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Ethidium Bromide–Dinucleotide Complexes. Evidence for Intercalation and Sequence Preferences in Binding to Double-Stranded Nucleic Acids

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Synopsis

The solution complexes of ethidium bromide with nine different deoxydinucleotides and the four self-complementary ribodinucleoside monophosphates as well as mixtures of complementary and noncomplementary deoxydinucleotides were studied as models for the binding of the drug to DNA and RNA. Ethidium bromide forms the strongest complexes with pdC-dG and CpG and shows a definite preference for interaction with pyrimidine–purine sequence isomers. Cooperativity is observed in the binding curves of the self-complementary deoxydinucleotides pdC-dG and pdG-dC as well as the ribodinucleoside monophosphates CpG and GpC, indicating the formation of a minihelix around ethidium bromide. The role of complementarity of the nucleotide bases was evident in the visible and circular dichroism spectra of mixtures of complementary and noncomplementary dinucleotides. Nuclear magnetic resonance measurements on an ethidium bromide complex with CpG provided evidence for the intercalation model for the binding of ethidium bromide to double-stranded nucleic acids. The results also suggest that ethidium bromide may bind to various sequences on DNA and RNA with significantly different binding constants.

INTRODUCTION

Ethidium bromide–nucleic acid complexes have been extensively studied by a variety of techniques.[1–7] It is generally accepted that the strong mode of binding of ethidium bromide (Figure 1) to double-stranded nucleic acids occurs by intercalation of the planar phenanthridinium ring between adjacent base pairs. The intercalation model was proposed by Lerman[8] and in the past decade a variety of drugs have been shown to interact with DNA in this manner. A variety of physicochemical measurements have been used to determine if a drug molecule intercalates into the DNA helix. Often a drug is assumed to intercalate if the observed effect is similar to the effect produced by ethidium bromide or the aminoacridine dyes. For

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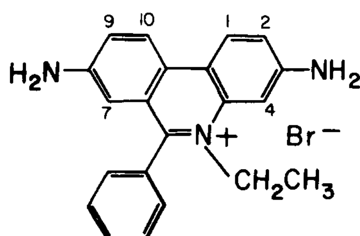

Fig. 1. Structural formula of ethidium bromide.

example, a decrease in the sedimentation coefficient and an increase in the viscosity of DNA upon the addition of the drug indicates intercalation.[9] The interaction of a drug with superhelical closed circular DNA is also a good means of determining if a drug intercalates.[10–12] The intercalation model is not universally accepted and other models have been proposed[13] including one in which the aromatic heterocyclic rings are located on the outside of the helix.[14] The present experiments were designed to determine the nature of the ethidium bromide–nucleic acid complex using deoxy and ribose dinucleotides as models for DNA and RNA, respectively. We have recently investigated the interaction of actinomycin D with a series of deoxydinucleotides.[15–16] Actinomycin D forms a complex with *two* pdG-dC dinucleotides in a cooperative fashion: we interpreted this as supporting evidence that actinomycin D forms an intercalated complex with DNA.[8,17,18] Our actinomycin D experiments[15,16] also suggested that the dinucleotides could be used as a good model system to investigate the nature of the drug–DNA complex. The present experiments support this observation and provide additional information concerning the possible sequence preferences of the interaction of ethidium bromide with nucleic acids.

EXPERIMENTAL

Ethidium bromide and the ribodinucleoside monophosphates were purchased from Sigma Chemical Company; the deoxydinucleotides were purchased from Collaborative Research, Inc. Ethidium bromide and the deoxydinucleotides were dissolved in a 5-m*M*, pH 7.1, PIPES buffer [piperazine-*N*-*N*′-bis(2-ethane sulfonic acid) monosodium·dihydrate, Calbiochem A grade]. The ribodinucleoside monophosphates were dissolved in a 5-m*M* potassium phosphate buffer in D_2O, pH(meter) = 7.0 in order to facilitate nuclear magnetic resonance experiments. For the visible spectral titrations 2-ml aliquots of the ethidium bromide stock solution were generally used; the nucleotides were added with a Hamilton microliter syringe through a preequilibrated rubber septum on the optical cell. The spectra were corrected for the dilution effect of the addition of the nucleotides. The concentration of the ethidium bromide solutions was determined spectrophotometrically, using $\epsilon_{480} = 5600$.[19] For the visible spectral titrations the concentration of the ethidium bromide was kept in the region where the absorbance was linearly dependent on concentration. The con-

