# Comparative Studies of the Binding of Ethidium Bromide and Its Photoreactive Analogues to Nucleic Acids by Fluorescence and Rapid Kinetics†

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ABSTRACT: The binding studies on the interaction of ethidium bromide with DNA have been hampered by its reversibility, which prevents direct isolation and thus characterization of the complex. The recent development of photoaffinity labeling has provided a means to circumvent this problem. However, to be useful as a probe for the parent compound, a photosensitive analogue must be shown to interact in vivo and in vitro just as the parent analogue. These studies demonstrate by steady-state and nonosecond fluorescence and stopped-flow kinetics that one of the azido analogues of ethidium, 8-azido-3-amino-5-ethyl-6-phenylphenanthridinium chloride, binds nucleic acids quite similarly to ethidium bromide. The interaction of the other azide, 3,8-diazido-5-ethyl-6-phenylphenanthridinium chloride, with DNA is qualitatively different from that of the monoazide and ethidium bromide. These results suggest that the monoazide would serve as an ideal probe for determining the actual target sites of ethidium bromide in vivo and in vitro.

The nature of the interactions between ethidium bromide and nucleic acids has been increasingly well characterized in recent years by a variety of physical–chemical techniques (Bresloff & Crothers, 1975; Reinhardt & Krugh, 1978). These interactions are important because of the conformational perturbations in the DNA helix arising from the intercalative-type interaction (Radloff et al., 1967; Waring, 1970; Reinert, 1973; Wang, 1974). Many of the biological properties of ethidium have been attributed directly to these specific interactions (Newton, 1957, 1974; Waring, 1964). In order to study the ethidium–nucleic acid complex, however, only indirect methods are feasible due to the free dissociation of the complex. Even the crystallographic techniques which represent the most direct physical–chemical approach are limited by the necessity of using small nucleic acid models, which may not represent adequately the in vivo nucleic acid binding sites (Jain et al., 1977). Currently, our laboratory has been involved in the development of photosensitive analogues of ethidium as probes for studying drug–nucleic acid interactions (Hixon et al., 1975; Graves et al., 1977). Ideally, the photoreactive analogue should interact just as the parent ethidium bromide prior to photolysis. However, once photolytic activation is initiated, these interactions are rendered irreversible through covalent attachment of the drug in situ (White & Yielding, 1977).

It is important to demonstrate that the interaction of ethidium monoazide with nucleic acids, both prior to and after photolysis, does simulate that of the parent ethidium bromide. This report deals with the comparison of the noncovalent interactions of ethidium bromide and ethidium mono- and diazides with calf thymus DNA, using steady-state and stopped-flow kinetics. The covalent adducts between ethidium monoazide and the dinucleoside monophosphates CpG, UpA, and ApU were compared with the noncovalent ethidium complexes with these nucleic acid models using nanosecond fluorometry. The results support the contention that the monoazide analogue is an ideal model compound for the characterization of ethidium–nucleic acid interactions.

## Materials and Methods

Calf thymus DNA was purchased from Calbiochem and used without further purification. The concentrations of the DNA solutions are stated in terms of nucleotide phosphorus calculated from an extinction coefficient of $\epsilon_{260} = 6600\ M^{-1}\ cm^{-1}$. The dinucleoside monophosphates UpA, ApU, and CpG were obtained from Sigma Chemical Co. and were used without further purification. Their concentrations were based on dried weight. Ethidium bromide was purchased from Calbiochem and recrystallized once in methanol prior to its use in the binding studies or as a precursor in the synthesis of the mono- or diazido analogues (Graves et al., 1977). Drug concentrations were determined spectrophotometrically by using the following extinction coefficients: ethidium bromide, $\epsilon_{476} = 5680\ M^{-1}\ cm^{-1}$; ethidium monoazide, $\epsilon_{458} = 5220\ M^{-1}\ cm^{-1}$; ethidium diazide, $\epsilon_{432} = 5850\ M^{-1}\ cm^{-1}$ (Graves et al., 1977). All compounds were dissolved in 5 mM potassium phosphate buffer (pH 7.0) unless stated otherwise.

Fluorescence spectra were obtained with an Aminco Bowman SPF-125 spectrofluorometer thermostated at 25 °C. The values of emission maxima reported here were taken from uncorrected emission spectra. Fluorescence titrations of DNA with ethidium and its azides were performed with excitation at 510 nm for ethidium, 495 nm for the monoazide, and 450 nm for the diazide. These wavelengths correspond to the isosbestic wavelengths of the ligands in the presence of DNA. The emission wavelengths were 590, 580, and 500 nm for ethidium, the monoazide, and the diazide, respectively. The titration procedures followed the techniques of Peacocke & Skerrett (1956) which maintained a constant drug concentration throughout a range of nucleic acid concentrations.

