INTERACTIONS OF SEVERAL POLYCYCLIC AROMATIC HYDROCARBONS WITH DNA BASE MOLECULES STUDIED BY ABSORPTION SPECTROSCOPY AND FLUORESCENCE QUENCHING

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Summary

The interactions of several polycyclic aromatic hydrocarbons (PAHs) including benzo[*a*]pyrene, benzo[*e*]pyrene, benz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, dibenz[*a*, *c*]anthracene and dibenz[*a*, *h*]anthracene with selected deoxyribonucleic acid (DNA) bases, *i.e.* adenine, thymine, cytosine and 5-methylcytosine, and a nucleoside, guanosine, have been studied employing UV–visible absorption spectroscopy, fluorescence quenching and electrochemical measurements. The results indicate that PAHs interact with DNA bases by forming moderately strong 1:1 charge transfer (CT) complexes in their ground state. The fluorescence of PAHs was only quenched by 5-methylcytosine, guanosine and cytosine, and this was at lower than diffusion controlled rates in dimethyl sulfoxide. The fluorescence quenching rate constants showed a better inverse correlation with the solvent reorganization energies than with the free energies of activation calculated using the Marcus model of electron transfer.

1. Introduction

The interaction of polycyclic aromatic hydrocarbons with deoxyribonucleic acid (DNA) has attracted a great deal of interest in the past few decades owing to its relevance to carcinogenic mechanisms. As early as 1946, polycyclic aromatic hydrocarbons (PAHs), which are normally insoluble in aqueous media, were found to be solubilized by aqueous DNA base solutions [1] and later by aqueous DNA solutions [2, 3]. These studies were extended by other investigators employing various techniques. Of particular interest is the work of Ts'o and Lu [4] who reported that benzo[*a*]pyrene (BaP) could be induced to react photochemically with DNA. The work reported by Hoffman and Müller [5], Cavalieri and Calvin [6] and Blackburn *et al.* [7] all points to the fact that BaP reacts photochemically

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with DNA through covalent bindings. Moore *et al.* [8] have shown the importance of the 6-position of BaP in the photochemical covalent binding of its excited triplet with DNA. All these studies suggest that PAHs interact with DNA or DNA bases in their ground state as well as in their excited state. Indeed, this was shown to be the case for systems containing pyrene or mutagenic drug compounds and several nucleotides [9, 10]. These compounds were found to interact with DNA or its bases in both ground and excited states; for some cases the interaction was enhanced to a great extent in the excited state.

The mode of interaction of PAHs with DNA or its bases may vary from case to case. Whether the nature of the interaction is photochemical or not, it is reasonable to postulate that an electron transfer might be involved at some stage during the reaction. That is, a weak charge transfer (CT) complex, either in the ground state or in the excited state, may initiate a whole series of reactions. These reactions could be initiated by the presence of oxidants such as those present in biological systems, cytochrome P-450 for example, or by the photochemical excitation of PAHs. Evidence for these mechanisms has been reported. The role of photochemical excitation was advocated in papers by Birks [11] and Morgan *et al.* [12], whereas the importance of one-electron involvement in *in vivo* reactions was advanced by Wilk *et al.* [13], Cavalieri *et al.* [14] and Nagata *et al.* [15]. The CT model was proposed by Mason [16] and by Szent-Györgyi [17]. According to Szent-Györgyi, many biological reactions ranging from drug interactions to carcinogenesis resemble charge transfer chemistry.

We have studied the photochemical and electrochemical properties of PAHs employing fluorescence, electrogenerated chemiluminescence [18, 19] and transient electrochemical techniques [20 - 23]. As part of our continued effort in this area we have studied the interactions of several PAHs with selected DNA base molecules and a nucleoside, guanosine, in both their ground and excited states, and the results are reported in this paper. From the results obtained, the Marcus model of electron transfer in excited state interactions is also evaluated. All the PAHs studied here show moderate ground state interactions. Some of these compounds are carcinogenic.

2. Experimental details

BaP (Aldrich, Gold Label), benzo[*e*]pyrene (BeP; Aldrich, 99%), dibenz[*a*, *h*]anthracene (DBahA; Aldrich, 97%), dibenz[*a*, *c*]anthracene (DBacA; Aldrich, 97%), 3-methylcholanthrene (3MC; Eastman Organic), 7,12-dimethylbenz[*a*]anthracene (DMBA; Eastman Organic) and benz[*a*]-anthracene (BA; Eastman Organic) were used as received. DNA bases including adenine (Aldrich, 99%), thymine (Aldrich, 97%), cytosine (Sigma) and 5-methylcytosine (Sigma), and a nucleoside, guanosine (Aldrich, 98%), were also used as received. Unless otherwise noted, either spectrograde

(Eastman Organic) or analytical reagent grade (Mallinckrodt) dimethyl sulfoxide (DMSO) was used as a solvent, after fractional distillation with the reflux ratio 5:1 under reduced pressure, for spectroscopic and electrochemical measurements. Polarographic grade tetra-*n*-butylammonium perchlorate (TBAP; Southwestern Analytical, Austin, Texas) was used as a supporting electrolyte for cyclic voltammetric measurements after drying at 90 - 100 °C under vacuum for at least 24 h. Solutions prepared for electrochemical measurements were degassed by 3 - 5 freeze–pump–thaw cycles on a high-vacuum line.

