considerable spectral difference in the 260 nm region before and after dissociation. As is seen in Fig. 6, a smooth 260 nm DNA band with no discernable shoulder is apparent. Upon dissociation with SDS, however, peaks at 242, 263, and 273 nm, characteristic of pyrene spectrum are now clearly evident, a definitive indication that dissociation has occurred. The disappearance of vibronic fine structure upon binding is another strong support for the tight binding at these sites.

No studies were made with poly(dA-dG):poly(dC-dT) due to its unavailability. Since this polynucleotide contains pyrimidine on one strand and purine on the other, it is expected that the binding behaviour will be similar to those of homopolymers.

Fig. 6 . Spectral features in the DNA region are compared for pyrene solubilized in 0.39 mM poly(dA-dT):poly(dA-dT) before (—) and after (···) dissociation with SDS.

Induced Circular Dichroism

Positive CD bands were induced at 344 and 328 nm for pyrene in native DNA solution as shown in Fig. 7, coinciding with the absorbance maxima of the type I mode. These Cotton effects disappear on addition of SDS, confirming the pyrene dissociation from DNA. In denatured DNA, pyrene induces only very weak CD maxima at these same wavelengths indicating the presence of residual duplex and optical inactivity of type II binding. This is to be expected if this mode is nonstereospecific.

Of the polynucleotides studied, only poly(dA-dT):poly(dA-dT) exhibits sizable induced CD whose molar ellipticities are in fact positive and larger than those of the native DNA as is apparent

Fig. 7 . Induced CD spectra (expressed in molar ellipticity) for pyrene in 4.2 mM DNA (---); 0.3 mM poly(dA-dT):poly(dA-dT) (···); 0.39 mM poly(dA-dC):poly(dG-dT) all contain 0.01M NaCl with neutral pH.

from Fig. 7. Poly(dA-dC):poly(dG-dT) shows small but detectable Cotton effects with the same sign indicating some type I binding as noted earlier. These observations conform with the absorption spectral results and give strong evidences for the existence of the base sequence and stereospecific nature of type I binding.

Thermal Denaturation Experiment

Melting measurements were also carried out in an attempt to differentiate these two distinct binding modes. The melting curves for the poly(dA-dT):poly(dA-dT) and poly(dA):poly(dT) and their pyrene-saturated solutions in 0.01 M NaCl are shown in Fig. 8. The melting temperatures for the poly(dA-dT):poly(dA-dT) and its pyrene saturated solutions are 41.6°C and 48.7°C, respectively whereas the corresponding ones for the poly(dA):poly(dT) are 52.1°C and 55.8°C. Thus pyrene binding to poly(dA-dT):poly(dA-dT) resulted in a 7.1°C increase in the melting temperature, almost twice that of 3.7°C induced by binding to poly(dA):poly(dT). It is tempting to correlate the relative increase in the melting temperature upon pyrene binding to its binding affinity. This, however, may not be valid. Although the melting temperature increase in poly(dA):poly(dT) is only 3.7°C, it is rather significant since it indicates that pyrene does indeed bind to poly(dA):poly(dT) despite the absence of any spectral shift. Furthermore, if account is taken of the fact that this polynucleotide solubilizes pyrene an order of magnitude less than its sequence isomer, it is clear that the binding of pyrene stabilizes duplex structure of poly(dA):poly(dT) more than that of poly(dA-dT):poly(dA-dT).

To show that the release of pyrene is intimately related to the duplex disruption, the absorbance at 344 nm was also monitored for pyrene in poly(dA-dT):poly(dA-dT). Indeed, the melting temperature of the pyrene release is identical to that of the polynucleotide denaturation as can be seen from the crossover of the two melting curves (Fig. 8). One feature worth noting is the steady but non-cooperative release of pyrene from the type I sites in the premelting region of the polynucleotide.

The absorption spectra for pyrene before and after melting are

Fig. 8. Thermal melting curves. (O) 0.39 mM poly(dA-dT):poly(dA-dT) monitored at 262nm; (●) pyrene-saturated 0.39 mM poly(dA-dT):poly(dA-dT) monitored at 262nm; (◀) 0.27 mM poly(dA):poly(dT) monitored at 258nm; (▶) pyrene-saturated 0.27 mM poly(dA):poly(dT) monitored at 258nm; (◻) pyrene-saturated 0.39 mM poly(dA-dT):poly(dA-dT) monitored at 344nm. All solutions contain 0.01M NaCl and heated at a rate of 0.2°C/min. α is defined as $(A_t - A_l) / (A_h - A_l)$, where A_h , A_l , and A_t are absorbances at high, low, and temperature t , respectively. For the 344nm melting curve the h and l are reversed.

shown in Fig. 9. The nearly 100% increase in intensity due to band narrowing as well as the wavelength shift after melting are quite apparent. It is noteworthy that this intensity corresponds to approximately 7 μ M pyrene in the denatured solution, more than 20 times its solubility in water, a strong evidence for the existence of binding to the single-stranded DNA.

