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FLUORESCENCE AND PHOTOELECTRON STUDIES OF THE INTERCALATIVE BINDING OF BENZ(A)ANTHRACENE METABOLITE MODELS TO DNA

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Summary: DNA binding of nonreactive metabolite models derived from benz(a)anthracene was studied. The molecules investigated include 1,2,3,4-tetrahydrobenz(a)anthracene (1), 5,6-dihydrobenz(a)anthracene (2), and 8,9,10,11-tetrahydrobenz(a)anthracene (3), as well as anthracene and phenanthrene.

Measurements of the effects of DNA binding upon fluorescence intensities and fluorescence lifetimes indicate that molecules 1 and 3 (K_A = 1.5 - 2.5x10^3 M^{-1}) bind more strongly to native DNA than does molecule 2 ($K_A \simeq$ 0.5x10^3 M^{-1}). Furthermore, molecules 1 and 3 bind to DNA much more effectively than do the two less sterically hindered π electron metabolite models, anthracene and phenanthrene. Photoelectron data suggests that the enhanced binding of molecules 1 and 3 is due to increases in polarizability. Experiments carried out with denatured DNA indicate that the binding of molecule 1 entails the greatest intercalation.

The finding that carcinogenic hydrocarbons form metabolites containing reactive epoxide groups(1) has stimulated studies of hydrocarbon metabolite binding to DNA(2,3). For a given hydrocarbon there are several pathways leading to different epoxide metabolites(4). Figure 1 shows some of the metabolites which are formed from the potent carcinogen 7,12-dimethylbenz(a)anthracene. Of these epoxides, 1,2-oxide-3,4-dihydrodiol-7,12-dimethylbenz(a)anthracene (a bay region epoxide) is most carcinogenic(5). Different epoxide metabolites formed from the same parent hydrocarbon exhibit different reactive properties(1,5). However, different metabolites also exhibit different physical binding properties, and these properties may influence carcinogenic activity.

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Using fluorescence and photoelectron techniques we have studied how stereoelectronic properties influence reversible π binding properties. The benz(a)anthracene derivatives shown in Fig. 1 as well as anthracene and phenanthrene have been used as nonreactive models of epoxide metabolites.

EXPERIMENTAL

Fluorescence experiments were performed with a Perkin Elmer 650-10 Fluorescence Spectrometer and a Photochemical Research Associates Model 2000 Nanosecond Fluorescence Spectrometer. Photoelectron spectra were measured with a Perkin Elmer PS 18 Photoelectron Spectrometer.

Samples of molecules 1 to 3 were prepared and purified using published methods(6). Anthracene, phenanthrene and calf thymus DNA were purchased from Sigma Chemical Company. DNA concentrations are reported in terms of PO_4^- molarity calculated from an average base-pair molecular weight of 617.8. This is based on a calf thymus DNA composition which is 60% A-T base pairs (7). The solvent system used consisted of methanol (15% by volume) in water. The solutions were maintained at a pH of 7.1 with 10^{-3} M sodium cacodylate. The hydrocarbon concentrations were in the range 10^{-6}-10^{-7}M. Except for one lifetime experiment all fluorescence studies were carried out after samples were flushed with N_2 for two hours.

One lifetime study of 3 was carried out in the absence of DNA but in saturated O_2. Experiments with denatured DNA involved heating solutions to 95°C for 5 min. At 260 nm this led to a hyperchromicity of 29%. Stern-Volmer plots were measured at the maxima in the uncorrected emission spectra. For molecules 1, 2, and 3 these occured at wavelengths of 392, 350, and 372 nm respectively. The excitation wavelengths used were 330, 312, and 300 nm respectively.

