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A SPECTROFLUORIMETRIC INVESTIGATION OF CALF THYMUS DNA MODIFIED BY BP DIOLEPOXIDE AND 1-PYRENYLOXIRANE

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Abstract

7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BP diolepoxide, 1) and 1-pyrenyloxirane (2) bind chemically to calf thymus DNA. The fluorescence efficiency of pyrenyl groups in mutagen modified DNA varies appreciably with its conformation and decreases in the order: pyrenes, modified denatured DNA and modified native DNA. A particularly interesting observation is that the fluorescence efficiency of mutagen modified DNA intensifies substantially upon denaturation. Our results suggest that the pyrenyl groups in mutagen modified DNA are intercalated between the base pairs of DNA. Since both 1 and 2 are powerful frame-shifting mutagens for S. typhimurium TA-98, the intercalative covalent binding of these compounds to DNA may provide a molecular basis for their mutagenic activity.

Polynuclear aromatic hydrocarbons are metabolically activated in vivo to oxiranes which will bind chemically to biological macromolecules (1). 7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, commonly known as BP anti-diolepoxide (BPDE, 1) binds selectively to the 2-amino group of guanine in DNA (2-3) and has been implicated in the chemical carcinogenesis of benzo[a]pyrene (4-6). Aryloxiranes are a group of chemicals which possess both mutagenic and carcinogenic activities (7-8). In connection with our interest in chemical carcinogenesis, we synthesized 1-pyrenyloxirane (PO, 2) (9) which is structurally related to 1. Rcently, Drinkwater, Miller, Miller and Yang demonstrated that both 1 and 2 efficiently uncoil the supercoiled SV-40 viral DNA and are powerful mutagens against S. typhimurium TA-98 (8), a strain of bacteria developed by Ames which is particularly sensitive to frame-shift mutagens (10). In our attempt to study the molecular mechanism of their mutagenic activity, we invest-

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Abbreviations: BP, benzo[a]pyrene; BPDE, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; PO, 1-pyrenyloxirane; PO-GMP, N^2-[1-(1-pyrenyl)-2-hydroxy-1-ethyl]-guanosine monophosphate; $\emptyset_f$, fluorescence quantum yield; k_q, rate constant for fluorescence quenching; k_{diff}, rate constant for a diffusion-controlled process.

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igated by fluorescence spectroscopy the interaction of 1 and 2 with native and heated denatured calf thymus DNA and found that the fluorescence efficiency of pyrenyl groups chemically bound to DNA varies appreciably with the conformation of DNA. Particularly, the fluorescence of the pyrenyl groups is attenuated when they are attached to native calf thymus DNA and may be substantially restored upon heat denaturation (Figure). These observations suggest that the major portion of chemically bound mutagens may be intercalated between the base pairs of native DNA, thus providing a molecular basis for their mutagenic action.

Methods

BPDE 1 (11) and PO 2 (9) were prepared in our laboratory. Native calf-thymus DNA was purchased commercially. Buffered solutions of DNA were prepared in deionized distilled water at the given ionic strength, either a cacodylate buffer containing 0.05 M in sodium cacodylate or a phosphate buffer containing 10^{-4} M each in NaH_2PO_4 and Na_2HPO_4 (pH 7.0). Denatured DNA was prepared by heating the buffered DNA solution at 100° for 12 min and then by rapid cooling in an ice bath. Binding of mutagens to DNA was carried out by adding 1 or 2 (18.4-23.6 μmole) in 0.3 ml of methanol to 10 ml of a buffered solution of DNA (600 μg/ml). Clear solutions were obtained with native DNA but not with denatured DNA. The mixture was incubated at 37.5±0.5° for 24 hr in the dark. The unbound mutagen and its hydrolysis product(s) were removed by exhaustive extraction (10-14 times) with buffer saturated ethyl acetate. The binding level of the mutagen to DNA was determined by uv-visible spectroscopy and calculated according to the work of Weinstein and coworkers (12). When pyrene instead of 1 or 2 was used in a control experiment, we found the binding level to be less than 0.01 nmole/ml, i. e., beyond the sensitivity of our instruments. After the denaturation of modified DNA, the solution was again extracted with ethyl acetate to remove any mutagen which may have detached itself from DNA during denaturation. The fluorescence and fluorescence excitation spectra were performed on a Perkin-Elmer MPF-4 Spectrofluorimeter with a thermostatic cell compartment (25.0±0.2°) and corrected spectrum accessories. The fluorescence quantum yields ($\emptyset_f$) of mutagen modified DNA and several reference compounds are listed in the Table.