centrations of the nucleotide solutions were also determined spectrophotometrically, using the extinction coefficients supplied by Collaborative Research and P-L Biochemicals. We estimate that the uncertainty in the extinction coefficients supplied by Collaborative Research is ±10%. Several of the deoxydinucleotides contained a small amount of an impurity that absorbed visible light even after the solutions were filtered through an 8-μm Millipore filter. The spectra were corrected for this impurity. The visible spectral titrations were performed on a Cary model 14 spectrophotometer with a Forma model 2095-2 temperature regulator. The spectra were recorded in a 1-cm cell, with the temperature regulated at 25°C. The visible spectra of the solutions used in the circular dichroism (CD) experiments were usually recorded in a 2-mm cell and were measured at the same (bath) temperature used for the CD measurements. The circular dichroism measurements were performed on a Cary-60 spectrophotometer with a Cary model 6002 circular dichroism attachment using 1-mm thermostatted cells. The θ values are direct readings from the recorder. The nuclear magnetic resonance spectra were measured on a JEOL PFT-100 100-MHz Fourier transform nmr spectrometer.

RESULTS

The change in the absorbance of ethidium bromide at 460 nm is plotted in Figure 2 against increasing deoxydinucleotide concentration for a series of deoxydinucleotides. The most salient feature of these data is the striking difference between the titration curve of ethidium bromide with pdC-dG as compared to any other deoxydinucleotide. The difference in the association of ethidium bromide with the sequence isomers pdC-dG and pdG-dC is evident in the data in Figure 2. The difference curves for the four self-complementary deoxydinucleotide complexes with ethidium bromide had essentially the same overall shape. All four curves had a minimum in the 460-nm region, a maximum at ~540 nm, and crossed through zero in the 495–510 nm region. However, the amplitudes of ΔA at the minimum and maximum points (~460 and ~540 nm) were quite dependent upon the nature of the dinucleotide as indicated by the data in Figure 2. The analysis of the data in terms of equilibrium constants is not straightforward. Double reciprocal plots and computer analysis show that the titration curves for the self-complementary deoxydinucleotides pdC-dG and pdG-dC are not hyperbolic. Within experimental error, the pdA-dT and pdT-dA titration curves at 25°C are well represented by a hyperbolic binding curve. A comparison of the nucleotide concentrations that produce a change in the absorbance equal to one-half of the extrapolated maximum change in absorbance provides a qualitative measure of the relative binding constants. Using this criterion, the data in Figure 2 show that pdC-dG binds the strongest of all the deoxydinucleotides tested. A comparison of the binding curves for the sequence pairs pdC-dG and pdG-dC, pdT-dG and pdG-dT (3 and 7), and pdT-dA and pdA-dT (4 and 6)

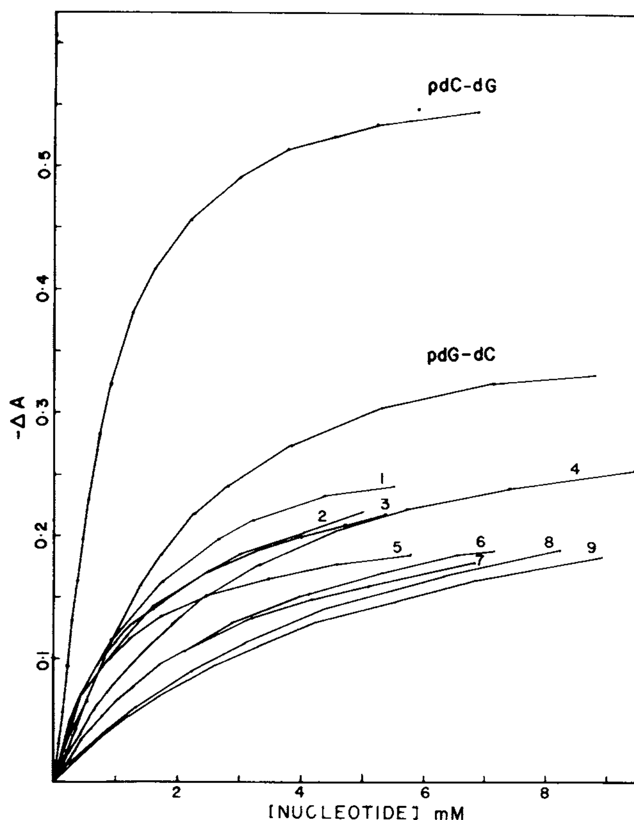

Fig. 2. Effect of various deoxydinucleotides on the absorbance of ethidium bromide at 460 nm (25°C). *1*—pdA-dG; *2*—pdG-dA; *3*—pdT-dG; *4*—pdT-dA; *5*—pdC-dA; *6*—pdA-dT; *7*—pdG-dT; *8*—5′-dGMP; *9*—5′-dAMP. The ethidium bromide concentration at the start of each titration was 2.7×10^{-4} *M*.