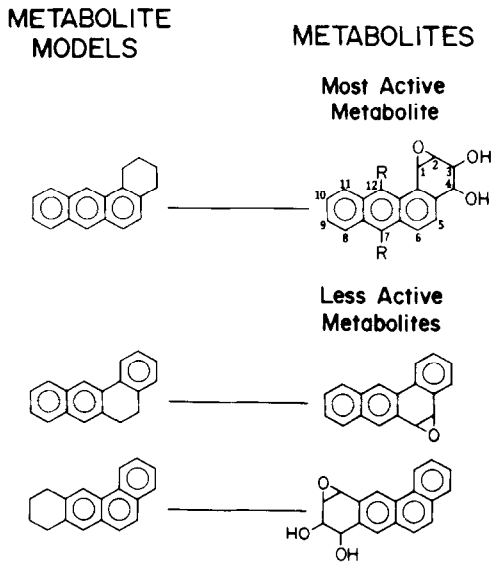

Fig. 1 Reactive metabolites formed from 7,12-dimethylbenz(a)anthracene and nonreactive metabolite models.

RESULTS AND DISCUSSION

The Stern-Volmer plots of Fig. 2 indicate that native DNA quenches the fluorescence of molecule 1 four times more effectively than it quenches 2 and 3. Figure 2 also indicates that denatured DNA ($[PO_4^-] = 4.5 \times 10^{-4}$ M) does not appreciably quench fluorescence from 1. These results provide strong evidence that the bay region metabolite model, 1, binds to DNA in a manner which involves significant intercalation.

Figure 3 contains results of lifetime studies of fluorescence emission from molecules 1 to 3 in the absence and in the presence of DNA. For 3, Fig. 3 shows a decay profile in the absence of DNA but in the presence of O_2 (2.7×10^{-4} M). The O_2 data shows how dynamic quenching shortens the lifetime of 3. For molecule 3, Fig. 3 also shows a decay profile in the presence of denatured DNA.

The decay profiles of molecules 1 and 2 do not change significantly when native DNA [$(PO_4^-) = 4.4 \times 10^{-4}$ M] is added. For molecule 3, however, DNA

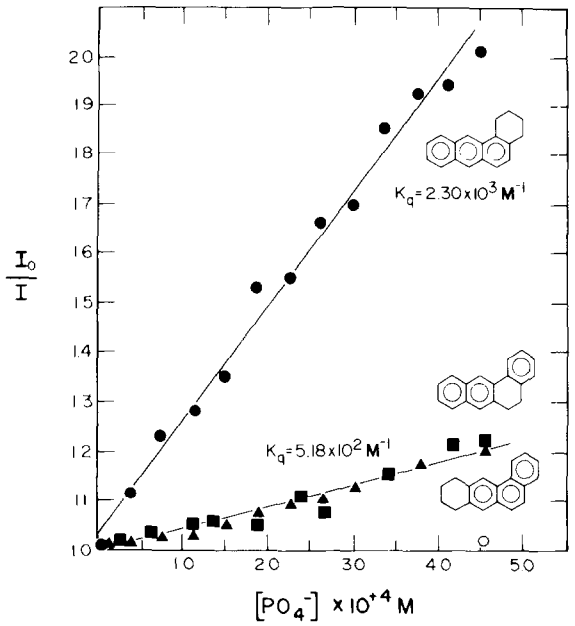

Fig. 2 Stern-Volmer plots and quenching constants derived from the fluorescence quenching of 1,2,3,4-tetrahydrobenz(a)anthracene (●), 5,6-dihydrobenz(a)anthracene (■), and 8,9,10,11-tetrahydrobenz(a)anthracene (▲) by DNA. The open circle shows I_0/I for 1,2,3,4-tetrahydrobenz(a)anthracene in denatured DNA ($[PO_4^-] = 4.4 \times 10^{-4}$ M).