Results and Discussion

Since the 2-amino group of guanine in DNA is the selective binding site of 1 in DNA (2), we synthesized 3 as the model compound in our spectrofluorimetric analysis (13). We found that the $\emptyset_f$ of pyrenyl groups decrease in the following order: pyrenes, pyrenyloxiranes 1 and 2, 3, modified denatured DNA and modified native DNA. Since aryloxiranes are known to undergo photolytic decomposition (14), 1 and 2 are thus expected to exhibit lower $\emptyset_f$ (0.32-0.36) than their hydrolysis products and pyrene (0.66-0.72). The lower $\emptyset_f$ of 3 is attributed to the intramolecular interaction between the two chromophores (15). The results on modi-

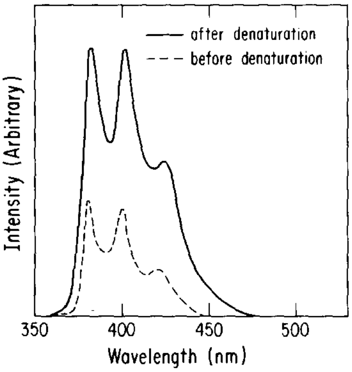
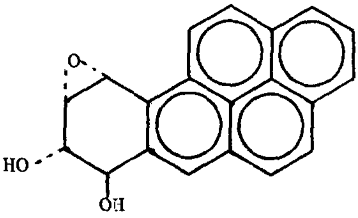


Figure. Corrected Fluorescence Spectra of BPDE 1 Modified Calf Thymus DNA in Phosphate Buffer before and after Denaturation



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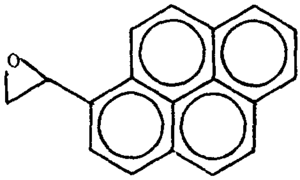

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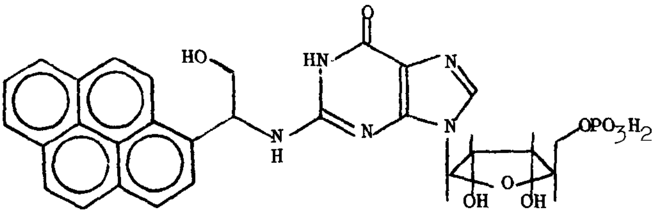

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fied DNA clearly indicate that molecular conformations exert a remarkable effect on the ϕ_f of bound pyrenyl groups, which varies from 22% to 67% of the model compound. A particularly interesting observation is that the fluorescence of mutagen modified DNA intensifies substantially upon denaturation, and the result of one such experiment is illustrated in the Figure. The ϕ_f of modified DNA after denaturation becomes comparable to or exceeds that of modified DNA which has been denatured prior to its treatment with mutagens. We attribute the

Table. Fluorescence Quantum Yields of Aromatic Mutagen Derivatives and Mutagen Modified DNA

Mutagen	Substrate	Method	Medium	Binding level (mutagen:base)	ϕ_f
Pyrene	---	--	ethanol	---	0.72[a]
BPDE, **1**[b]	---	--	ethanol	---	0.32
PO, **2**[b]	---	--	hexane	---	0.36
PO-GMP, **3**	---	--	phosphate[c]	---	0.13
BPDE, **1**	Native DNA	--	cacodylate[d]	1:143	0.028
BPDE, **1**	Denatured DNA	A[e]	cacodylate	1:143	0.054
BPDE, **1**	Denatured DNA	B[f]	cacodylate	1:111	0.051
BPDE, **1**	Native DNA	--	phosphate	1:119	0.030
BPDE, **1**	Denatured DNA	A[e]	phosphate	1:119	0.087
BPDE, **1**	Denatured DNA	B[f]	phosphate	1:106	0.065
PO, **2**	Native DNA	--	phosphate	1:61	0.036
PO, **2**	Denatured DNA	A[e]	phosphate	1:61	0.070
PO, **2**	Denatured DNA	B[f]	phosphate	1:72	0.074