Absorption spectra were recorded using a Cary 219 spectrophotometer. Fluorescence quenching experiments were carried out using an Aminco–Bowman spectrophotofluorometer, which had a bandwidth of 5.5 nm for both excitation and emission monochromators. Fluorescence lifetimes were measured with an Optitron model NF-100 nanosecond fluorometer equipped with an Optitron NR-100 nanosecond radiator [24]. The fluorescence decay signal from a Hammamatsu R928 photomultiplier tube was monitored using a Tektronix 5S14N sampler plugged into a Tektronix 5115 storage oscilloscope. The sampling oscilloscope was interfaced with an Apple II+ microcomputer through an Applab A/D and D/A card purchased from Interactive Microwares Incorporated (State College, Pennsylvania). The sampling oscilloscope was driven by the computer and the data were stored on a floppy disk. These data were later used for deconvolution with a published algorithm [25] to obtain lifetimes. Cyclic voltammograms were recorded using a Princeton Applied Research (PAR) model 173 coupled with a PAR model 175 universal programmer. The electrochemical cell had a conventional three electrode configuration with a platinum disk working electrode ($A \approx 0.0228$ cm^2), a platinum wire counterelectrode and a saturated calomel electrode (SCE) as a reference for the measurements of the oxidation potentials of DNA bases. For the measurement of the reduction potentials of PAHs, a silver wire pseudo-reference electrode was used with an internal reference compound present (tri-*p*-tolylamine (TPTA); $E_{p,ox} = 0.918$ V *versus* SCE).

The radii of the compounds were estimated from their densities; these were determined by weighing a given amount of a compound into a volumetric flask, followed by another weighing procedure after the flask was filled up to the mark with a solvent. Absolute ethanol was used as the solvent for PAHs while distilled water was used for DNA bases. Since the densities of these solvents are known at the experimental temperature, the volume of the compound can be readily calculated. Once the density is known, the radius r can be obtained using the equation

$$\frac{4}{3}\pi r^3 dN = w \tag{1}$$

where d is the determined density, N is the number of molecules used for the measurements and w is the weight of the compound.

3. Results and discussion

3.1. Ground state interaction

The interaction of PAHs with DNA bases in the ground state was investigated using the assumption that they form 1:1 CT complexes with bases. Bases are assumed to be donors whereas PAHs are acceptors. The equilibrium constants of these complexes were obtained by measuring absorbance spectra of a series of solutions containing a given amount of a PAH and various concentrations of base or nucleoside molecules. A typical example of such spectra is shown in Fig. 1(a) for the 3MC–guanosine system. As can be seen in the figure, the absorption of 3MC in the short wavelength region decreased upon addition of DNA bases owing to the decrease in its equilibrium concentration, while new absorption appears in the long wavelength region owing to the formation of the complex (Fig. 1(b)). These new absorbance data were treated with the modified Benesi–Hildebrand equation [26]

$$\frac{1}{\Delta A}\,\frac{[Q]_0}{[A]_0+[Q]_0} = \frac{1}{K_c^{AQ}\epsilon^{AQ}}\,\frac{1}{[A]_0+[Q]_0} + \frac{1}{\epsilon^{AQ}} \qquad (2)$$

which was originally derived for nuclear magnetic resonance measurements for a complex with 1:1 stoichiometry [27]. Here ΔA is the change in absorbance in long wavelength regions owing to complex formation, $[A]_0$ and $[Q]_0$ are the "added" concentrations of PAH and base respectively, K_c^{AQ} is the formation constant and ϵ^{AQ} is the hypothetical molar absorptivity of a pure complex at the wavelength at which the measurement is made. When the $(1/\Delta A)\{[Q]_0/([A]_0+[Q]_0)\}$ *versus* $\{1/([A]_0+[Q]_0)\}$ plot is made, one can easily determine ϵ^{AQ} as well as K_c^{AQ} from the slope and the intercept. Typical plots are shown in Fig. 2 for systems containing BA as an acceptor for various donor molecules. The near-perfect linearity of these plots indicates that the complex should have 1:1 stoichiometry. Other