indicates that ethidium bromide binds to the dinucleotides with a pyrimidine–purine sequence stronger than to their respective sequence isomers in these dinucleotide pairs. The association of the ethidium bromide and the self-complementary ribodinucleotide monophosphates provides even more striking results as shown in Figure 3. Once again the CpG sequence shows the strongest binding of the four self-complementary dinucleotides, although all four of these dinucleotides will form a complex with ethidium bromide. The difference curves for these complexes are shown in Figure 4. These curves have approximately the same overall shape except that the magnitude of the induced spectral change for complex formation of ethidium bromide with CpG is much larger than with the other nucleotides (at 25°C). A consideration of the nucleotide concentrations that produce a half-maximum change in the absorbance indicates that ethidium bromide binds stronger to CpG when compared to GpC. A more detailed analysis in terms of binding constants requires a number of assumptions and will not be presented.

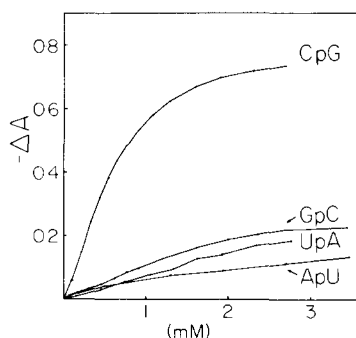

Fig. 3. Effect of the self-complementary ribodinucleoside monophosphates on the absorbance of ethidium bromide at 460 nm (25 °C). The ethidium bromide concentration at the start of each titration was 2.7×10^{-4} *M*.

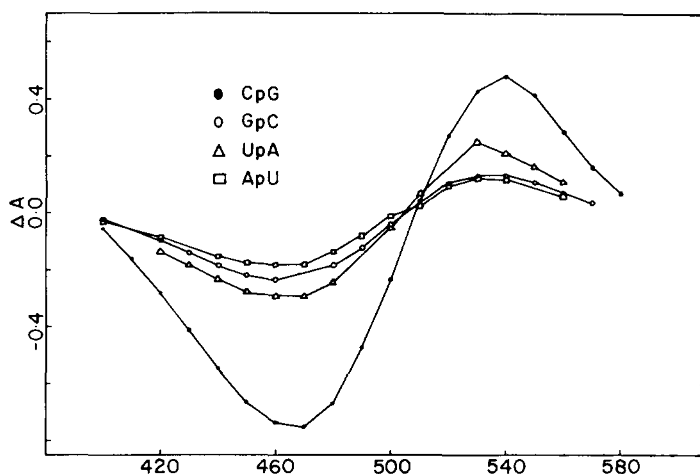

Fig. 4. Difference curves for the ethidium bromide complexes with the ribodinucleoside monophosphates. The curves were calculated point by point from a spectrum obtained at the end of each titration. The final nucleotide concentrations were CpG (2.7 m*M*), GpC (2.7 m*M*), UpA (4.7 m*M*), and ApU (6.3 m*M*).

Preliminary studies on complexes of actinomycin D with selected deoxydinucleotides had shown that circular dichroism provides potentially useful information about these systems (H. E. Auer and T. R. Krugh, unpublished results). Circular dichroism studies on ethidium bromide–dinucleotide complexes are especially interesting because ethidium bromide by itself is optically inactive. When ethidium bromide binds to double stranded nucleic acids a characteristic induced circular dichroism spectrum is observed in the 300–600 nm region.[7,20–24] The induced circular dichroism spectra of ethidium bromide in the 300–600-nm region for complexes with the self-complementary nucleotides dC-dG, CpG, and UpA are shown in Figures 5 and 6. These spectra are very similar to the published CD spectra for ethidium bromide bound to double-stranded DNA and

