BNL--45087

DE92 010153

Photoinduced electron transfer and fluorescence mechanisms in covalently linked polynuclear aromatic-nucleotide complexes

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ABSTRACT

The fluorescence of polycyclic aromatic hydrocarbon-nucleic acid complexes is quenched by photoinduced electron transfer mechanisms in aqueous solutions at ambient temperatures. These effects are illustrated with the biologically important compound benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), a mutagenic and carcinogenic metabolite of the environmental pollutant benzo[a]pyrene, which forms covalent mutagenic lesions with 2'-deoxyguanosine (dG) residues in DNA. The dependence of the fluorescence yield and fluorescence decay times of the covalent model adduct (+)-*trans*-BPDE-N^2-dG as a function of temperature and methanol/water composition are described. Because of the sensitivity of the fluorescence of the pyrenyl residue to the polarity of the microenvironment, the magnitude of the fluorescence yield can be used to distinguish between highly hydrophobic (e.g. intercalation) and other more solvent-exposed BPDE-nucleic acid binding sites.

1. INTRODUCTION

Hydrophobic polycyclic aromatic hydrocarbon (PAH) molecules readily form noncovalent complexes with DNA[1]. After metabolic activation to epoxide derivatives[2], covalent addition products (adducts) are formed as well[3]. For example, benzo[a]pyrene, one of the most widely studied environmental PAH pollutants, is metabolized *in vivo* to over 30 different oxygenated derivatives[2]. The most tumorigenic metabolite is the diol epoxide (+)-*anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) which forms covalent adducts with DNA by predominantly reacting with the exocyclic amino group (N^2) of guanine via its 10-position (reviewed in ref. 3). However, in a competing reaction, BPDE also reacts efficiently with water to form the biologically inactive tetraol hydrolysis products BPT (7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrene, Fig. 1). In cellular environments, some of the covalent BPDE-DNA lesions, if they are not removed by the enzymatic repair machinery, interfere with the normal DNA replication and are believed to cause mutations and cancer[2,3].

Fluorescence techniques are frequently employed to trace and identify carcinogenic PAH-DNA adducts and to study the interactions of PAH metabolites with DNA[4-12]. However, at ambient temperatures, the fluorescence of both noncovalently and covalently bound PAH moieties is strongly quenched[1,7,8,10,11,12]. For example, the fluorescence of covalent BPDE-DNA adducts is ≈ 70 times lower than the fluorescence yield of BPT in aqueous solution in the absence of nucleic acids[10]. The pyrene-like fluorescence of BPT is readily quenched by DNA[12] and by 2'-deoxyguanosine[13] (dG) upon physical complex formation.

Recently, employing transient flash photolysis techniques and BPT/dG mixtures and the adduct BPDE-dG (in which BPDE is covalently linked via its 10-position to the N^2 group of dG) as model systems, we have shown that the quenching mechanism involves a photoinduced electron transfer from dG to BPT or from the dG residue to the covalently linked pyrenyl residue in the BPDE-dG adducts. In this work, the solvent- and temperature-dependence of the fluorescence decay times and yields of the covalently linked BPDE-deoxyguanosine complexes

are described, and these characteristics are shown to be consistent with the photoinduced electron transfer mechanism. This mode of non-radiative decay is likely to be the dominant fluorescence quenching mechanism in covalent and noncovalent PAH-nucleic acid complexes.

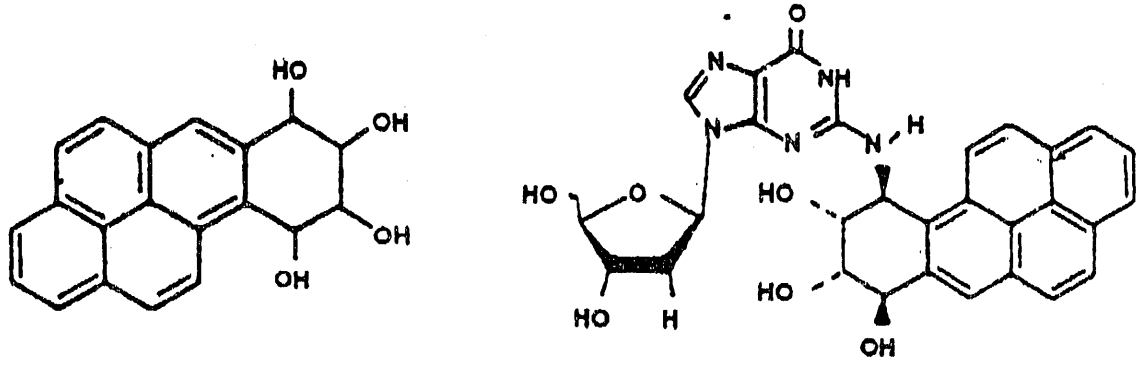

Fig. 1: Structures of BPT and the BPDE-dG adduct.