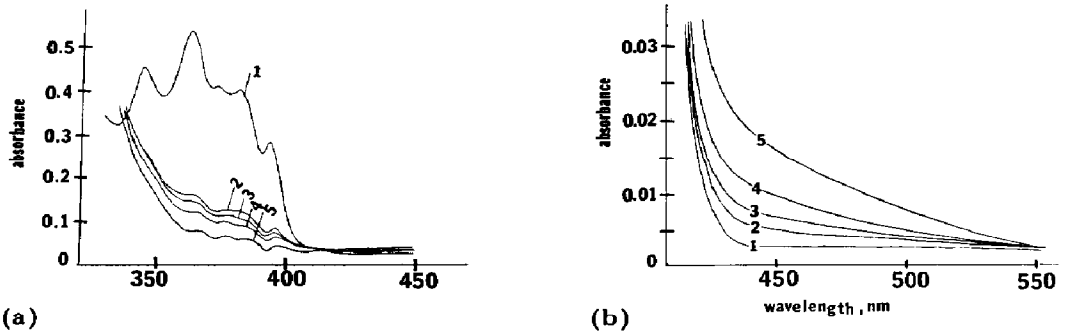

Fig. 1. (a) Absorption spectra of a solution of 1.0×10^{-5} M 3MC in the presence of the following concentrations of guanosine in DMSO: curve 1, zero; curve 2, 5.0 mM; curve 3, 10.0 mM; curve 4, 25.0 mM; curve 5, 50.0 mM. (b) Absorption spectra of a solution of 1.0 mM 3MC in the presence of the following concentrations of guanosine in DMSO: curve 1, zero; curve 2, 50.0 mM; curve 3, 150.0 mM; curve 4, 200.0 mM; curve 5, 250.0 mM.

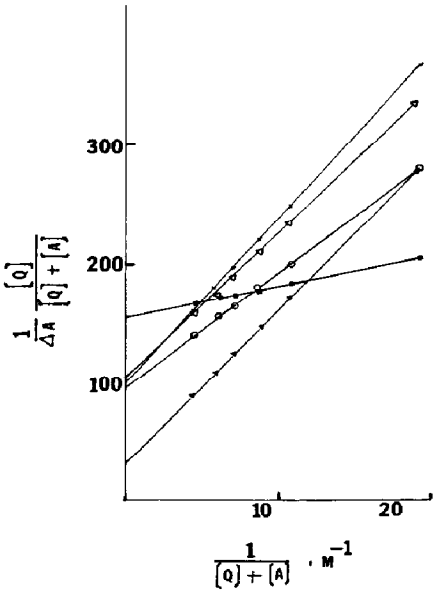

Fig. 2. Plots of eqn. (2) for systems containing BA and various bases. Measurements were made at 410 nm with a BA concentration of about 1.0 mM for added concentrations of bases of 50 - 250 mM: ■, BA with 5-methyl cytosine; ▲, BA with cytosine; ○, BA with thymine; △, BA with guanosine; ×, BA with adenine.

systems showed similar results with varied slopes and intercepts. Association constants K_c^{AQ} and molar absorptivities ϵ^{AQ} thus obtained are listed in Table 1 together with the wavelengths at which the measurements were made; these wavelengths are significantly longer than those at which hydrocarbons and bases absorb. The results obtained from blank experiments indicate that an increase in absorbances is observed only when both donor and acceptor molecules are present. We chose to use the longer wavelengths for the measurements to avoid possible interferences from the absorption of parent molecules, although one can readily calculate K_c^{AQ} from the decrease in absorbances due to the addition of base molecules (see Fig. 1(a)).

The results listed in Table 1 indicate that the interaction of PAHs with DNA bases in the ground state is generally stronger than for the "weak" charge transfer complexes [26]. Also, it is apparent from Table 1 that no general trend exists. However, the interaction is reasonably strong, although the exact nature of the interaction is not understood. As can be seen from Fig. 1, the absorption maxima for the complexes were broad; it was difficult to obtain the CT energies. Also, the fact that the association constants do not correlate with other parameters such as redox potentials may indicate that entropy effects for the complexation arising from geometric rearrangements could be more important than other phenomena. Theoretical and kinetics studies may help to improve our understanding of the nature of interactions, since only the change in free energies related to association reactions is investigated in our study.