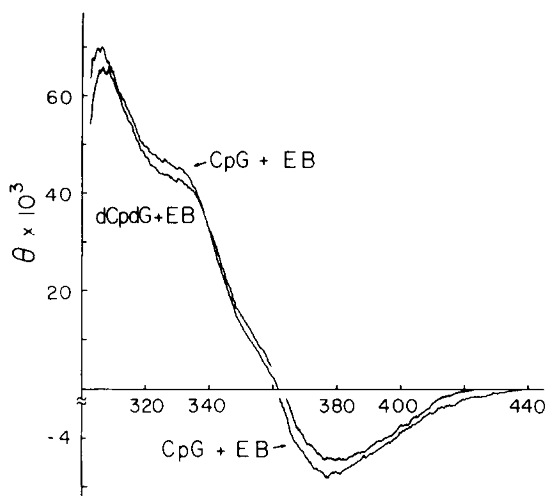

Fig. 5. Induced CD spectra of ethidium bromide upon addition of CpG and dC-dG in a 1-mm thermostatted cell at 3°C. The samples were 0.75 mM EB and 2.3 mM CpG and 0.75 mM EB and 3.2 mM dC-dG. Both samples were dissolved in a D_2O potassium phosphate buffer (5 mM) pD = 7.4 (pH meter + 0.4). The θ values are direct readings from the recorder. Note that the scale of the ordinate has been expanded below the zero level.

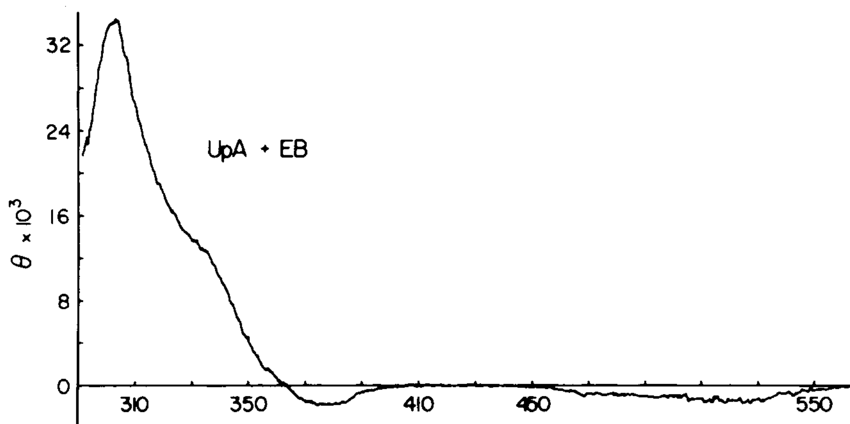

Fig. 6. Induced CD spectrum of ethidium bromide (0.41 mM) upon addition of UpA (2.0 mM) in a 1-mm cell at 0°C. A similar sample containing ethidium bromide (0.41 mM) and ApU (2.4 mM) did not exhibit an induced CD spectrum in the 300–600-nm region.

RNA, providing a good indication that the geometries of the complexes formed between ethidium bromide and the dinucleotides are very similar to the geometry of the DNA and RNA ethidium bromide complexes. Under the conditions used in Figure 6 the nucleotide ApU does not induce a CD spectrum in ethidium bromide. The observation of a CD spectrum appears to be correlated with the shift in the absorption maximum of ethidium

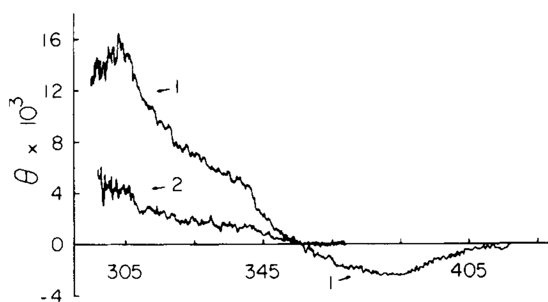

Fig. 7. Induced CD spectra of solutions of ethidium bromide (0.37 m*M*) with 8 m*M* pdT-dA (curve *1*) and 8 m*M* pdA-dT (curve *2*) at 2°C in a 1-mm cell.

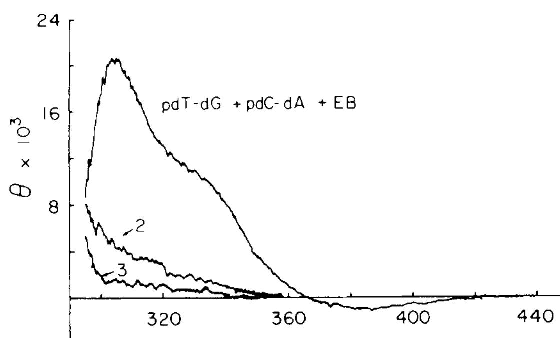

Fig. 8. Induced CD spectra of ethidium bromide (0.41 m*M*) upon addition of the *complementary* nucleotides pdT-dG (1.2 m*M*) + pdC-dA (1.2 m*M*) in a 1-mm cell at 0°C. A mixture of the *noncomplementary* nucleotides pdT-dG + pdA-dC (both 1.2 mM) and ethidium bromide (0.41 m*M*) gave rise to curve *3* under identical conditions used above. Curve *2* is the induced circular dichroism spectrum for a solution of ethidium bromide (0.41 m*M*) and the *complementary* nucleotides pdG-dT (2.7 m*M*) + pdA-dC (2.6 m*M*) containing the purine–pyrimidine sequence.