2. EXPERIMENTAL

The (+)-BPDE enantiomer was obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. The BPDE-dG adduct ((+)-*trans*-BPDE-N^2-dG, in which the exocyclic amino group of guanine adds *trans* relative to the ring-opened epoxide group at the C10 position of BPDE, Fig. 1) was prepared as described elsewhere[14]. This adduct was purified by reverse phase HPLC methods employing a Hypersil-ODS 250x10 mm column (Keystone Scientific, Inc., Bellefonte, PA), and 0-90% linear gradients of methanol in 20 mM sodium phosphate buffer solution in 60 minutes (flow rate 3.0 ml/min). The BPDE-dG adducts are characterized by pyrene-like vibronic absorption spectra with maxima near 315, 330, and 345 nm. Its concentrations were established by using a molar extinction coefficient of 29,000 $M^{-1}cm^{-1}$ at 345 nm.

The covalent BPDE-oligonucleotide adduct d(5'-CACATG^{BPDE}TACAC) and similar oligonucleotide adducts, in which the single guanine is modified at the exocyclic amino group by *trans* addition, was prepared as described recently[15]; this adduct has the same *trans*-N^2-dG stereochemical characteristics as the BPDE-dG adduct, but the modified guanine is flanked by dT on both the 5'-sides and 3'-sides in this oligonucleotide.

The shorter fluorescence decay profiles ($\leq$ 15 ns) were determined at port U9B of The National Synchrotron Light Source at the Brookhaven National Laboratory using single photon time-correlated techniques as described by Laws and Sutherland[16]; the time between successive synchrotron light pulses was = 19 ns. The longer lifetimes were determined with a conventional 40 kHz nitrogen flash discharge lamp (Photochemical Research Associates, London, Ontario) and single-photon counting electronics similar to those used at Brookhaven[16]. A model 1902 Spex Fluorolog single-photon counting fluorometer (Spex Industries, Edison, NJ) was employed to measure relative fluorescence yields as a function of temperature. In these measurements, the BPDE-dG adducts were dissolved in 70% ethylene glycol/30 % (v/v) water mixtures. The variable temperature experiments were carried out with the aid of a Cryogenic Workstation (Model RC 152, Cryo Industries, Salem, NH).

3. RESULTS

The relative fluorescence yield of BPDE-dG adducts increases by a factor 30-35 as the temperature is lowered from room temperature to 100-120 K. This rise is particularly sharp in the region of 190 - 270 K (■, Fig. 2); in this temperature range, the viscosity of the mixture increases, and solidification occurs at about 195 K. The fluorescence decay profiles are mono-exponential below 270 K, increase from 26 ns at 270 K to about 300 ns at 100 K (▲, Fig. 2), and closely follow the temperature dependence of the fluorescence yields.

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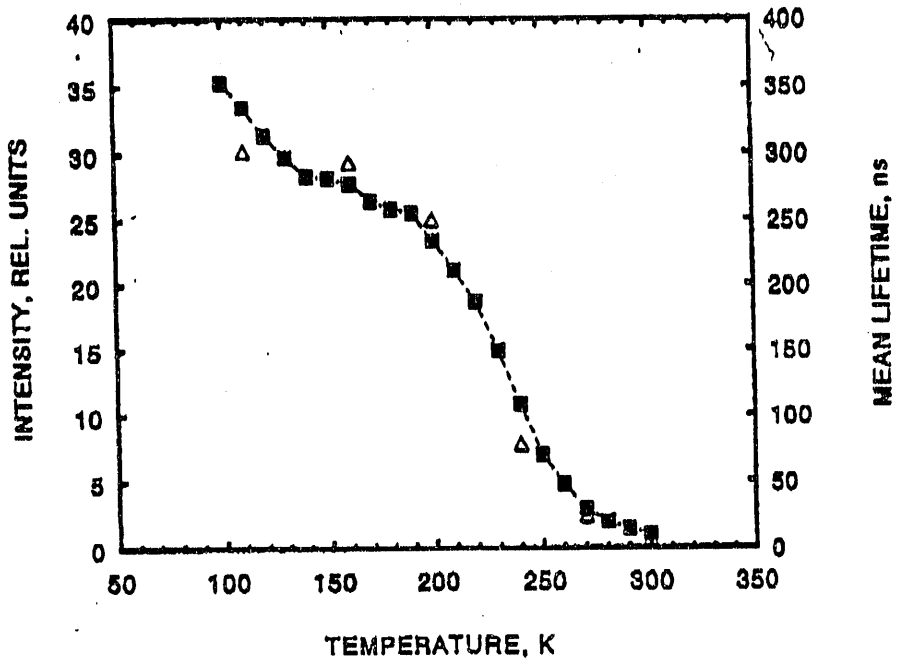

Fig. 2: Temperature dependence of the fluorescence yield (■) and the mean fluorescence decay time (▲) of BPDE-dG adducts (1.0 μM) in a 70% ethylene glycol/30% water mixture.