Fluorescence lifetimes were determined at 4 °C with a single photon-counting pulsed fluorometer (Harvey & Cheung, 1977) equipped with an Ortec 9532 light pulser operated at 3 atm of nitrogen. Samples were excited at 500 nm, and the emission was isolated with a Corning 3-144 filter followed by a 570-nm cutoff filter. Decay data were transferred by teletype to a Sigma 7 computer where the data were deconvoluted by a nonlinear least-squares search based on the Marquardt al-

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Table I: Fluorescence and Equilibrium Binding Parameters of Calf Thymus DNA–Ethidium Complexes[a]

| | ethidium | | ethidium monoazide | | ethidium diazide | |
|---|---|---|---|---|---|---|
| | free | bound | free | bound | free | bound |
| absorption max (nm) | 475 | 520 | 456 | 495 | 430 | 447 |
| emission max (nm) | 600 | 590 | 590 | 580 | 500 | 507 |
| rel intensity[b] | 1 | 17 | 1 | 17.5 | 1 | 0.068 |
| $K_1$ ($M^{-1}$) | | $2 \times 10^6$ | | $1.1 \times 10^6$ | | $0.93 \times 10^6$ |
| $n_1$ (mol of ligand/phosphate) | | 0.20 | | 0.27 | | 0.18 |

[a] The results for the complexes between DNA and mono- and diazides are for the dark complexes prior to photolysis. [b] Intensity relative to emission peak of free ligand.