TABLE 1

Association constants of PAHs (in units of litres per mole) with various DNA bases in ground states[a]

PAH	*DNA base*				
	Guanosine	*Adenine*	*Thymine*	*Cytosine*	*5-Methyl-cytosine*
1 Benzo[*a*]pyrene (450)[b]	1.1	8.7	2.1	4.8	10.0
	(32.6)[c]	(4.6)	(6.6)	(2.6)	(30.0)
2 Benzo[*e*]pyrene (390)	1.6	2.6	5.8	6.0	4.4
	(400)	(34)	(159)	(433)	(30.4)
3 Dibenz[*a*, *h*]anthracene (450)	1.1	3.0	3.1	3.3	11.2
	(45.0)	(12.4)	(13.6)	(14.0)	(0.51)
4 Dibenz[*a*, *c*]anthracene (390)	2.0	22.5	0.55	4.2	1.8
	(348)	(7.6)	(531)	(168)	(19.7)
5 Benz[*a*]anthracene (410)	3.6	1.3	10.0	2.7	60.2
	(5.0)	(10.2)	(10.2)	(30.6)	(6.4)
6 7,12-Dimethylbenz[*a*]anthracene (450)	1.4	18.2	1.5	15.7	6.3
	(192)	(1.2)	(81.8)	(1.7)	(63.0)
7 3-Methylcholanthrene (430)	3.7	4.1	2.6	1.3	1.4
	(27.8)	(11.9)	(13.5)	(31.6)	(6.8)

[a]Approximately 1.0 mM PAH used for about 10 - 100 mM base or nucleoside in DMSO at 25 °C.
[b]The numbers in parentheses are the wavelengths at which the measurements were made (in nanometres).
[c]The numbers in parentheses indicate molar absorptivities of CT complexes in litres moles per centimetre.

Finally, it should be pointed out that the molar absorptivities reported in Table 1 should be accepted with caution. Molar absorptivities of CT complexes have been reported to be dependent on the concentrations of acceptor and/or donor compounds. Problems in this area are discussed in the literature [28].

3.2. Fluorescence quenching

The excited state interactions of PAHs with DNA bases were measured by fluorescence quenching experiments. Since fluorescence intensity measurements can be affected by the inner filter effect due to the absorption tail of DNA base or nucleoside molecules in the general area of PAH excitation, we obtained fluorescence quenching rate constants from fluorescence lifetime measurements. Also, the formation of ground state complexes should result in a reduction of the absorbances of the PAHs, and this would give false Stern–Volmer plots for fluorescence quenching. The inner filter effect is avoided by using lifetime data, because lifetimes reflect quantum yields of fluorescers regardless of the intensity of the excitation light sources used. From the Stern–Volmer equation [29]

$$\frac{\tau_0}{\tau} = 1 + k_q \tau_0 [Q]_0 \tag{3}$$

the quenching rate constant k_q is readily calculated. Here τ_0 and τ are respectively the lifetimes of PAHs in the absence and the presence of the quencher Q.

Typical excitation and fluorescence decay profiles are shown in Figs. 3(a) and 3(b) for BaP; the phase plane plot is shown in Fig. 3(c). The inverse of the slope of the plot in Fig. 3(c) is the lifetime in nanoseconds [24, 25]. The lifetimes of PAHs thus determined are listed in Table 2.

The Stern–Volmer plots using lifetimes were quite linear indicating dynamic quenching of PAH fluorescences by DNA bases or nucleosides. The highest concentration of DNA bases or guanosine was approximately 0.10 M for these experiments. Excellent linearities up to the donor concentrations of 0.10 M seen here and also in the absorbance plots suggest that the self-association of DNA bases or nucleosides which is known to occur by a stacking mechanism in aqueous solution may not be important in DMSO. The quenching rate constants obtained from Stern–Volmer plots are listed in Table 3. DNA bases such as adenine and thymine did not show any quenching effect on PAH fluorescences. While the fluorescence decay curves of most PAH–DNA base systems had a single component, those for systems containing cytosine had double component decay curves. The fast and slow decaying components were sufficiently different to allow the estimation of quenching constants from the slow decaying components. We have attempted neither to deconvolute the double component system nor to investigate the nature of the fast decaying component. We surmise that this fast decaying emission might have originated from an exciplex.

TABLE 2

Electrochemical and spectroscopic data and radii

Compound	$E_{p,red}$ *versus SCE* (V)	$E_{p,ox}$ *versus SCE* (V)	ΔE_p (mV)	E_s[a] (eV)	τ (ns)	r (Å)
1 Benzo[*a*]pyrene	−1.749	–	60	2.978	21.5	9.11
2 Benzo[*e*]pyrene	−2.082	–	56	3.151	42.7	7.05
3 Dibenz[*a*, *h*]anthracene	−1.947	–	60	3.129	24.7	9.34
4 Dibenz[*a*, *c*]anthracene	−1.964	–	65	3.247	43.3	9.0[b]
5 Benz[*a*]anthracene	−1.874	–	60	3.159	38.5	8.49
6 7,12-Dimethylbenz[*a*]anthracene	−1.925	–	59	3.093	23.1	6.80
7 3-Methylcholanthrene	−2.045	–	62	3.089	15.5	8.74
8 Guanosine	–	1.222	–	–	–	4.78
9 Cytosine[c]	–	–	–	–	–	3.89
10 5-Methylcytosine	–	1.173	–	–	–	5.47

[a]Average energies of 0–0 bands of absorption and fluorescence spectra.