bromide (EB) as it forms a complex with the nucleotides. The UpA + EB solution had a λ_{max} = 513 nm (0°C) while the ApU + EB solution had λ_{max} = 488 nm. The association of pdT-dA and pdA-dT with ethidium bromide also demonstrates the apparent correlation of the shift in λ_{max} beyond ~495 nm and the observation of a CD spectrum. A solution of 8-m*M* pdT-dA and 0.37-m*M* EB had λ_{max} of 485 nm at 25°C and essentially no induced CD spectrum. Upon cooling to 2°C the absorption maximum shifts to 515 nm, and a CD spectrum is observed (curve *1* of Figure 7). On the other hand, a solution of 8-m*M* pdA-dT and 0.37-m*M* EB has a λ_{max} of ~485 nm at 25°C and shifts only to 498 nm at 2°C with the concommitant induction of the CD spectrum shown in curve *2* of Figure 7.

The role of complementarity is nicely demonstrated by a comparison of the visible and circular dichroism spectra of mixtures of complementary and noncomplementary nucleotides. The induced circular dichroism spectrum of ethidium bromide in a solution of the complementary nucleotides pdT-dG and pdC-dA is shown in Figure 8 and is in sharp contrast to the very weak

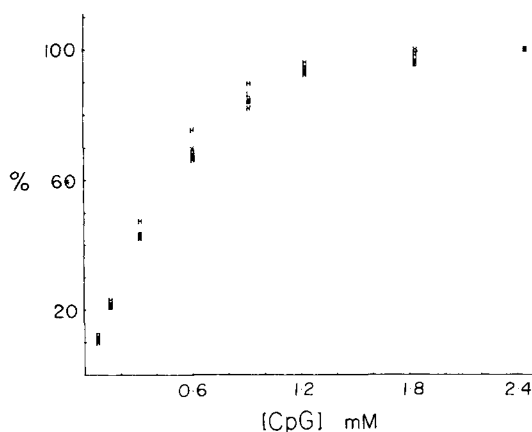

Fig. 9. Comparisons among the fractional changes in the visible spectrum of ethidium bromide at 460 and 540 nm ($\Delta A/\Delta A_{max}$) and the fractional CD observed (θ/θ_{max}) at 310, 320, and 330 nm. The symbols used are: *H*, vis(460); *N*, vis(540); *X*, CD(310); *L*, CD(320); *O*, CD(330). The data were scaled by defining the observed values of ΔA and θ at 2.4 m*M* CpG as ΔA_{max} and θ_{max}. The experiments were performed at 2°C in a 1-mm cell with an ethidium bromide concentration of 4.6×10^{-4} *M*.

CD spectrum observed for a solution of ethidium bromide with the noncomplementary nucleotides pdT-dG and pdA-dC (curve *3* of Figure 8). A solution of the complementary dinucleotides pdG-dT and pdA-dC (purine–pyrimidine sequence) with ethidium bromide gave rise to curve *2* of Figure 8. The absorption maximum of ethidium bromide was 508 nm for the mixture of the complementary pyrimidine–purine sequence dinucleotides pdT-dG + pdC-dA; for the noncomplementary mixture pdT-dG + pdA-dC, λ_{max} was 489 nm; and for the mixture of the purine–pyrimidine sequence dinucleotides pdG-dT + pdA-dC, λ_{max} was 498 nm. There was no appreciable induced circular dichroism observed for solutions of ethidium bromide and any *one* of the nucleotides used in Figure 8.

For the titration of ethidium bromide with CpG the fractional change in the visible spectra ($\Delta A/\Delta A_{max}$) is fairly well correlated with the corresponding fractional CD observed (θ/θ_{max}) as shown in Figure 9. The 460-nm data and the 540-nm data from the visible spectra tend to be on either side of the CD data taken at 310, 320, and 330 nm. Within experimental error, the fractional increase in the CD data at each of these wavelengths was the same; this indicates that the CD bands centered at 305–310 nm and ~330 nm are simultaneously induced throughout the titration. The shift in the absorption maximum is also correlated to the fractional change in the visible spectrum ($\Delta A/\Delta A_{max}$) and the fractional CD observed (θ/θ_{max}).