Thermal denaturation measurements were not carried out for the guanine containing polynucleotides due to their high melting temperatures.

Spectral Characteristics of Anti-BPDE

It is of interest to see if the predominance of the type I binding of pyrene to poly(dA-dT):poly(dA-dT) is also exhibited by anti-BPDE, the suspected ultimate carcinogenic metabolite. Unfortunately the quantities available were not sufficient to

Fig. 9 . Absorption spectra of pyrene in 1.2 mM aqueous DNA solution before (—) and after (---) thermal denaturation. Measured in 2 mm pathlength cuvettes.

carry out the described solubilization studies. Spectral observations, however, were made by adding small amounts of anti-BPDE in THF to various polynucleotide solutions. As anti-BPDE rapidly hydrolyzes to BPT in DNA solution and only a small fraction ends in covalent bonding, the spectra observed should represent the binding characteristics of anti-BPT. Indeed spectral observations with anti-BPT give identical results. Spectra for anti-BPDE in 1.2 mM native and denatured DNA along with poly(dA-dT):poly(dA-dT) and poly(dG-dC):poly(dG-dC) solutions are shown in Fig. 10. A 353 nm band is apparent in the native DNA

Fig. 10 . Spectral characteristics of anti-BPDE in various polynucleotide solutions. Anti-BPDE in THF was added to DNA solutions. (···) 1.2 mM DNA/anti-BPDE=60, 0.1% THF; (-·-·-) 1.2 mM denatured DNA /anti-BPDE=60, 0.1% THF; (—) 0.3 mM poly(dA-dT):poly(dA-dT)/anti-BPDE=38, 0.4% THF; (---) 0.3 mM poly(dG-dC):poly(dG-dC)/anti-BPDE=38, 0.4% THF.

Fig. 11 . Absorption spectra of covalently bonded anti-BPDE in 0.3 mM poly(dG-dC):poly(dG-dC)/0.01 M NaCl (···); 1.2 mM aqueous DNA solution (- - -); 1.2 mM denatured DNA solution (- · - ·); 0.3 mM poly(dA-dT):poly(dA-dT)/0.01 M NaCl (—). 3 μ L of 20 mM anti-BPDE in THF was added to 3 mL of DNA solution and then extracted five times with 20 mL of ether after the mixture was let sitting for 30 minutes at room temperature.

solution, 10-nm red shifted from that of denatured DNA, again suggesting the prominence of type I binding in the duplex form. Of the polynucleotides tested, only poly(dA-dT):poly(dA-dT) shows a similar maximum, indicating that the preference for the type I mode in this polynucleotide is also observed for anti-BPT. The predominance of type II binding for poly(dG-dC):poly(dG-dC) is also evident from the spectral similarity with the denatured DNA. The presence of the 343 nm peak, characteristic of free BPT, in the native DNA spectrum is the consequence of slightly higher solubility of this hydroxyl containing metabolite in the aqueous phase as well as the presence of 0.1% THF. The more prominent presence of this maximum in poly(dA-dT):poly(dA-dT) spectrum is due to a higher THF content of 0.4% in this solution.

The extent of covalent adduct formation as well as its spectral characteristics are also assessed by subjecting a 3-mL anti-BPDE+DNA mixture to five 20-mL ether extractions. Results for the two polynucleotides as well as the native and denatured DNAs are presented in Fig. 11. It is seen that the denatured DNA covalently binds almost as much anti-BPDE as the native DNA, although the spectral maxima of the former are about 3-nm red shifted (349 vs 346 nm). Poly(dA-dT):poly(dA-dT) forms negligible covalent adduct and poly(dG-dC):poly(dG-dC) exhibits significant

chemical lesion with anti-BPDE as expected. It is noteworthy that although poly(dG-dC):poly(dG-dC) exhibits little type I physical binding, its spectral characteristics of the chemical adducts are identical to those of the native rather than the denatured DNA. These results taken together suggest that type I physical binding is not essential for chemical adduct formation and the microenvironment surrounding the pyrene moiety covalently bonded to single-stranded DNA is different from its binding to duplex form.