[b]Estimated value.

[c]Not oxidized within the anodic background potentials of DMSO.

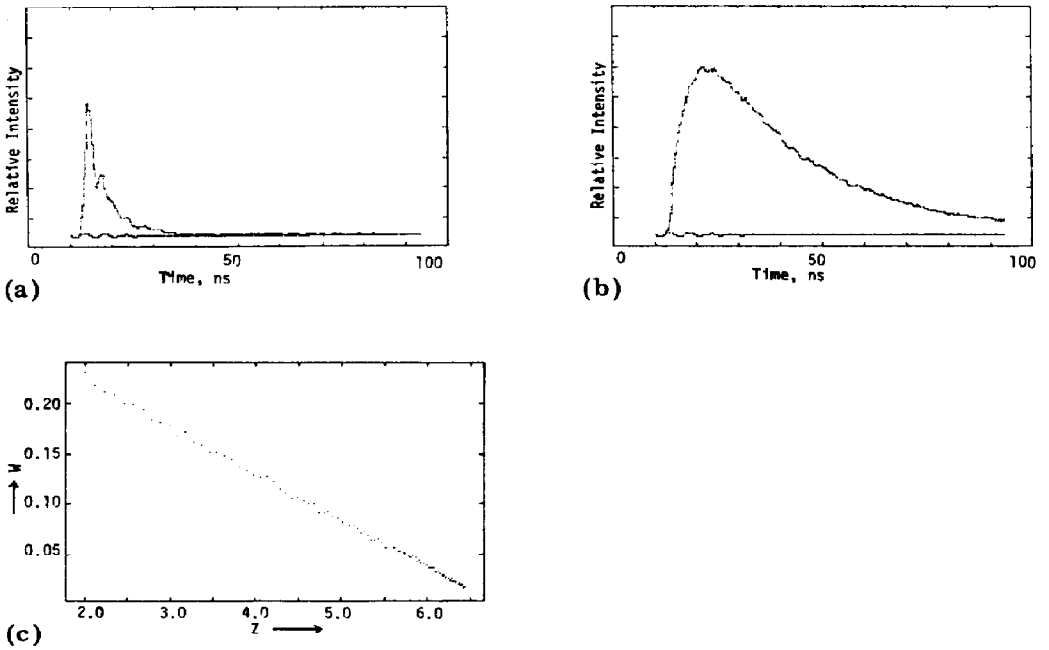

Fig. 3. The fluorescence decay profile and the phase plane plot of BaP (1.0×10^{-5} M) in DMSO: (a) excitation pulse; (b) fluorescence decay and (c) phase plane plot. The sample was excited at 285 nm and the fluorescence was detected at 410 nm. Baselines are also shown in (a) and (b). The scale of the y axes of (a) and (b) is 50 millivolts per division.

TABLE 3

Rate constants for the quenching of PAH fluorescences by various DNA bases[a]

PAH	*DNA base*		
	Guanosine	*Cytosine*[b]	*5-Methyl-cytosine*
1 Benzo[*a*]pyrene	3.06×10^8	—	9.38×10^8
2 Benzo[*e*]pyrene	1.24×10^8	—	6.80×10^8
3 Dibenz[*a*, *h*]anthracene	1.41×10^7	3.92×10^7	1.09×10^8
4 Dibenz[*a*, *c*]anthracene	3.50×10^8	—	5.19×10^8
5 Benz[*a*]anthracene	2.11×10^8	7.08×10^7	5.28×10^8
6 7,12-Dimethylbenz[*a*]anthracene	—	—	6.77×10^8
7 3-Methylcholanthrene	—	1.78×10^7	1.15×10^9

[a] In units of litres per mole per second.
[b] Estimated from double-component decay curves.

The quenching rate is generally lower than the diffusion-controlled rate which is calculated to be approximately 5.4×10^9 l mol^{-1} s^{-1} in DMSO at room temperature. It is interesting to note that adenine and thymine, which did not show any quenching, are not oxidizable within the background

potential of the solvent, DMSO. Both guanosine and 5-methylcytosine, which showed quenching for most PAHs, have oxidation potentials within the background. Cytosine, which shows a low quenching ability, is not oxidized within the range. These observations indicate that the oxidation potentials of the donor molecules are related to their quenching ability. Since oxidation potentials reflect electron donating ability, we decided to examine the electron transfer mechanism for quenching phenomena according to the Marcus theory of electron transfer.