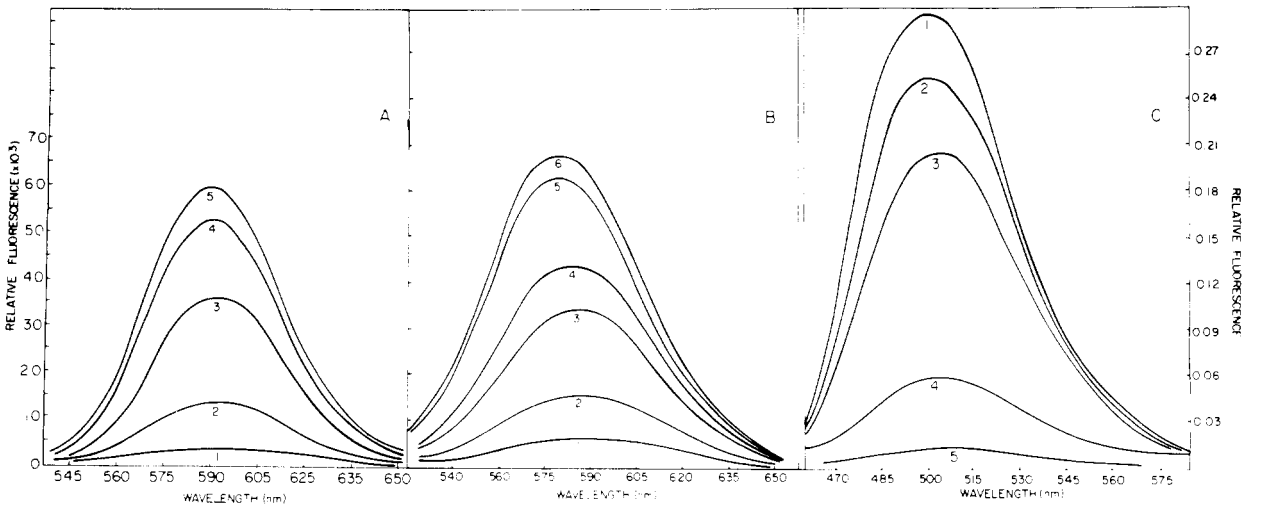


FIGURE 1: Fluorescence spectra resulting from the interaction of ethidium bromide (A), ethidium monoazide (B), and ethidium diazide (C) with calf thymus DNA. In (A), curve 1, free ethidium bromide ($2.71 \times 10^{-5}$ M) was titrated with varying concentrations of DNA as shown by curves 2, 3, 4, and 5, denoting DNA concentrations of $1.77 \times 10^{-5}$, $5.32 \times 10^{-5}$, $1.33 \times 10^{-4}$, and $5.32 \times 10^{-4}$ M, respectively. Curve 5 represents fully bound ethidium bromide with saturating concentrations of DNA. (B) shows the titration curve of ethidium monoazide (free at $2.18 \times 10^{-5}$ M, curve 1) with DNA added in curves 2, 3, 4, 5, and 6 to give DNA concentrations of $2.02 \times 10^{-5}$, $6.05 \times 10^{-5}$, $1.51 \times 10^{-4}$, $4.03 \times 10^{-4}$, and $6.05 \times 10^{-4}$ M, respectively. Fully bound ethidium monoazide is shown as curve 6. (C) shows the titration of ethidium diazide (free at $2.95 \times 10^{-5}$ M, curve 1) with DNA added in curves 2, 3, 4, and 5 to give concentrations of $1.56 \times 10^{-5}$, $3.12 \times 10^{-5}$, $1.56 \times 10^{-4}$, and $4.68 \times 10^{-4}$ M, respectively. Fully bound ethidium diazide is represented by curve 5. The experimental conditions are described under Materials and Methods.

gorithm, a procedure similar to that proposed by Grinvald & Steinberg (1974). The goodness of fit between the observed data and the chosen functions was determined by the criteria of the chi-square ratio ($\chi_R^2$) and the autocorrelation function of the residuals.

The kinetics of ligand binding to DNA were measured at 4 °C with a Durrum D-110 stopped-flow spectrometer operated in the fluorescence mode. The spectrometer was interfaced with a DEC PDP-8/i minicomputer for digitizing kinetic traces. Individual kinetic experiments were analyzed by a Sigma 7 computer by fitting the traces to single- or multiple-exponential functions using a nonlinear least-squares program based on the Marquardt algorithm, as described previously (Garland & Cheung, 1979). The chi-square ratio ($\chi_R^2$) was used to determine whether or not an individual trace could be fitted by a chosen function (Garland & Cheung, 1979). The results of each kinetic experiment reported in Table II represent the mean and standard deviation of four to six individual traces.

Procedures involving the dark interaction of the azido analogues were carried out under a photographic safelight due to the photosensitivity of the analogues. Open shutter time was kept to a minimum although successive spectral scans in the 350–700-nm range showed no photolytic effects on the azide analogues. Covalent adducts between the monoazide and the dinucleoside monophosphates were obtained as described previously for monoazide and DNA covalent adducts (unpublished experiments) with 2 mM nucleotide and 20 $\mu$M azide.

## Results

*Steady-State Fluorescence.* The steady-state spectral properties of the monoazide and diazide complexed with DNA are compared in Table I and Figure 1 with the corresponding parameters for their parent compound. Qualitatively, the fluorescence parameters of the monoazide are similar to those of the ethidium complex. A 10-nm blue shift was observed for each of the bound species, and this spectral shift was accompanied by a large intensity enhancement, suggesting that each fluorescent moiety was protected similarly from solvent molecules. A different spectral distribution was found for the diazide complex. A 7-nm red shift and a large reduction in emission intensity was observed in the presence of DNA.

Further experiments in this laboratory, examining solvent effects on these ethidium analogues, demonstrated that methanol solutions of ethidium bromide and ethidium monoazide showed enhanced fluorescence when compared to their aqueous solutions. However, a methanol solution of the diazide demonstrated a decreased fluorescence compared to its aqueous solution. Olmstead & Kearns (1977) have suggested that the fluorescence enhancement observed for ethidium bromide binding to DNA is due to the environmental effects on the primary amino substituents. Since the diazido analogue has no primary amines, it is difficult to determine the precise