The CD spectrum for an EB + dG-dC solution is shown in Figure 10. Although the magnitude of the induced *circular dichroism* of *the* EB + dG-dC *solution* (λ_{max} = 505 nm at 3°C) is much less than for the correspon-

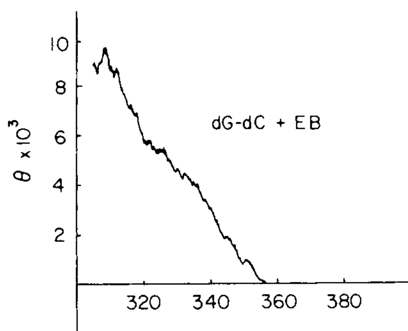

Fig. 10. Induced CD spectrum of ethidium bromide (0.75 m*M*) upon addition of dG-dC (2.7 m*M*) in a 1-mm cell at 3°C.

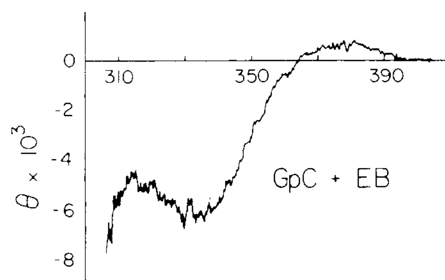

Fig. 11. Induced CD spectrum of an ethidium bromide (0.75 m*M*) solution with 3.1 m*M* GpC at 3°C in a 1-mm cell.

ding EB + dC-dG solution shown in Figure 6 (with $\lambda_{max} \cong 515$nm), there still appears to be positive bands at ~310 and ~330 nm (Figure 10). The induced CD spectrum of an EB + GpC solution is shown in Figure 11 and is in contrast to all the other CD spectra recorded to date. The small θ values should be compared to the CD spectrum of an EB + CpG solution (Figure 6). The GpC–EB complex exhibits a small positive band at ~380 nm and a negative band at 330 nm.

The nuclear magnetic resonance spectra of ethidium bromide, the nucleotide CpG, and the EB + CpG solution are shown in Figure 12. The H1 and H10 protons of ethidium bromide are doublets ($J_{H1,H2} \cong J_{H9,H10} \cong$ 8.5 Hz) but appear in the spectra as the downfield triplet due to overlap of two of the peaks. The asymmetric triplet next to the H1 and H10 protons is assigned to the meta and para protons of ethidium bromide while the upfield peak in the aromatic region is the H7 proton. These assignments are in agreement with the previous work of Kreishman et al.[25] In forming the EB–CpG complex it is obvious that several of the ethidium resonances are shifted upfield. These shifts are a result of the magnetic shielding effects of the guanine and cytosine rings and may be used to determine the geometry of the complex (for a discussion and illustration of these techniques see Refs. 16 and 26). The H7 proton has been shifted upfield at least 0.8 ppm (and disappears under the nucleotide resonances) while the

H1 and H10 protons are shifted upfield ~0.6 ppm. The *meta* and *para* protons of the phenyl ring are relatively unchanged while the H2, H4, H9, and *ortho* protons are shifted upfield by varying amounts. In addition, the methyl resonance is shifted upfield ~0.1 ppm. The magnitude and direction of these shifts are only consistent with the stacking of the guanine and phenanthridinium rings. The presence of 1 *M* NaCl does not significantly alter either the CD or the nmr spectra (although it does diminish the apparent binding constants both here and with the binding of ethidium bromide to DNA and RNA). Finally, when ethidium bromide binds to double-stranded DNA and RNA the helix is stabilized with respect to thermal denaturation.[1-7,20] This effect has also been observed for the binding of ethidium bromide to CpG by directly observing the NH proton of guanine in a solution containing 0.02 *M* EB and 0.04 CpG in H_2O, pH = 7.0. (T. R. Krugh, manuscript in preparation). In a solution containing only CpG the NH proton rapidly exchanges with solvent water protons and is unobservable. If this proton forms a hydrogen bond, then the exchange rate is diminished and a broad low-field resonance appears. The observation of the NH proton almost certainly indicates the formation of Watson-Crick base pairs between the complementary cytosine and guanine bases on opposite nucleotides (e.g., Ref. 27). In the present experiments the ethidium acts as a nucleation center for helix formation between complementary nucleotides and therefore effectively stabilizes the helix.