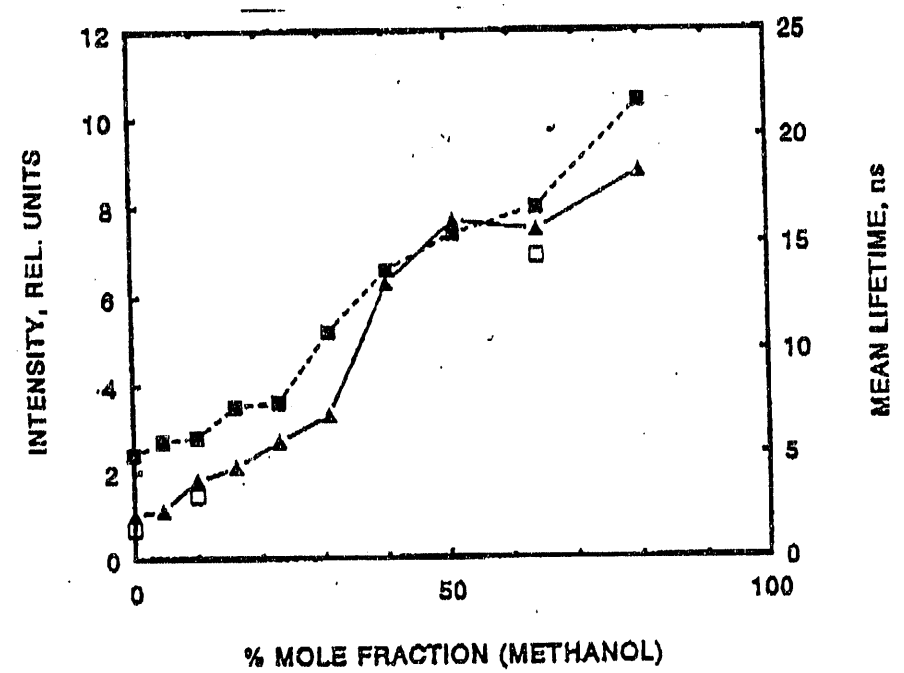

Fig. 3: Relative fluorescence yields of the BPDE-dG adduct (▲, 0.24 μM) and d(5'-CACATG^{BPDE}TACAC) oligonucleotide adduct (■, 1.2 μM) in methanol/water mixtures of different compositions (24 °C). Mean fluorescence decay times of the BPDE-dG adduct (□) taken from Table 1. Both solutions were de-oxygenated by nitrogen bubbling. In air-saturated solutions, only the yields at the two highest methanol concentrations are somewhat lower than in the nitrogen-saturated solutions.

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At 150-160 K, there is a sharp discontinuous jump in the fluorescence yield by a factor of 2-3 (not shown in Fig. 2). This effect is due to a solvent phase transition, which is evidenced by the appearance of cloudiness and increased light scattering. Such discontinuities in the same temperature range are also observed with other samples of fluorophores. In contrast, the fluorescence decay times of BPDE-dG adducts do not exhibit any discontinuities as the temperature traverses this phase transition region near 150 K (▲, Fig. 2). Therefore, the discontinuities in the fluorescence yields have been eliminated in the presentation of the data in Fig. 2 by matching the fluorescence yields on either side of the phase transition at 150-160 K.

The variations in fluorescence yields of (+)-*trans*-BPDE-N^2-dG adducts and of a (+)-*trans*-BPDE-N^2-dG adduct incorporated in the single-stranded modified oligonucleotide 5'-d(CACATG^{BPDE}TACAC), as a function of the mole fraction of methanol in a methanol/water mixture, are shown in Fig. 3. In pure water, the yield of the oligonucleotide adduct is 2.4 times higher than that of the nucleoside monomer BPDE-dG adduct; otherwise, the fluorescence yields of these two adducts exhibit rather similar increases as the mole fraction of methanol is increased.

The fluorescence decay profiles of the BPDE-dG adduct were measured in water and in two different water/methanol mixtures at the National Synchrotron Light Source at Brookhaven. Two typical fluorescence decay profiles are shown in Figs. 4 and 5 (no methanol, and 80% MeOH/20% H_2O mixture (v/v), respectively). The time-dependent decay, I(t), was interpreted in terms of bi-exponential decay profiles as follows[16]:

$$I(t) = A_1\exp\{-t/\tau_1\} + A_2\exp\{-t/\tau_2\} \qquad (1)$$

in which the sum of the amplitudes is normalized to unity ($A_1 + A_2 = 1.0$), and the two lifetimes are denoted by τ_1 and τ_2. The residuals (bottom inset) and autocorrelation function (top right) are also shown in Figs. 4 and 5. In general, because of the small quantities of samples which were available, and the limited beam time, it was not feasible to accumulate a sufficiently large number of counts to improve the statistics beyond the limits shown. Furthermore, because of the conformational heterogeneities of these BPDE-dG adducts (see below), the fluorescence decay profiles are likely to be multiexponential rather than bi-exponential. Therefore, it was decided to limit the fits to a two-component expression (Eq. 1), in spite of the rather indifferent fits as shown by the autocorrelation functions, and bearing in mind the intrinsic limitation of this two-exponential approach and the relatively low number of counts. Some typical results obtained from fits of Eq. 1 to the data, as well as the mean lifetimes ($\tau_{mean} = A_1\tau_1 + A_2\tau_2$) are summarized in Table 1.

Table 1: **Fluorescence decay parameters of BPDE-dG adducts* in different methyl alcohol/water mixtures.**

MeOH, mole fraction	τ_1, ns,(A_1)	τ_2, ns,(A_2)	τ_{mean}**, ns
0	0.86 (0.37)	2.0 (0.63)	1.6
10%	0.89 (0.16)	3.8 (0.84)	3.3
64%	1.3 (0.06)	15 (0.94)	14

*In these particular experiments, the BPDE-dG adduct contained a phosphate group at the 5'-end of dG. However, the fluorescence properties are not affected by the presence of this phosphate group (data not shown).
**$\tau_{mean} = A_1\tau_1 + A_2\tau_2$. The values for the reduced χ^2 values are for the three sets of data (top to bottom): 0.918, 0.790, 0.856.

The mean fluorescence decay times are superimposed on the relative fluorescence yield data in Fig. 3. In

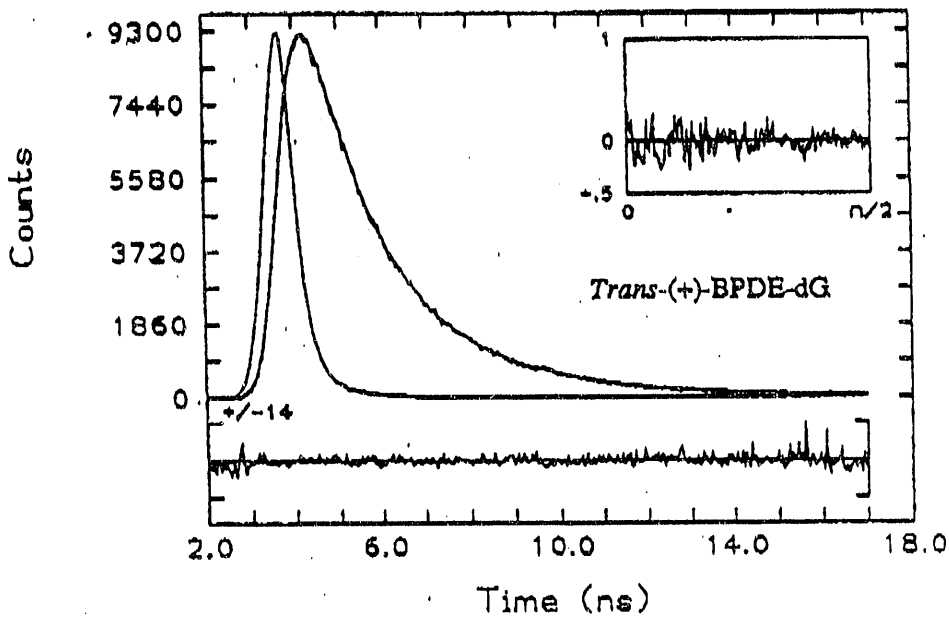

Fig. 4: Fluorescence decay profile of a BPDE-dG (1.0 μM) adduct in water. See text and Table 1 (analysis of data according to Eq. 1) for details.