3.3. Fluorescence quenching mechanism — the Marcus model of electron transfer

Several mechanisms for fluorescence quenching including the trivial, Förster energy transfer and electron transfer mechanisms are conceivable. Under the experimental conditions used here, the electron transfer mechanism should be the most likely candidate since the lowest singlet excited state energies of quenchers are higher than those of PAHs. In order to examine this mechanism, we calculated the free energies of activation for a postulated quenching mechanism

$$^1A^* + Q \longrightarrow (A^-Q^+)^* \tag{4}$$

employing the Marcus model of electron transfer [30]. According to Marcus, the most important parameter in determining the free energy of activation for an electron transfer reaction is the solvent reorganization energy due to the absence of bond cleavage. The free energy of activation for an electron transfer reaction in the Marcus model has the form

$$\Delta G^{\neq} = w^r + \frac{\lambda}{4}\left(1 + \frac{\Delta G_R^{o\prime}}{\lambda}\right)^2 \tag{5}$$

with

$$\Delta G_R^{o\prime} = \Delta G^{o\prime} + w^p - w^r \tag{6}$$

Here w^r is the work required to bring the reactants together to the most probable separation distance R in the intersection region of the potential energy surfaces, *i.e.* the activated complex, w^p is the corresponding work to move the products apart, $\Delta G^{o\prime}$ is the standard free energy of reaction in the medium and λ is a reorganization term. The λ term is the sum of an inner contribution λ_i (from changes in coordination in each inner coordination shell) and an outer contribution λ_o (from solvent reorganization). The important assumption made by Marcus is that λ_o is much larger than λ_i. The value of λ_o is calculated from the equation

$$\lambda_o = \left(\frac{1}{2r_a} + \frac{1}{2r_b} - \frac{1}{r_{ab}}\right)\left(\frac{1}{\epsilon_{op}} - \frac{1}{\epsilon_s}\right)e^2 \tag{7}$$

where r_a and r_b represent the radii of a PAH and a base molecule respectively, r_{ab} is simply the sum of r_a and r_b, ϵ_{op} and ϵ_s are respectively the

optical and static dielectric constants ($\epsilon_{op} = n^2$ where n is the refractive index), and e is the electronic charge. It is also assumed in the calculation that w^r and w^p are negligible.

Several experimental parameters are required to carry out the Marcus calculation; they were determined by spectroscopic and electrochemical methods and are listed in Table 2. Since an electron transfer from a base molecule to an excited PAH is assumed, the $\Delta G^{o\prime}$ values should be computed from the oxidation potentials of the DNA bases and the reduction potentials of the excited PAHs. The oxidation and reduction potentials of the bases and PAHs were determined using cyclic voltammetry. Examples are shown in Fig. 4 for the oxidation of 5-methylcytosine (curve a) and the reduction of DMBA (curve b). The reduction potential of an excited PAH can be estimated by the method of Rehm and Weller [31]. Thus, the $\Delta G^{o\prime}$ value is estimated from their equation

$$\Delta G^{o\prime} = E_{p,ox} - (E_{p,red} + E_s) \tag{8}$$

where $E_{p,ox}$ and $E_{p,red}$ are cyclic voltammetric peak potentials of the oxidation of a base and the reduction of a PAH respectively, and E_s is the energy of the lowest singlet excited state estimated from the O–O bands of the absorption and emission spectra of the PAH and DMSO. Table 4 lists the results of these calculations using molecular radii, oxidation potentials, reduction potentials, energies of the lowest singlet excited states of PAHs, and the dielectric constant ($\epsilon = 45$) and refractive index ($n = 1.4795$) of the solvent, DMSO. The calculation could not be performed on cytosine, because it was not oxidized within the anodic background of DMSO.

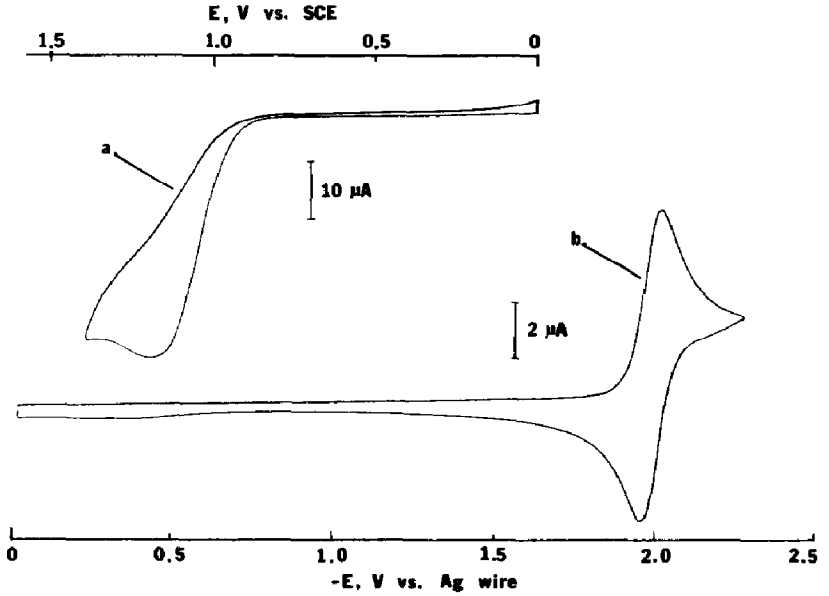

Fig. 4. Cyclic voltammograms of (a) 3.6 mM 5-methylcytosine, (b) 0.19 M DMBA, with 0.1 M TBAP as a supporting electrolyte, in DMSO at a scan rate of 100 millivolts per second at a platinum disk electrode.