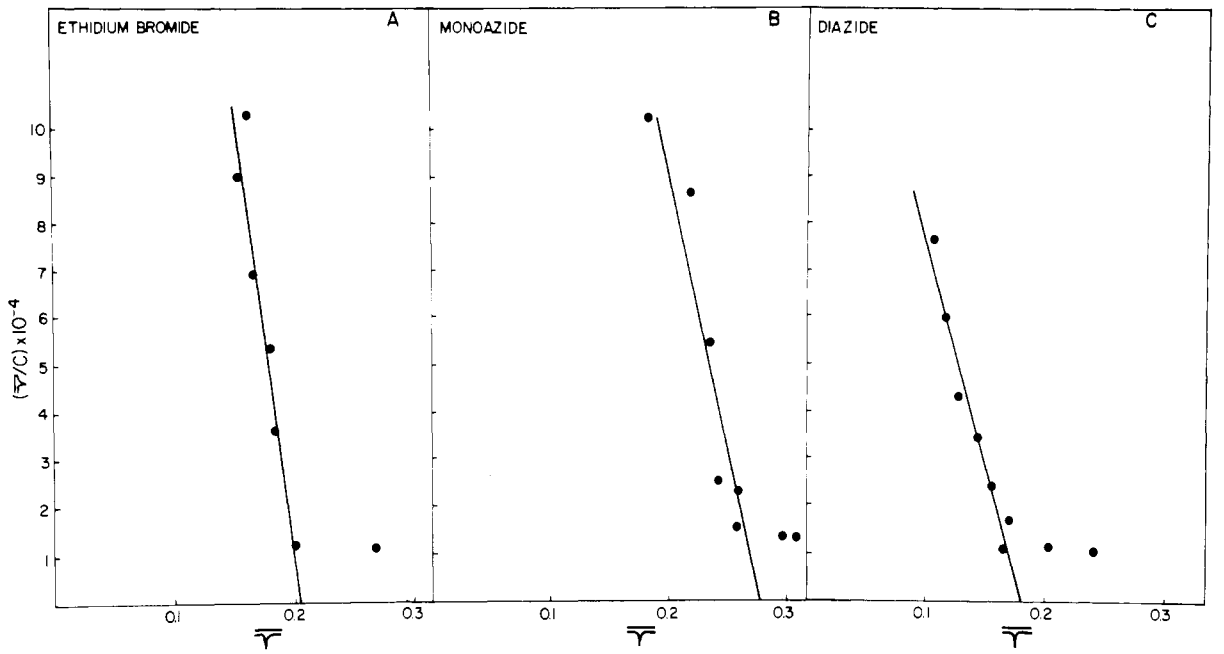


FIGURE 2: Scatchard analysis of the DNA binding with $2.46 \times 10^{-5}$ M ethidium bromide (A), $2.00 \times 10^{-5}$ M ethidium monoazide (B), and $3.15 \times 10^{-5}$ M ethidium diazide (C). Linear regression analysis yielded the following correlation coefficients: 0.98 for ethidium bromide, 0.93 for ethidium monoazide, and 0.98 for ethidium diazide. Experimental conditions were as described in the legend to Figure 1.

Table II: Kinetic Parameters of the Interaction of Calf Thymus DNA with Ethidium and Ethidium Azides[a]

| reaction | $k_{obsd}^{+}$ ($s^{-1}$) | $a^{+}$ | $k_{obsd}^{-}$ ($s^{-1}$) | $a^{-}$ |
|---|---|---|---|---|
| association | | | | |
| ethidium | 217 ± 15 | +0.58 ± 0.02 | 33 ± 3 | +0.42 ± 0.02 |
| monoazide | 211 ± 24 | +0.43 ± 0.02 | 27 ± 3 | +0.57 ± 0.02 |
| diazide | >400 | −0.65 ± 0.08 | 35 ± 6 | +0.35 ± 0.05 |
| dissociation | | | | |
| ethidium | 0.2 ± 0.02 | −0.54 ± 0.03 | 0.03 ± 0.04 | −0.46 ± 0.03 |
| monoazide | 0.15 ± 0.01 | −0.1 ± 0.05 | | |
| diazide | 100 ± 20 | +0.2 ± 0.03 | 0.03 ± 0.06 | +0.2 ± 0.03 |

[a] Reactions were carried out in 10 mM Tris, pH 8.0 and 4 °C. Association reaction: cuvette concentrations (after mixing) were 20 μM ligand and 100 μM DNA. Dissociation reaction: premixed ligand–DNA (20 and 100 μM, respectively) was reacted with a large excess of actinomycin D. The reactions were carried out in the dark. Since it is known that the photolytic reaction between azides and DNA requires minutes for completion, the observed rates for the azides are those of the dark reaction. $k_{obsd}^{+}$ and $k_{obsd}^{-}$ are the observed first-order rate constants of the fast and slow components, respectively. $a^{+}$ and $a^{-}$ are the normalized amplitudes associated with the fast and slow components. Positive or negative values of the amplitudes indicate increased or decreased fluorescence, respectively. The results of each kinetic experiment represent the mean and standard deviation of four to six individual traces. The chi-square ratio ($\chi_R^2$) values for all of the above fits were in the range of 1.0–1.35.

differences or similarities of the DNA binding between the diazide and ethidium.