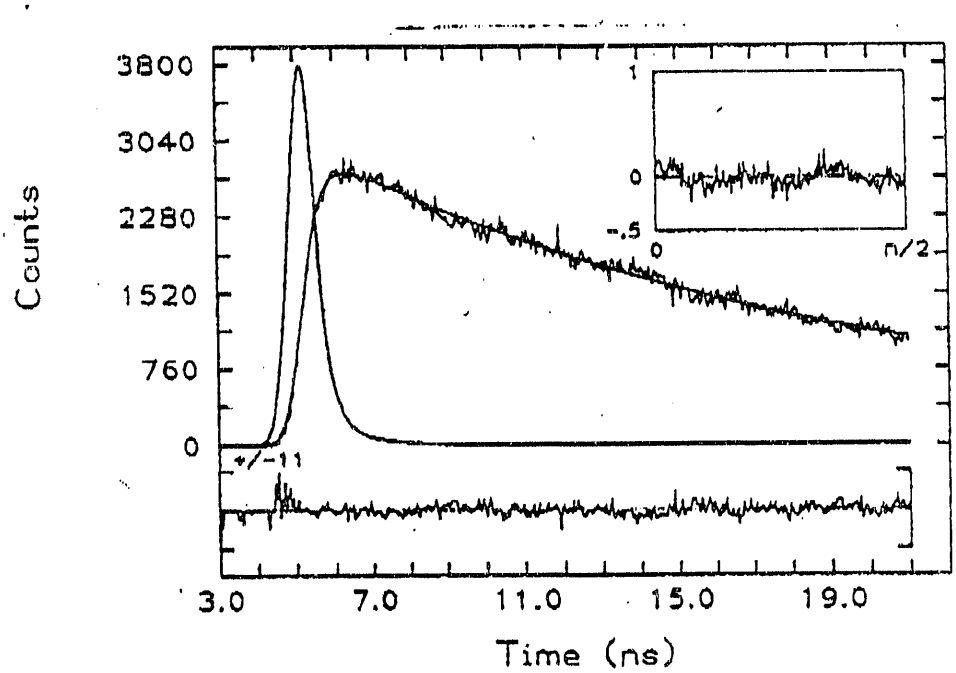

Fig. 5: Fluorescence decay profile of a BPDE-dG adduct in a methanol/water mixture (mole fraction of methanol: 64%). See text and Table 1 (analysis of data according to Eq. 1) for details.

spite of the crudeness of the data and the inherent limitations of the two-exponential analysis, there is a good correlation between the fluorescence yield data and τ_{mean} obtained at different MeOH mole fractions.

4. DISCUSSION

4.1 Solvent dependence.

The polarity of the solvent environment can have a profound effect on photoinduced electron transfer rates and efficiencies, and thus on the quenching of excited states and fluorescence yields[17-22]. The polarity of the solvents affects both the ion pair Coulomb interaction energy as well the solvation energies of the negative and positive radical ions. In general, the higher the polarity, the greater the probability that solvent-stabilized radical ions of overall lower energy will be formed, thus preventing recombination and regeneration of the fluorescence-emitting singlet excited states.

In the case of the N-(deoxyguanosin-8-yl)-2-aminofluorene adduct, an increase in the water content of a 1-propanol/water mixture causes the fluorescence yield to decrease and the emission maximum to shift progressively to the red[21]. Red-shifted exciplex emission has also been observed in the case of covalently linked pyrene-$(CH_2)_n$-N,N-dimethylaniline complexes[23]. In the case of the BPDE-dG adduct, however, the molecular pyrene-like emission spectrum remains unchanged as the mole fraction of methanol in an aqueous methanol solution is decreased[13]. This suggests that, in this case, the solvent-stabilized radical ions recombine back to the ground state by non-radiative routes.

The fluorescence yield increases observed as the effective dielectric constant of the solvent is decreased (Fig. 3), is characteristic of electron transfer-based fluorescence quenching mechanisms[17,18,20,21,22]. It is interesting to note that in the case of the BPDE-dG adduct, the relative mean fluorescence decay time is approximately proportional to the relative fluorescence yield at different MeOH mole fractions. Similar behavior was noted with the covalently linked donor/acceptor N-(deoxyguanosin-8-yl)-2-aminofluorene system[21]. However, in the noncovalently bound anthracene/diethylaniline charge transfer complex, the fluorescence yield of the complex increases more sharply than the lifetime as the dielectric constant of the solvent is decreased[17]; this effect was attributed by Knibbe et al. to differences in the probabilities of exciplex/contact ion pair formation, and the probabilities of decay of this exciplex by a fluorescence mechanism. The approximate correspondence between the fluorescence lifetimes and fluorescence yields exhibited by the covalently linked PAH-guanosine moieties (Fig. 3 and ref. 21), suggests that photoinduced electron transfer, followed by solvent-stabilization of the radical ion pairs, competes directly with fluorescence decay mechanisms. As the mean dielectric constant of the solvent mixture is decreased, the probability of solvent-stabilization of the ion pair, with a concomitant lowering of the energy below that of the fluorescence-emitting exciplex level, decreases and the fluorescence yield increases.

In the case of the BPDE-dG adduct, there is a sharp rise in the fluorescence yield in the 30-50% range of mole fractions of methanol (Fig. 3). Similar effects have been observed in other fluorescence emitting charge-transfer complexes in acetonitrile- and dimethyl sulfoxide/water mixtures[22]. These effects might be due to displacements of water by MeOH molecules from the first solvation shells in this range of mole fractions (selective solvation effects[22]). Such a change in solvation is expected to affect contributions of the inner and outer sphere reorganization energies to the overall free energy of photoinduced electron transfer[20,22,24,25].