TABLE 4

Free energies of activation and reorganization energies in electron volts

PAH	*DNA base*		
	Guanosine	*Cytosine*	*5-Methyl-cytosine*
1 Benzo[*a*]pyrene	0.133	—	0.0952
	(0.548)[a]	(0.666)	(0.486)
2 Benzo[*e*]pyrene	0.229	—	0.187
	(0.569)	(0.676)	(0.516)
3 Dibenz[*a*, *h*]anthracene	0.157	—	0.117
	(0.546)	(0.666)	(0.484)
4 Dibenz[*a*, *c*]anthracene	0.108	—	0.0730
	(0.548)	(0.667)	(0.487)
5 Benz[*a*]anthracene	0.108	—	0.0734
	(0.552)	(0.667)	(0.492)
6 7,12-Dimethylbenz[*a*]anthracene	1.172	—	0.133
	(0.574)	(0.679)	(0.522)
7 3-Methylcholanthrene	0.241	—	0.195
	(0.549)	(0.667)	(0.490)

[a]The numbers in parentheses are the solvent reorganization energies.

An attempt to correlate the free energies of activation thus calculated for electron transfer processes and $\log k_q$ was not satisfactory, as can be seen in Fig. 5. Although there is a definite trend the plots are highly scattered. However, it is true that 5-methylcytosine is always a better fluorescence quencher than guanosine, which in turn is a more effective quencher than cytosine. This is to be expected from their oxidation potentials. It should also be noticed that neither adenine nor thymine quench the hydrocarbon fluorescence. These DNA bases were not oxidized within the anodic background of the solvent, as has been already pointed out. All these observations are consistent with what the Marcus theory predicts.

One interesting aspect is that the solvent reorganization energy λ correlates better with $\log k_q$ than does the Marcus free energy, as shown in Fig. 6. This suggests that the difference in solvation energies before and after the electron transfer appears to be more important than the thermodynamics of the process itself. To show that this is indeed the case, we also attempted to plot $\log k_q$ *versus* $\Delta G^{\circ\prime}$ calculated using the method of Rehm and Weller, and the result (not shown) turned out to be even more scattered. The Marcus model, which incorporates both Born's solvation energy [32] and the thermodynamics of the system involved, might have given too much weight to the thermodynamics in this case. Another reason why the Marcus theory fails to correlate with the observed bimolecular quenching rates could be that the measured oxidation potentials of DNA bases may not reflect the true oxidation potentials. We believe that the reduction potentials of PAHs are reasonably accurate because the reduction waves were all

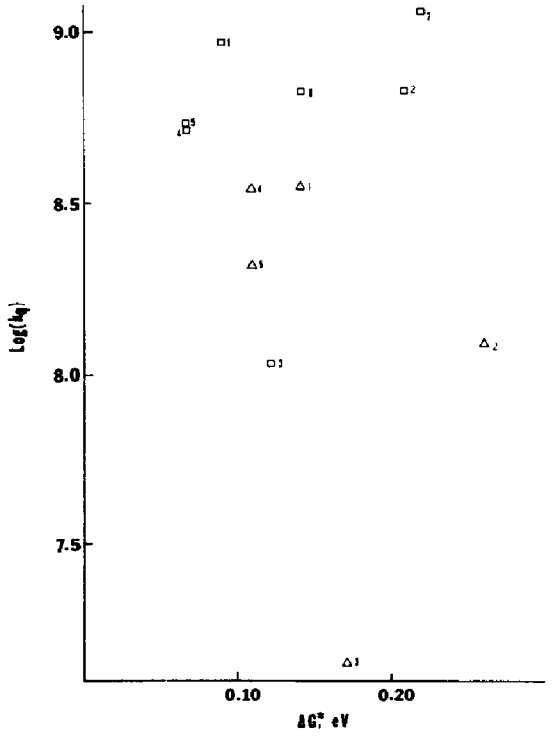

Fig. 5. Plot of $\log k_q$ *vs.* $\Delta G^{\neq}$ for various PAH–DNA base pairs. The two DNA bases shown in this plot are 5-methylcytosine (□) and guanosine (△). The numbers indicate PAHs as listed in Table 3.