The binding parameters of these ligands to DNA were deduced from fluorescence titration results and Scatchard plots; the results are given in Table I and Figure 2. Under the present experimental conditions the binding of ethidium could be resolved into two components: one with a high intrinsic binding constant ($K_1$) of $2 \times 10^6$ $M^{-1}$ and a value of $n$ ($n_1$) equal to 0.20 mol of ligand per phosphate group and the other with a low intrinsic binding constant which was not evaluated in the present work because of insufficient data. The present value of $K_1$ is in agreement with the single binding constant for ethidium derived from absorbance (Waring, 1965) and fluorescence (LePecq & Paoletti, 1967) measurements. The binding data of the monoazide from the initial portion of the titration curve gave $K_1 = 1.1 \times 10^6$ $M^{-1}$ and $n_1 = 0.27$ mol of ligand per phosphate group. This binding is comparable to the binding of the parent compound. Because of insufficient data points, no attempt was made to estimate the affinity of the other class of binding sites. The diazide binding also appeared to be similar to that of ethidium as far as the binding affinity was concerned: $K_1 = 0.93 \times 10^6$ $M^{-1}$ and $n_1 = 0.18$ mol of ligand per phosphate group.

*Kinetics of Binding.* The binding and emission results suggested that the monoazide may interact noncovalently with DNA in the dark in a manner similar to ethidium. Further support for this contention came from the results of stopped-flow kinetic studies. The stopped-flow trace resulting from the reaction of ethidium with DNA is compared with that of the monoazide in Figure 3A, while the corresponding trace for the diazide is shown in Figure 3B. The traces for ethidium and the monoazide could not be fitted to a single-exponential function but did fit a two-exponential function, yielding two first-order rate constants. The trace of the diazide was also biphasic and had to be fitted to a two-exponential function, again yielding two first-order rate constants but with amplitudes of opposite sign.

The dissociation of the bound ligands was studied through displacement by actinomycin. The results of these kinetic experiments are summarized in Table II. The reactions of the monoazide and ethidium with DNA showed relatively large increases in the emission signal on binding, suggesting that the monoazide intercalates in a manner similar to ethidium. The two apparent first-order rate constants were the same for