[a] Degassed pyrene in ethanol was used as the secondary standard, B. Stevens and M. Thomaz, *Chem. Phys. Lett.* (1968), 1, 535-536. The accuracy of ϕ_f is ±10% of values given. [b] The ϕ_f of the corresponding glycol obtained from the hydrolysis of the oxirane is similar to that of pyrene, 0.66-0.71. [c] 10^{-4} M each in Na_2HPO_4 and NaH_2PO_4. [d] 0.05 M in sodium cacodylate. [e] Method A, the mutagen modified native DNA was denatured by heating. [f] Method B, heat denatured DNA was modified with the mutagen.

discrepency between the ϕ_f of **3** and mutagen modified denatured DNA to its residual secondary structure.

We have found that nucleotides such as *d*-GMP and *d*-TMP are efficient quenchers of the fluorescence of pyrene ($k_q = 1.2 \times 10^9\ sec^{-1}M^{-1}$); however, native calf thymus DNA is a much more efficient quencher [$k_q = 4.8 \times 10^{10}\ sec^{-1}M^{-1}$ (in nucleotide unit) at [pyrene] $= 6 \times 10^{-7}$ M]. Since nucleotides and nucleic acids have higher excited energy levels than pyrene, the quenching of excited aromatic compounds by nucleotides or DNA requires a substantial interaction between the two π-electronic systems and the bimolecular encounter quenching of excited pyrene by DNA cannot exceed the diffusion-controlled limit (k_{diff} in H_2O at 25° is estimated to be $6.6 \times 10^9\ sec^{-1}M^{-1}$) (16). The results indicate that static quenching must be in effect, i. e., pyrene must be in the proximity of base-pairs of DNA prior to its excitation or intercalated between them. In a recent report by Geacintov and Prusik (17), they suggested that the fluorescence of BPDE modified DNA is derived from the pyrenyl groups non-intercalated between the base pairs of DNA. Our independent study indicates that there are multiple binding sites in the mutagen modified DNA; however, the major portion of the pyrenyl groups is non-fluorescent and thus remains intercalated between the base pairs. Weinstein and his coworkers reported that there is little conformation change in the structure of calf thymus DNA by a low level of binding of BPDE 1 (18). Drinkwater, Miller, Miller and Yang (8) demonstrated that 1 and 2 uncoil the supercoiled SV-40 DNA with an efficiency similar to that of ethidium bromide, indicating that these oxiranes intercalate efficiently into the double strand of native DNA. A portion of these intercalated mutagens becomes subsequently chemically bound to DNA, and our spectrofluorimetric investigation indicates that the major portion of the chemically bound mutagen molecules remains intercalated between the base pairs of DNA.

S. typhimurium TA-98 is a hisD-3052 mutant and the base sequences in that region of both the mutant and revertant have been mapped by Yourno and Isono (19). The hisD-3052 region is particularly rich in G:C bases; the mutant corresponds to a single deletion of a G:C pair from a triplet of G-G-G (or C-C-C), and the revertant is caused by an additional deletion of G-C (or C-G) doublet from a nearby (G-C)$_4$ [or (C-G)$_4$] sequence (19). In view of the higher solublizing power of guanine derivatives over adenine derivatives for pyrene and other polynuclear aromatic hydrocarbons (20) and the highly selective binding of 1 to guanine in DNA (2-3), the intercalative covalent binding of these aryloxiranes may provide a molecular basis for their frame-shifting mutagenic activity in S. typhimurium TA-98, which will be elaborated in a subsequent publication (21).

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