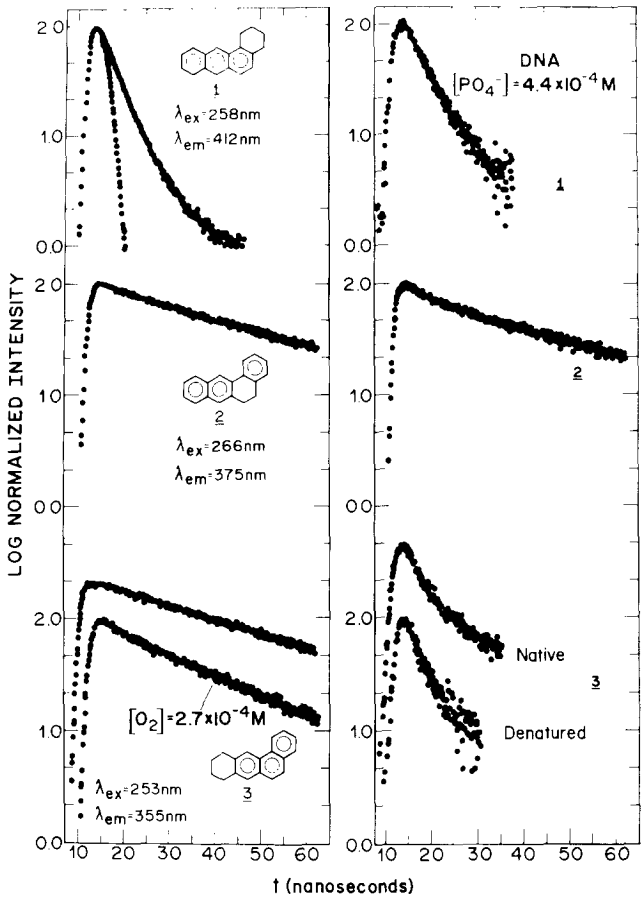

Fig. 3 Fluorescence decay profiles for 1,2,3,4-tetrahydrobenz(a)anthracene, 5,6-dihydrobenz(a)anthracene and 8,9,10,11-tetrahydrobenz(a)anthracene in the absence (left) and in the presence (right) of DNA ($[PO_4^-]$ = 4.4×10^{-4} M). For 1 and 2 the decay profiles on the right were measured in native DNA. For 3 the profiles were measured in both native and denatured DNA. For 3 a decay profile in the presence of O_2 (2.7×10^{-4} M) is given on the left. An instrument response curve is given in the upper left corner.

greatly alters the decay profile. This indicates that 3 binds significantly in the ground state to DNA. The decay profile exhibits two lifetimes. the shorter associated with bound molecules, the longer associated with free molecules.

The strong quenching exhibited by molecule 1 in Fig. 2 and the similarity of the decay profiles in the presence and in the absence of DNA suggest that binding of this molecule to DNA greatly reduces its quantum yield, Q_{Bound}. Other strongly intercalating hydrocarbons which exhibit this behavior are pyrene and 7,8,9,10-tetrahydroxytetrahydrobenzo(a)pyrene(8). Furthermore,

fluorescence lifetimes of 1 are short (<30nsec), indicating that dynamic quenching in the presence of DNA is negligible. Since Q_{Bound} is approximately zero, an estimate of the association constant, K_A, of molecule 1 is given directly by the quenching constant, $K_A \simeq K_q = 2.3 \times 10^3\ M^{-1}$.

Obtaining an approximate DNA binding constant, K_A, for molecule 3 involved deconvolution of the decay profile of 3 in the presence of native DNA. This analysis (9) indicated that at $[PO_4^-] = 4.4 \times 10^{-4}$ M the emission of 3 follows the decay law

$$I(t) = [I(o)/725.3][610.6e^{-t/3.7} + 114.7e^{-t/28.6}].$$

The ratio of photon intensities ($I_{Bound}/I_{Free} = 0.69$) associated with the bound and free states was obtained by integrating the long and short lifetime components separately. An approximate value of the association constant is then given by the equation

$$K_A = \frac{[Bound]}{[Free]\,[PO_4^-]} = \frac{I_{Bound}}{I_{Free}} \frac{Q_{Free}}{Q_{Bound}} \frac{1}{[PO_4^-]}.$$

Here Q_{Free}/Q_{Bound} is the ratio of quantum yields for the bound and free states. For $Q_{Free}/Q_{Bound} = 1$, a value $K_A = 1.56 \times 10^3 M^{-1}$ was obtained. A choice of $Q_{Free}/Q_{Bound} = 1.54$, yielded $K_A = 2.4 \times 10^3\ M^{-1}$. When these latter values were employed to calculate the Stern-Volmer quenching constant, K_q, a value of $5.2 \times 10^2\ M^{-1}$ was obtained, in agreement with the results of Fig. 2.