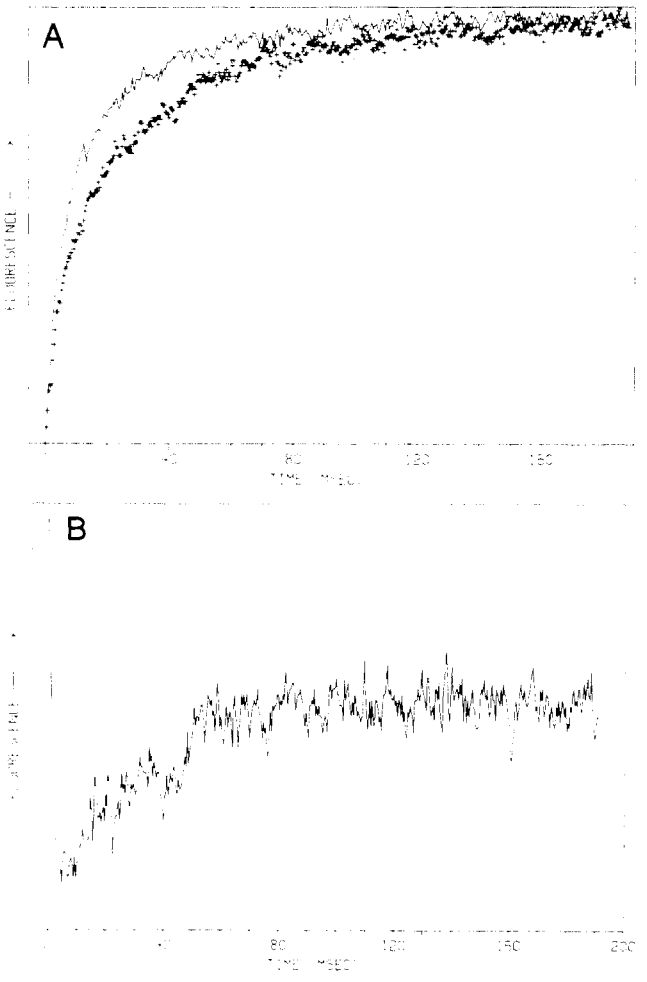


FIGURE 3: Typical kinetic traces of the reaction of DNA with ethidium and its azides. (A) The reaction of 100 $\mu$M DNA with 20 $\mu$M ethidium bromide (—) or 20 $\mu$M ethidium monoazide (+) in 10 mM Tris, pH 8.0 and 4 °C; $\chi_R^2$ = 1.0 and 1.1, respectively. Excitation wavelength was 488 nm for the bromide and 437 nm for the azide; emission was isolated with a 510-nm cutoff filter. (B) The reaction of 100 $\mu$M DNA with 20 $\mu$M ethidium diazide; same conditions as in (A), with excitation at 437 nm and emission isolated with a 510-nm cutoff filter; $\chi_R^2$ = 1.1.

each ligand, but the relative amplitudes of the two components were different.

*Fluorescence Lifetimes of Dinucleoside Monophosphate Complexes.* The lifetime of ethidium complexed with dinucleoside monophosphates and that of the covalent adducts which were formed subsequent to photolysis between the monoazide and these nucleotides were measured. A typical lifetime curve for the covalent adduct between CpG and the monoazide is displayed in Figure 4. The other lifetime results are shown in Table III with the ratio of the coefficients associated with each of the observed lifetimes and with the value of the chi-square ratio. Free ethidium in water solution is known to have a single lifetime of ~2 ns (Olmstead & Kearns, 1977). The 21-ns lifetime observed for the CpG–ethidium complex is characteristic of the intercalation complex formed by this pyrimidine (3′–5′) purine dimer with the ligand. UpA and ApU showed a 2-ns component, suggesting the presence of significant amounts of free ligand. The relatively short lifetime (7.7 ns) observed for the ApU complex suggested that the bound ligand was more exposed to solvent in this complex than in the intercalation complex formed with CpG. The long lifetime of the UpA complex was intermediate between that of the other two species, and the decay pattern of this complex actually did not fit a two-lifetime model, as shown by the

Table III: Fluorescence Lifetime of Ethidium–Dinucleotide Complexes and Covalent Ethidium–Dinucleoside Monophosphate Adducts[a]

| | $\tau_1$ (ns) | $\tau_2$ (ns) | $C_1/C_2$ | $\chi_R^2$ |
|---|---|---|---|---|
| | | Ethidium | | |
| CpG | | 20.8 | | 1.3 |
| UpA | 2.1 | 17.4 | 3.1 | 3.2 |
| ApU | 2.4 | 7.7 | 9.1 | 1.3 |
| | | Monoazide Adduct | | |
| CpG | 2.2 | 19.3 | 0.9 | 1.0 |
| UpA | 1.4 | 15.2 | 6.7 | 4.2 |
| ApU | 1.6 | 6.2 | 8.6 | 1.7 |

[a] Measurements were carried out at 4 °C in 0.15 M NaCl and 0.15 mM sodium citrate, pH 7.0, with 2 mM nucleotide in the presence of 20 $\mu$M ligand. $C_1/C_2$ is the ratio of amplitudes associated with the short ($\tau_1$) and long ($\tau_2$) lifetimes: $F(t) = C_1 \exp(-t/\tau_1) + C_2 \exp(-t/\tau_2)$.

relatively high value of the chi-square ratio (3.2). This finding indicates that the long lifetime (17.4 ns) is likely resolvable into two components, which would suggest the presence of two different forms of the UpA–ethidium complex in solution.

Similar lifetime results were found for the monoazide–dinucleoside monophosphate covalent adducts. The presence of a 2.2-ns short component for the CpG adduct reflects the lower binding affinity of CpG for the monoazide in the dark, which was demonstrated from titration studies. The uncomplexed monoazide upon photolysis apparently did not react with the dinucleoside monophosphate but remained in solution as an unbound species. The possibility of a second form of the UpA–monoazide adduct was also suggested by the high value of the chi-square ratio (4.2), just as in the case of the UpA–ethidium complex. These results can be taken as evidence that both ethidium and its monoazide bind similarly to the three dinucleoside monophosphates. The binding kinetics of the interactions between either ligand and the dinucleoside monophosphates could not be measured. Even at 4 °C the reactions were so rapid that a large intensity enhancement was observed within the mixing time of the stopped-flow spectrometer and this signal could not be resolved.