4.2 The temperature dependence

The parallel behavior of the fluorescence yields and lifetimes as a function of temperature (Fig. 2), suggest that photoinduced electron transfer fluorescence quenching competes with fluorescence decay. In this respect, the solvent polarity and the temperature dependencies of these two fluorescence decay parameters are similar.

The observed upper lifetime limit of ≈ 300 ns for BPDE-dG adducts at ≈ 100 K is the same as that of the tetraol BPT in the same solvent and at the same temperature (data not shown). Thus, below 160-190 K, the electron transfer rate appears to be comparable, or lower in magnitude than the fluorescence decay rate.

Besides electrostatic effects, other solvent effects, such as the temperature-dependence of the viscosity, could be important in determining the magnitude of the electron transfer rate constants. The BPDE-dG adduct is conformationally mobile due to rotations about the C10-N^2 and N^2-C^2 bonds. This gives rise to fluctuations in the relative orientations and average center-center distances between the covalently bound BPDE and dG moieties which, in turn, may strongly influence the rates and yields of electron transfer[26]. Changes in solvent viscosities as a function of temperature, should also affect this conformational mobility, and thus the probabilities of electron-transfer as evidenced by variations in the fluorescence yields. Furthermore, the probabilities of conformations in which there are stronger hydrophobic π-stacking interactions between the dG and BPDE moieties are likely to decrease as the mole fraction of methanol increases. Thus, the rising fluorescence yields with decreasing temperature may be attributed to any one or a combination of these effects.

The formation of solvent-stabilized ion radicals may be associated with a small activation energy[24] which would also give rise to an increase in the fluorescence yield as the temperature is lowered. In general, the temperature dependence of fluorescence quenching processes by photoinduced electron transfer are expected to be complex and difficult to interpret[27,28]. The strong temperature dependence of the fluorescence lifetimes and yields of the BPDE-dG system (Fig. 3) resembles the temperature dependence of the fluorescence decay times of a covalently linked free base porphyrin-quinone complex in 2-methyl-tetrahydrofuran[29]. In that case, the temperature dependence of photoinduced electron transfer was interpreted in the light of Marcus' theory[25]; a sharp change in the temperature dependence of the electron transfer rate constant was noted near 160 K; however, it was not possible to determine whether this transition is due to a change from a non-adiabatic electron transfer mechanism in the higher temperature range, to an adiabatic solvent-controlled regime in the lower temperature range[29]. In view of these complexities, no further attempts were made to interpret the temperature dependence of the fluorescence quantum yield and lifetimes (Fig. 2) as a function of temperature.

4.3 Comparisons of fluorescence yields of different systems

The fluorescence yields of BPDE-dG and other covalent complexes in water relative to the fluorescence yield of the tetraol BPT are compared in Table 2.

Table 2. Relative fluorescence yields in water at 24 °C.

SUBSTANCE	Yield, %
BPT (Tetraol)	100
(+)-*trans*-BPDE-N^2-dG Adduct	0.8
(+)-*trans*-BPDE-N^6-dA Adduct	66
d(5'-CTATTG^{BPDE}TTATC), (+)-*trans* Adduct	1.9
d(5'-CTATAG^{BPDE}ATATC), (+)-*trans* Adduct	4.2

The fluorescence yield of the BPDE-dG adduct in water at 24 °C is over a 100 times smaller than that of BPT (the fluorescence decay time of BPT in deoxygenated aqueous solutions is 200 ns[10]). This strong quenching of the fluorescence in the covalently linked BPDE-dG adducts is attributed to photoinduced electron transfer. As

detailed elsewhere[13], the photoinduced electron transfer from the guanosyl moieties to the pyrenyl moieties in covalently linked BPDE-dG adducts is consistent with the redox characteristics of the pyrene ring system[30] and of guanosine[31,32]. The oxidation potential of adenosine is higher than that of guanosine[31,32]; we observe that the fluorescence of covalent BPDE-adenosine adducts ((+)-*trans*-BPDE-N^6-dA) is only slightly lower than the fluorescence yield of the tetraol BPT (Table 2). Apparently, photoinduced electron transfer rates are much smaller in the BPDE-adenosine than in the BPDE-guanosine adducts; these observations are consistent with the differences in the oxidation potentials of these two purine derivatives.

Finally, because the fluorescence yields of BPDE-dG adducts are highly sensitive to the polarity of the local microenvironment, these yields depend on the nature of the nucleotides immediately adjacent to the BPDE-dG adduct in different oligonucleotides. This is illustrated in Table 2. First, the pyrenyl residue fluorescence yield is about 2.4 times greater in the oligonucleotide d(5'-CTATTG^{BPDE}TTATC) than in the BPDE-dG adduct. In the d(5'-CTATAG^{BPDE}ATATC) adduct, in which the BPDE chromophore is flanked by adenine residues, the yield is higher than in the ---TG^{BPDE}T--- sequence. Since the purine adenine residues are bulkier than the pyrimidine thymine residues, these results are consistent with a lower water activity, and thus a higher fluorescence yield in the ---AG^{BPDE}A--- sequence. In summary, these results suggest that the photoinduced electron transfer and fluorescence quenching phenomenon can serve as a useful tool to probe the local polarity and solvent-accessibility of PAH-nucleic acid complexes. In this manner, it might be possible to distinguish between intercalation-type complexes (relatively hydrophobic binding sites), and other types of complexes with greater degrees of exposure to the aqueous solvent environment.