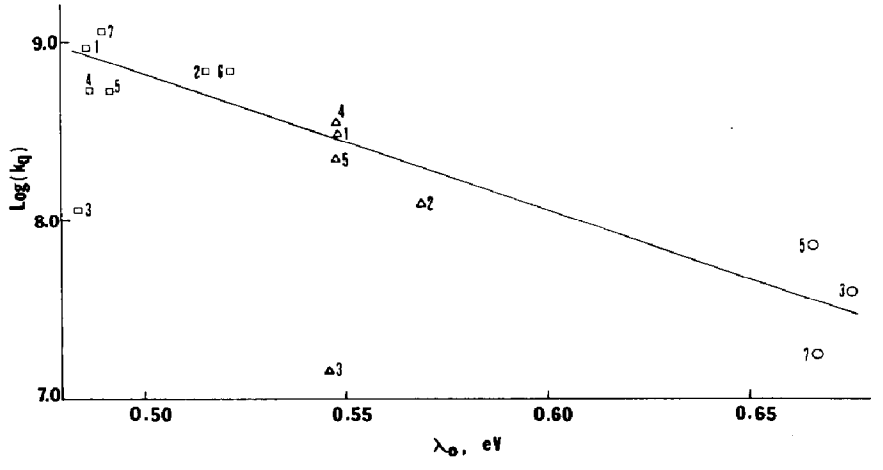

Fig. 6. A plot of $\log k_q$ *vs.* λ for various PAH–DNA base pairs. The DNA bases are 5-methylcytosine (□), guanosine (△) and cytosine (○). See Table 3 for the labelling of the PAHs. A least-squares line is drawn for the data points with the exception of those for DBahA (3).

electrochemically reversible as can be seen in Fig. 4(b). Further, the differences ΔE_p in cathodic and anodic peak potentials for given waves for PAH reductions are all approximately 60 mV, as listed in Table 2, in other

words the theoretically expected values [33] for reversible one-electron transfer. However, no reversal (cathodic) peaks were observed for the oxidation of guanosine and 5-methylcytosine, indicating the irreversibility of the electron transfer process. It should be noted that electrochemical potentials do not represent true thermodynamic parameters unless the processes involve reversible electron transfer. Thus, it is likely that our inability to measure true oxidation potentials might have contributed to the poor correlations of the Marcus free energies for activation with the observed quenching rates.

Another contribution to the poor correlation could have resulted from inaccuracies associated with the measurements of the molecular radii. Our method of estimating molecular radii does not take into account the effect of solvent interaction with solute molecules. That is, the molecular radii of compounds which strongly interact with solvent molecules may appear to be smaller than they actually are. As pointed out by one of the referees, it is difficult to understand why the radius of DMBA is smaller than that for BA. Also, the radius of guanosine seems smaller than expected. Perhaps these molecules interact with the solvent more strongly than do other compounds, or they may exist in an aggregated form in the particular solvent in which the measurements were made. Whatever the reason might be, it is clear that the molecular radius will affect the Marcus parameters. It is also possible that radii estimated in one solvent may not be the same as in the other because of the difference in their respective solvation structures. The assumption, however, that both PAHs and DNA bases are spherical appears to be reasonable, since λ takes only molecular radii and solvent properties into consideration.

Although the Marcus free energies of activation do not correlate well with the quenching rate constants, we still believe that electron transfer is involved in quenching the PAH fluorescence with DNA bases. The solvent reorganization, on which Marcus' model for outer sphere electron transfer is based, could be the rate determining step for the quenching process. It is thus reasonable to conclude that PAH fluorescences are quenched by DNA bases or guanosine via electron transfer.

Finally, it should be pointed out that electron transfer processes may be too complex to be explained using simple models. We believe that overlaps (λ_i) between donor and acceptor molecules may also be important parameters for determining the kinetics of the reaction. In Born's and thus Marcus' model all these molecules are treated as being spherical.

4. Conclusions

The PAHs studied here showed a moderate tendency to interact with DNA bases to form weak 1:1 charge transfer complexes in their ground state. Although the Marcus free energies of activation were poorly correlated with the quenching rate constants, it is reasonable to conclude that electron

transfer is involved in quenching processes, because the solvent reorganization energies showed a reasonably good correlation with the bimolecular quenching rates.

The implication of reaction (4), or that corresponding to the ground state, is that the CT complex may be formed as an intermediate, followed by the formation of a covalent binding. We expect the CT complex in the excited state (an exciplex) to have a high probability of decaying to the chemical products. From this point, the chemistry shown by this pair may not be different from that initiated electrochemically. Various adducts have been detected by either chemical or electrochemical activations [22].

It would be interesting to investigate whether varying the chain length of nucleosides and nucleotides would make any difference to their interactions with PAHs that differ in biological activity. Work is in progress in our laboratory in this area.

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