## Discussion

The present fluorescence lifetime results show that the environment of ethidium bound to dinucleoside monophosphates is very similar to that of the fluorescent moiety of ethidium monoazide which is covalently attached to the dinucleoside monophosphates upon photoactivation. The similarity was observed with three different dinucleoside monophosphates which are known to have different degrees of preference for ethidium. Recent X-ray crystallographic studies of the solid-state complexes of ethidium bromide with 5-iodo-CpG and 5-iodo-UpA showed that ethidium was both intercalated and stacked over the miniature double helical complex (Jain et al., 1977; Tsai et al., 1975). These fluorescence lifetime data suggest that the CpG–drug complex appeared to be predominantly intercalative in nature. However, in the case of UpA, the interaction of ethidium and its monoazide appeared not to be entirely through intercalation as demonstrated by the lifetimes which were significantly shorter than the 20–21 ns observed for CpG. The lifetime data suggest the presence of two species of ethidium–UpA complexes and of ethidium monoazide–UpA adducts under our experimental conditions. The ApU interaction with ethidium and its monoazide appeared to be essentially nonintercalative. These results are supported by the observations of Reinhardt & Krugh (1978) and Kastrup et al. (1978) which demonstrated a pyrimidine–purine base specificity for ethidium–DNA binding by

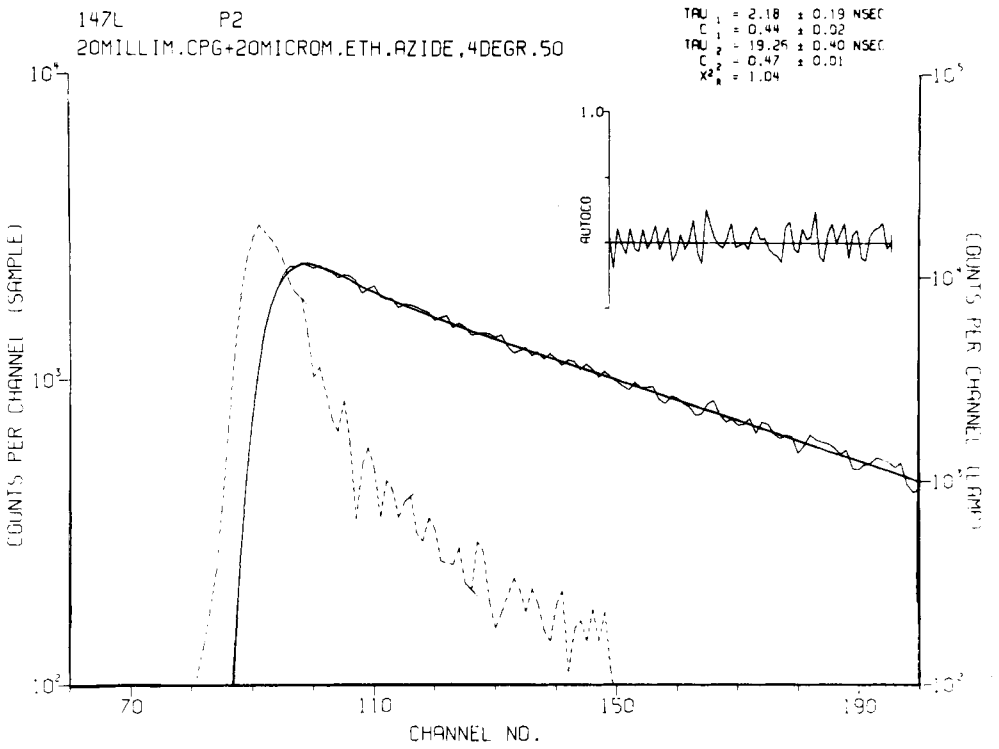


FIGURE 4: Fluorescence decay of 20 $\mu$M ethidium monoazide covalently linked to 20 mM CpG in 0.15 M NaCl and 0.15 mM sodium citrate at pH 7.0 and 4 °C. The observed decay is fitted to a two-exponential function (solid curve) with the autocorrelation of the residuals shown in the inset. The dashed curve represents the lamp pulse profile. Timing calibration: 0.36 ns/channel.

fluorescence lifetime measurements.