5. CONCLUSIONS

The observed solvent polarity and temperature dependence of the fluorescence yields is fully consistent with a photoinduced electron transfer fluorescence quenching mechanism. This effect is likely to play an important role in the decay of excited singlet states of most PAH compounds bound noncovalently or covalently to DNA and other nucleic acids.

6. ACKNOWLEDGEMENTS

We would like to thank Professor V.A. Kuzmin (Moscow) for many stimulating discussions. The work on photoinduced electron transfer was supported by a grant from the Office of Health and Environmental Research, Department of Energy (DE-FGO2-86ER60405); research on oligonucleotide base-sequence effects is supported by the Department of Energy, grant DE-FGO2-88ER60674. The National Synchrotron Light Source (NSLS) at Brookhave National Laboratory is supported by the Office of Chemical Research and the Office of Materials Research, United States Department of Energy (USDOE). The fluorometer at port U9B of the NSLS is supported by the Office of Health and Environmental Research, USDOE.

7. REFERENCES

1. N.E. Geacintov, T. Prusik and J.M. Khosrofian, *J. Am. Chem. Soc.*, "Properties of benzo[a]pyrene-DNA complexes investigated by fluorescence and triplet flash photolysis techniques", 98, pp. 6444-6452, 1976.

2. A.H. Conney, "Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons", *Cancer Res.* 42, pp. 4875-4917, 1982.

3. B. Singer and D. Grunberger, *Molecular Biology of Mutagens and Carcinogens*, Plenum Press, New York, 1983.

4. P. Vigny and M. Duquesne, "Luminescence as a tool in hydrocarbon carcinogenesis", *J. Lum.* 18/19, pp. 587-592, 1979.

5. K. Vahakangas, A. Haugen, and C.C. Harris, "An applied synchronous fluorescence spectrophotometric assay to study benzo[a]pyrene diol epoxide-DNA adducts", *Carcinogenesis* 6, pp. 1109-1115, 1985.

6. R. Jankowiak and G.J. Small, "Fluorescence Line Narrowing: A high-resolution window on DNA and protein damage from chemical carcinogens", *Chem. Res. Toxicol.* 4, 256-269, 1991.

7. N.E. Geacintov and S.K. Kim, "Applications of luminescence techniques in studies of polycyclic carcinogen-nucleic acid interactions", in *Luminescence Techniques in Chemical and Biochemical Analysis, Practical Spectroscopy Series*, Vol. 12, W.R.G. Baeyens, D.D. Keukeleire and K. Korkidis, eds., Marcel Dekker, Inc., New York, pp. 317-339, 1991.

8. P. R. LeBreton, "The intercalation of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene metabolite model compounds into DNA, in: *Polycyclic Hydrocarbons and Carcinogenesis*, R.G. Harvey, ed., ACS Symposium No. 283, The American Chemical Society, Washington, D.C., pp. 209-238, 1985.

9. S.K. Kim, N.E. Geacintov, H.C. Brenner and R.G. Harvey, "Identification of conformationally different binding sites in benzo[a]pyrene diol epoxide-DNA adducts by low temperature fluorescence spectroscopy", *Carcinogenesis* 10, pp. 1333-1335, 1989.

10. N.E. Geacintov, D. Zinger, V. Ibanez, R. Santella, D. Grunberger, and R.G. Harvey, "Properties of covalent benzo[a]pyrene diol epoxide-DNA adducts investigated by fluorescence techniques", *Carcinogenesis* 8, pp. 925-935, 1987.

11. O. Undeman, P.O. Lycksell, A. Gräslund, T. Astlind, A. Ehrenberg, B. Jernström, F. Tjerneld and B. Nordén, "Covalent complexes of DNA and two stereoisomers of benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide studied by fluorescence and linear dichroism", *Cancer Res.* 43, pp. 1851-1860, 1983.

12. V. Ibanez, N.E. Geacintov, A.G. Gagliano, S. Brandimarte and R.G. Harvey, "Physical binding of tetraols derived from 7,8-dihydroxy-9,10-epoxy-benzo[a]pyrene to DNA", J. Am. Chem. Soc. 102, 5661-5666, 1980.