With denatured DNA ($[PO_4^-] = 4.4 \times 10^{-4} M$), Fig. 3 indicates that molecule 3 exhibits a decay profile similar to that observed in the presence of native DNA. Because native DNA provides a better host for strong intercalative interactions than denatured DNA(10), the lifetime studies of 3 suggest a native DNA-hydrocarbon complex structure which does not involve extensive base sandwiching. It appears that 3 becomes partially wedged into native DNA and that the π overlap alters the lifetime of 3. However, the overlap is much less complete than for molecule 1. This is consistent with the results in Fig. 2.

DNA has only a weak effect on the fluorescence intensity and the decay profile of 2. From the quenching data in Fig. 2 we estimate that the value of K_A for molecule 2 is at least four times smaller than the values for 1 and 3.

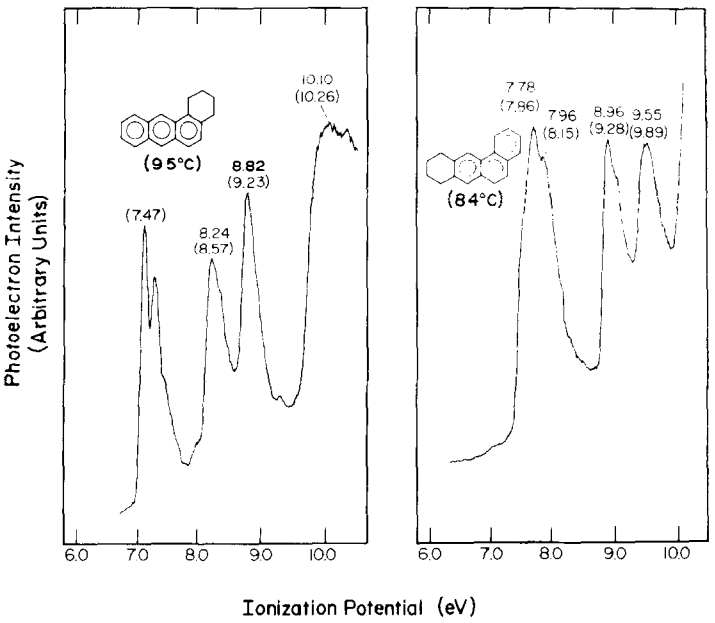

Fig. 4 He(I) photoelectron spectra of 1,2,3,4-tetrahydrobenz(a)anthracene and 8,9,10,11-tetrahydrobenz(a)anthracene. Assignments are given along with probe temperatures. Numbers in parentheses are ionization potentials taken from ref. 14 for corresponding orbitals in anthracene and phenanthrene.

The effects of DNA upon the fluorescence intensities and decay profiles of anthracene and phenanthrene were also studied. The results indicate that the K_A for anthracene is about four times lower than for 1 and that the K_A for phenanthrene is about two times lower than for 3. This is surprising because the aliphatic rings in molecules 1 and 3 are expected to hinder binding.

Figure 4 provides a reasonable explanation for this result. The enhanced binding of 1 and 3 is consistent with earlier studies of methyl substitution effects upon the base stacking of nucleosides(11). In general the increased binding of the methyl substituted nucleosides is related to increases in the polarizabilities of the nucleotide bases (11,12). This is also consistent with photoelectron data indicating that methyl substitution destabilizes as many as eight of the highest occupied orbitals in nucleotide bases(13). The same effect appears to influence π interactions of hydrocarbons with nucleotide bases.

Figure 4 contains photoelectron spectra of molecules 1 and 3. In a manner similar to the methyl substitution of nucleotide bases, the presence of aliphatic rings in molecules 1 and 3 destabilizes all four of the highest occupied orbitals compared to corresponding orbitals occurring in anthracene and phenanthrene(14). In both 1 and 3 a net destabilization of the four highest occupied π orbitals is more than 0.8 eV.

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