The two apparent first-order rate constants that were observed for the binding of both ethidium and the monoazide to DNA were essentially identical, but their relative amplitudes were quite different. The heterogeneity of binding sites and the relatively low $P/D$ ratio used in the experiments preclude a firm interpretation. A likely explanation would be that the two observed rates represent ethidium binding to the two pyrimidine (3′–5′) purine sites of DNA, dC-dG, and dT-dA, as is suggested by the lifetime results that were obtained with dinucleoside monophosphates. Interactions with other base sequences (Krugh & Nuss, 1979) at high binding ratio cannot be excluded. Since the observed dissociation rate constants are some 2 orders of magnitude smaller than the observed binding rate constants (see Table II) and the binding reactions were carried out under identical concentrations for both ligands, it seems reasonable to suggest that the binding rate constants for the association of the two ligands are equal for each of the two sites. The difference in the relative amplitudes of ethidium and its monoazide could then be due to (1) differences in the quantum yield of the two ligands bound to the dC-dG and dT-dA sites, (2) differences in the dissociation rates of the ligands bound to at least one of the sites, or (3) a combination of both possibilities. The results in Table I indicate a very small difference in the relative quantum yield between the two bound species, a finding which is in accord with the recent observation of Bolton & Kearns (1978). The dissociation of bound ethidium showed two first-order processes, whereas only one process was detected for the dissociation of bound monoazide. This difference in the dissociation kinetics is reflected in the small difference of the observed binding constants for the two ligands (Table I). These considerations indicate that while there are subtle differences between the interaction of ethidium and its monoazide with DNA, the overall binding is similar for both ligands. While subtle differences between the ligands are not unexpected, they become detectable when a combination of both equilibrium and kinetic techniques is used to study the same system. The recent work of Bolton & Kearns (1978) shows that 80% of ethidium molecules covalently linked to tRNA have a very long lifetime, and the remaining 20% have a short lifetime. These results and their demonstration that the ethidium sites and the monoazide sites are mutually exclusive provide further support for our present conclusions.

The reaction of the diazide with DNA is qualitatively quite different from that of the other ligands. For the association reaction, the fast component of the biphasic trace had a negative amplitude while the slow component had a positive amplitude. In addition, the fast component had a rate constant in excess of 400 $s^{-1}$, which was at least twice as fast as the corresponding component of the other ligands. These results could be interpreted in terms of a sequential two-step mechanism: formation of an "outside bound" diazide–DNA complex followed by a first-order process possibly involving partial intercalation of the phenanthridinium ring. This mechanism could account for the large quenching of the diazide emission in the presence of DNA. The equilibrium constant between the two bound forms of the diazide complex suggested by this simple two-step mechanism would be the ratio of the observed forward and reverse rate constants: 35/0.43 = 80. The dissociation rates of the bound diazide are ~2 orders of magnitude faster than those involving ethidium and the monoazide. These kinetic results clearly indicate that the binding mechanism of the diazide is not similar to that of the other ligands. This conclusion is corroborated by fluorescence spectral results which showed that the binding of the diazide to DNA is accompanied by a red spectral shift and a large reduction of fluorescence intensity. The spectral changes of the other two ligands resulting from binding to DNA were in the opposite direction. In contrast, similar changes were observed in the absorption spectra (hypochromicity and red shift) of all three ligands in the presence of DNA (unpublished experiments). This finding suggests that in the diazide interaction with DNA, fluorescence detects either different or

additional processes that are not monitored by absorbance measurements. The apparent similarity in the equilibrium binding properties between ethidium and the diazide is fortuitous and should not be taken as evidence that the two ligands interact with DNA in a similar way, since they bind to different sites, as suggested by the kinetic results.

The photosensitive drug analogues are especially interesting for probing biological drug actions. This laboratory has observed that the ethidium monoazide, on photolysis in situ, is many times more effective than its parent compound (40–100-fold, depending on the system) in producing frameshift mutations in *Salmonella* (Yielding & Firth, 1980), in producing mitochondrial mutations in yeast (Fukunaga et al., 1980), in producing antitrypanosomal activity (unpublished results), and in provoking DNA repair in human lymphocytes (Cantrell & Yielding, 1977). These and the present studies suggest that the monoazide may be more effective due to its amplification of the ethidium bromide activity through covalent attachment. The present studies may also explain, to some extent, why the diazide is somewhat less effective than the monoazide but sitll more effective than the parent ethidium. The diazide may have a small portion intercalated covalently, and this interaction should appear as enhanced ethidium activity. However, the monoazide should all be bound covalently in the same orientation as the parent ethidium, thus magnifying the ethidium activity many-fold.

In summary, we have shown that the interaction of ethidium with DNA is mechanistically similar to that of its monoazide. This information which was derived from equilibrium and kinetic fluorescence studies provides additional support for using the monoazide as a photoaffinity label to target the action of ethidium on nucleic acids.

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