13. L. Margulis, P.F. Pluzhnikov, B. Mao, V.A. Kuzmin, Y.J. Chang, T.W. Scott and N.E. Geacintov, "Photoinduced electron transfer in a covalent benzo[a]pyrene diol epoxide-2'-deoxyguanosine complex", *Chem. Phys. Lett.*, in press.

14. S.C. Cheng, B.D. Hilton, J.M. Roman and A. Dipple, "DNA adducts from carcinogenic and noncarcinogenic enantiomers of benzo[a]pyrene dihydrodiol epoxide", *Chem. Res. Toxicol.* 2, pp. 334-340, 1989.

15. N.E. Geacintov, M. Cosman, B. Mao, A. Alfano, V. Ibanez and R.G. Harvey, "Spectroscopic characteristics and site I/site II classification of cis and trans benzo[a]pyrene diol epoxide enantiomer-guanosine adducts in oligonucleotides and polynucleotides", *Carcinogenesis* 12, pp. 2009-2108, 1991.

16. W.R. Laws and J.C. Sutherland, "The time-resolved photon counting fluorometer at the National Synchrotron Light Source", *Photochem. Photobiol.* 44, pp. 343-348, 1986.

17. H. Knibbe, K. Röllig, F.P. Schäfer and A. Weller, "Charge-transfer complex and solvent-shared ion pair in fluorescence quenching", *J. Chem. Phys.* 47, pp. 1184-1185, 1967.

18. A. Weller, "Photoinduced electron transfer in solution: exciplex and radical ion pair formation free enthalpies and their solvent dependence", *Z. Physik. Chem. N.F.* 133, pp. 93-98, 1982.

19. J.P. Palmans, M. van der Auweraer, A.M. Swinnen, and F.C. De Schryver, "Intermolecular exciplex formation between pyrene derivatives and 1,2-dimethylindole," *J. Am. Chem. Soc.* 106, pp. 7721-7728, 1984.

20. J. Santamaria, "Solvent and salt effects", in: *Photoinduced Electron Transfer, Part B: Experimental Techniques and Medium Effects*, M.A. Fox and M. Chanon, eds., Elsevier, Amsterdam, pp. 483-540, 1988.

21. L.P.A. van Houte, R. van Grondelle, J. Retèl, J.G. Westra, D. Zinger, J.C. Sutherland, S.K. Kim and N.E. Geacintov, "Excited state properties of the N-(deoxyguanosin-8-yl)-2-aminofluorene adducts", *Photochem. Photobiol.* 49, pp. 387-394, 1989.

22. H. Rau and G. Greiner, "Electron transfer in mixed solvents", *Z. Physik. Chem.* 170, pp. 73-85, 1991.

23. H. Staerk, W. Kühnle, R. Treichel and A. Weller, "Magnetic field dependence of intramolecular exciplex formation in polymethylene-linked A-D systems", *Chem. Phys. Lett.* 118, pp. 19-24, 1985.

24. N. Mataga, "Photochemical charge transfer phenomena - picosecond laser photolysis studies", *Pure & Appl. Chem.* 56, pp. 1255-1268, 1984.

25. R.A. Marcus and N. Sutin, "Electron transfers in chemistry and biology", *Biochim. Biophys. Acta* 811, pp. 265-322, 1985.

26. Y. Sakata, H. Tsue, Y. Goto, S. Misumi, T. Asahi, S. Nishikawa, T. Okada and N. Mataga, "Observation of large orientation effect on photoinduced electron transfer in rigid porphyrin-quinone compounds", *Chem. Lett.*, pp. 1307-1310, 1991.

27. J.E. Baggott, "The temperature dependence of photoinduced electron transfer reactions", in: *Photoinduced Electron Transfer, Part B: Experimental Techniques and Medium Effects*, M.A. Fox and M. Chanon, eds., Elsevier, Amsterdam, pp. 420-451, 1988.

28. J.E. Baggott and M.J. Pilling, "Electron donor-acceptor interactions in the quenching of aromatic

hydrocarbons Fluorescence", *J. Chem. Soc. Faraday Trans.* 1, 79, pp. 221-234, 1983.
20. U. Heinpel, M. von Maltzan and C. von Borczyskowski, "Temperature dependence of the electron transfer rates of free base porphyrin-quinone compounds", *Z. Physik. Chem.*, 170, pp. 107-116, 1991.
10. T. Kubota, K. Kano, B. Uno and T. Konse, "energetics of the sequential electroreduction and electroprotonation steps of benzenoid hydrocarbons", *Bull. Chem. Soc. Japan* 60, pp. 3865-3877, 1987.
11. M. Veradi, Zs. Feliér and E. Pungor, "Determination of purine bases by means of chromatovoltammetry", *J. Chromatogr.* 90, pp. 259-265, 1974.
22. B.V. Jovanovic and M.G. Simic, "One-electron redox potentials of purines and pyrimidines", *J. Phys. Chem.* 90, pp. 974-978, 1986.

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