DISCUSSION

The data clearly show that ethidium bromide is able to disciminate and preferentially bind to deoxydinucleotide and ribodinucleoside monophosphate sequence isomers. The CpG binding curve is unique in both the deoxy and ribose dinucleotide titrations at 25°C; this nucleotide binds the strongest of all the nucleotides tested. The ribodinucleoside monophosphates generally appear to bind slightly stronger than their deoxy analogs, but the data presented in Figures 2 and 3 involve deoxydinucleotides (e.g., pdC-dG) and ribodinucleoside monophosphates (e.g., CpG) so that we cannot directly compare the deoxy and ribodinucleotide data because the terminal phosphate of the dinucleotides increases the solubility of the compounds and decreases the strength of the interaction. The sigmoidal shape of the titration curves indicates that ethidium bromide forms a complex with more than one CpG molecule in a *cooperative* manner. The similarity of the circular dichroism and visible spectra of the complexes of ethidium bromide with certain of the dinucleotides in the present study to the spectra reported in previous studies with DNA and RNA provides a good indication that the dinucleotides (both deoxy and ribo) serve as good model compounds for the nucleic acids. Two complementary nucleotides must bind to an ethidium molecule in order to form a double helix, and thus it is inappropriate to discuss the complexation of ethidium bromide with the dinucleotides in terms of only a single binding constant. All of the

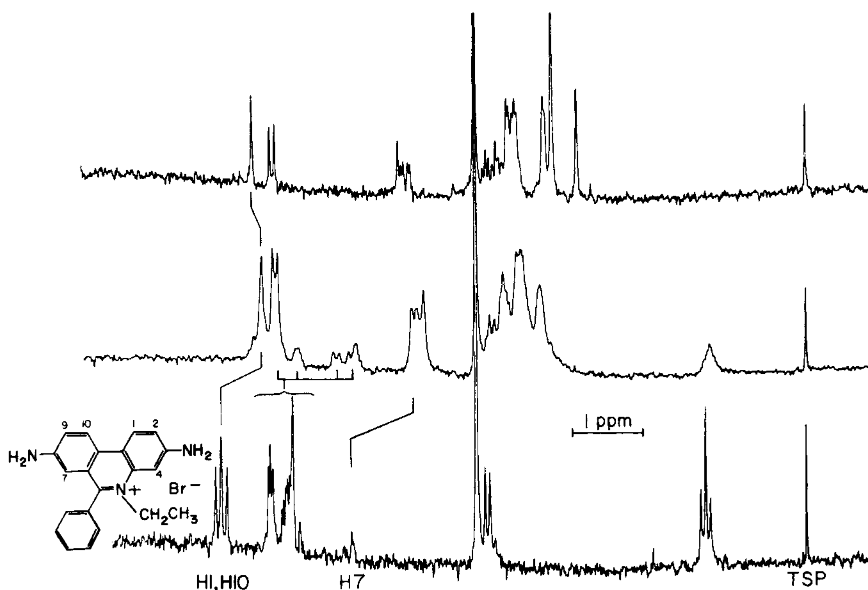

Fig. 12. 100-MHz proton Fourier transform nmr spectra of 0.9 mM CpG (top); 2.3 mM CpG + 0.75 mM EB (middle); and 0.75 mM EB (bottom). The spectra were run at 25°C with a 180–τ–90 (accumulate) pulse sequence to minimize the residual HDO peak. The use of this pulse sequence accounts for the small H7 peak since it has a longer T_1 value than the other EB ring protons. The movement of the peaks has also been followed by the incremental addition of the CpG. The samples were dissolved in a D_2O potassium phosphate buffer (5 mM) and the pD was adjusted to 7.4 (pH meter reading + 0.4). The small amount of dimerization present in the ethidium bromide solution (bottom spectrum) does not significantly affect the appearance of the spectrum. The induced chemical shifts of the various ethidium protons given in the text were calculated on the basis of the infinite dilution chemical shifts.

ethidium–nucleotide complexes are stabilized at low temperature, which is why the CD spectra were recorded at 0–3°C. Even at these low temperatures, the noncomplementary dinucleotides did not induce a CD spectrum (e.g., Figure 8) or shift the absorption maximum beyond ~490 nm, although they do form complexes with ethidium. On the other hand, the complexes of ethidium bromide with the complementary dinucleotides pdC-dG, pdG-dC, CpG, GpC, UpA, pdT-dA, and pdA-dT do exhibit a circular dichroism spectra in the 300–400-nm region. The magnitude of the induced CD for these complexes appears to be correlated with both the fractional change in the absorbance at 460 nm and the shift in the absorption maximum of the ethidium bromide–dinucleotide solutions. This behavior is consistent with the ethidium bromide molecule acting as a nucleation center for the formation of a helix by two complementary dinucleotides. The results obtained with the complementary mixtures of dinucleotides (Figure 8) also buttress the conclusion that complementary nucleotides form a minihelix around an ethidium bromide molecule. The nmr spectra (Figure 12) of the solutions used for the visible and circular