DISCUSSION

Spectroscopic evidence suggests that two binding modes are operative when pyrene binds to DNA in neutral solutions. Type I is characterized by a 10-nm red shift, requirement for duplex DNA, large hyperchromicity for pyrene spectrum upon dissociation with SDS, and large positive induced CD; type II, on the other hand, is characterized by little or no shift in the spectrum, no requirement for the duplex form, small hyperchromicity after SDS dissociation, and little or no induced CD. Sites I are predominant in duplex DNA and in poly(dA-dT):poly(dA-dT) whereas sites II are found with the denatured DNA and other polynucleotides such as poly(dA):poly(dT) and three different types of guanine containing copolymers which solubilize pyrene to a lesser extent. From the analogous spectral characteristics of BPT+DNA physical adducts and comparison with those of covalent adducts, it is reasonable to conclude that type I physical binding is not essential and may be unimportant for the chemical lesion.

As mentioned in the introduction that there are evidences to suggest that for BPT, the type I binding is intercalative while the type II sites are external. Linear dichroism measurements with pyrene (17) also support the notion that the type I binding of pyrene is intercalative in nature. Our results thus seem to indicate that of the ten intercalative binding sites in DNA, the pyrene moiety prefers exclusively the dA-dT (and/or dT-dA) sequence(s) in neutral solutions. The fact that the predominant covalent linkage between the anti-BPDE and DNA occurs at the guanine base as well as the nonessential requirement for the duplex form suggest that the intercalative mode may have little,

if any, to do with the mutagenic pathway of benzo(a)pyrene. A binding model between anti-BPDE and DNA can thus be envisaged: anti-BPDE binds preferentially at the dA-dT (and/or dT-dA) sequence(s) by intercalative mode while for other sequences external modes are favored but with lower affinity. Little or no covalent linkage occurs at most of the intercalative sites and the anti-BPDEs are rapidly hydrolyzed to their tetraols, thus serving as a detoxification process. Covalent lesions occur mainly at the external sites, which are presumably in the minor groove (18) where the 2-amino group of guanine is exposed. Such a model can thus explain the puzzling fact that although anti-BPDE predominantly binds to DNA intercalatively, only a small fraction (~2-6%) ends up with covalent linkage to guanine bases and exhibits external characteristics as all the guanine containing sequences prefer outside binding. Since thymine base quenches about a factor of ten more efficiently than the other three bases (19) and since pyrene moiety predominately intercalates at the dA-dT (and/or dT-dA) sequence(s), the phenomenon of almost complete quenching of pyrene-like fluorescence when BPT binds to DNA can also be nicely accounted for. The stereoselective covalent binding of anti-BPDE may be the consequence of the geometric constraint of the minor groove to allow only certain orientation to be trapped as well as differences in cis versus trans epoxide addition.

Recently Meehan et al. (10) presented evidences to support the non-covalent intercalation of anti-BPDE into the stacked base pairs of DNA and suggested that the covalent binding of this metabolite to DNA is preceded by such an event. Although their experimental findings support our model, their conclusion disagrees with ours. It is noted, however, that their conclusion was partially based on the finding that many intercalating agents display a preferential physical binding to the G-C-rich sequences (20). This is precisely what we have found not to be the case for pyrene moiety in neutral solutions. There are also reports (21-22) which indicate an unwinding angle of 26-30° when anti-BPDE covalently bound to SV40 DNA, suggesting an intercalated chemical adduct. The fact that physically bound anti-BPDE unwinds the helix by half that reported for the covalent complex (10),

however, leaves open the possibility that such large unwinding angle may have its origin in something other than intercalation.

It is interesting to note that the covalent adduct of anti-BPDE with denatured DNA exhibits a bathochromic shift with respect to that of the duplex adduct, underscoring the fact that the external mode of duplex binding may differ from that on the single strands. This is reasonable as pyrene moiety can stack on the bases in the single strand whereas the pyrene chromophore in the minor groove will be little affected by the transition moments of the bases due in part to the distance as well as the near perpendicular orientation of the interacting chromophores. Linear dichroism experiments (11, 23) suggest that in the anti-BPDE + duplex DNA covalent adducts, the pyrene plane makes an angle of 45 degrees or more with the base pair and the fluorescence quenching experiments with acrylamide (23-24) indicate that pyrene is only partially accessible to the solvent. All these are consistent with a model in which the pyrene moiety leans inside the minor groove with an angle much like a ladder leans against a wall. There are also some indications that the hydroxyls at the 7,8-positions in anti-BPDE may impose some steric hindrance towards intercalation (25-26). One may go one step further and speculate that these groups may also have anchoring effects on the ladder-like binding.

Our present results cannot differentiate between the binding abilities of dA-dT and dT-dA sequences. Attempts were made to use dinucleotides pdA-dT and pdT-dA as well as tetranucleotides d(AATT) and d(TTAA) for such comparative studies but without success. Little double helical form exists even at 0°C because of their low melting temperatures. Higher oligomers are needed for such a purpose. Although NMR studies are feasible at the dinucleotide and tetranucleotide levels, we do not have the facilities for such undertaking.

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