dichroism studies conclusively show that the complex formation involves a stacking of the ethidium bromide phenanthridinium ring and the nucleotide bases. These experiments thus provide strong support for the intercalation model of the ethidium bromide complex with nucleic acids. Any form of "outside binding"[14] of the ethidium to the nucleotides is inconsistent with the present results. Although more experiments are required to determine the exact type of complex formed, the present data are consistent with the model proposed by Fuller and Waring[28] in which the phenanthridinium ring is intercalated between adjacent base pairs.

The pdG-dC and GpC binding curves also appear to have a sigmoidal shape, which is consistent with the formation of an intercalated type complex, especially at low temperatures. It is more difficult to investigate the pdT-dA and pdA-dT complexes with ethidium bromide because these nucleotides tend to bind weaker than their respective guanine–cytosine nucleotide analogs. In the formation of an intercalated complex with the adenine–thymine or adenine–uracil nucleotides, four hydrogen bonds will be formed between the two self-complementary dinucleotides. The guanine–cytosine dinucleotides can form six hydrogen bonds between the complementary bases. On this basis we would expect to observe a smaller degree of cooperativity (and reduced binding affinity) in the interactions of the drugs with the adenine–thymine or adenine–uracil dinucleotides as compared to the guanine–cytosine dinucleotides. As a result, the experiments with ethidium bromide reported here provide the most insight when comparing sequence isomers such as CpG and GpC or pdT-dA and pdA-dT. In both the visible and circular dichroism experiments, ethidium bromide has demonstrated a preference for binding the pyrimidine–purine sequence dinucleotides (such as pdC-dG) as opposed to the purine–pyrimidine sequence dinucleotides (e.g., pdG-dC). This generalization holds for mixtures of the dinucleotides as well. We wish to emphasize that it is clear from both the present data and other studies with DNA and RNA[1–6,29] that ethidium bromide will bind to sequences other than pyrimidine–purine sequences. However, the present data suggest that ethidium bromide may bind to the various sequences available as intercalation sites on DNA and RNA with significantly different binding constants.

Experiments dealing with ethidium bromide intercalation into DNA or RNA are not expected to be conclusive with respect to the question of whether ethidium bromide exhibits a single binding constant for all the possible base sequences or whether there is a significant range in the binding constants for the various sequences. The standard use of Scatchard plots to determine the number of binding sites and the binding constants works well only when the binding constants are either identical or very different (e.g., Refs. 30–32). It is easy to show that the binding constants could easily vary over a range of a factor of 20 or more and produce Scatchard plots that closely approximate the reported Scatchard plots. The analysis with DNA and RNA is additionally complicated by the apparent electrostatic binding, which may be suppressed by the addition of salt ($>0.1\ M$).

However, the salt also drastically influences the binding constants and the thermodynamic changes associated with the binding to DNA[6] and RNA.[20] The net result is that experiments with native DNA or RNA under physiological conditions are unable to determine if various sequences bind ethidium bromide stronger than others. Experiments with synthetic DNA's of defined sequence should help to answer this question. The possibility that relatively simple molecules may possess sequence specificity may turn out to be important in the physiological action of the drugs. With respect to the unusually strong binding of ethidium bromide to the dinucleotides dC-dG and CpG it is interesting to note that ethidium bromide can act as a cooperative effector to reverse the salt-induced conformational change in a synthetic DNA that contains only dC-dG and dG-dC sequences, poly(dG-dC)·(dG-dC).[33] Preliminary experiments with proflavine and quinacrine·HCl did not produce effects comparable to the cooperative binding of ethidium bromide to poly(dG-dC)·(dG-dC) at high salt concentration.[33]

Finally, the present experiments continue to demonstrate the utility of the dinucleotides, especially the self-complementary dinucleotides, as models for DNA and RNA in the study of drug-nucleic acid complexes. The present techniques can provide guidance as to which nucleotides form the strongest drug–nucleotide complexes and suggested that the self-complementary pyrimidine–purine sequences would be likely to co-crystallize with ethidium bromide. Sobell and co-workers (personal communication) subsequently obtained single crystals of the co-crystalline complex of ethidium bromide with UpA and CpG for X-ray crystallographic studies. We have also observed (unwanted) co-crystallization of ethidium bromide with CpG during an nmr titration, and with UpA and dG-dC from solutions that had been used for optical titrations.

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