안트라센 유도체-합성DNA의 결합형태와 에너지전달과정에서 구아닌 염기의 아민기의 역할

趙昶範 · 孫觀銖 · 韓盛旭[†] · 丁孟浚[††] · 丁賢淑[†††] · 李佶俊*

영남대학교 화학과
[†]경운대학교 환경공학과
[††]상주대학교 화학공학과
[†††]계명문화대학 식품과학과
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Binding Properties of Anthryl Derivatives to Synthetic Polynucleotide and the Role of Guanine Amine Group in the Energy Transfer

Chang Beom Cho, Gwan-Su Son, Sung Wook Han[†], Maeng-Jun Jung[††],
Hyun-Suk Chong[†††], and Gil-Jun Lee*

*Department of Chemistry and Institute of Natural Science, Yeungnam University,
Kyoungsan 712-749, Korea*
[†]*Department of Environmental Engineering, Kyungwoon University, Kumi 735-850, Korea*
[††]*Department of Chemical Engineering, Sangju National University, Sangju 742-711, Korea*
[†††]*Department of Food Science, Keimyung College, Taegu 704-200, Korea*
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요 약. 안트라센 유도체-합성DNA의 결합형태를 여러 가지 분광학적인 방법들을 통해서 알아보았다. Methylamine과 methylethylenediamine. 즉, 길이가 다른 곁사슬을 가지는 안트라센 유도체들이 poly[d(A-T)2]와 poly[d(G-C)2]의 이중나선에 결합했을 때의 분광학적인 특성은 흡광. 원편광스펙트럼에서 봉우리의 장파장으로 이동, 흡광도의 감소를 볼 수 있으며, 선편광스펙트럼에서는 DNA흡광 영역에서 강한 음의 봉우리가 나타나는 것으로 요약할 수 있다. 이러한 현상들은 안트라센 유도체들이 위의 두 가지 DNA의 염기쌍 사이에 삽입이 되어있다는 것을 증명한다. 곁사슬의 길이에 따른 분광학적인 특성에는 별 차이가 없는 것으로 보아 곁사슬의 길이는 결합형태에 영향을 미치지 않는다는 것을 알 수 있다. 또한, 강한 에너지의 전달이 poly[d(A-T)2]와 poly[d(I-C)2]에는 일어나지만 poly[d(G-C)2]에는 일어나지 않는 것으로 보아 poly[d(G-C)2]의 작은 홈쪽에 돌출해 있는 아민기가 DNA염기로부터의 에너지의 전달을 방해하고 있는 것으로 판단된다.

ABSTRACT. The binding mode of anthryl derivatives to synthetic polynucleotides were investigated by various spectroscopic methods. The spectroscopic properties of anthracene with methylamine and methylethylenediamine side chains, complexed with poly[d(A-T)2] and poly[d(G-C)2], can be summarized as a red-shift, with a strong hypochromism in the absorption spectrum, similar induced CD spectra, and a strong negative LD spectrum with an LD^r magnitude comparable to the DNA absorption region. These observations indicate that anthracene moiety is intercalated between the nucleo-bases of poly[d(A-T)2] and poly[d(G-C)2]. The side chains did not alter the spectroscopic properties, demonstrating that the binding mode was not affected by them. A strong energy transfer was observed from poly[d(A-T)2] and poly[d(I-C)2] but not from poly[d(G-C)2], as reported by Kumar et al. (J. Am. Chem. Soc. (1993) 115, 8547). Since the binding mode is the same for all the polynucleotides, the amine group of the guanine base, which protrudes into the minor groove of poly[d(G-C)2], is concluded to disrupt the energy transfer.

INTRODUCTION

The binding properties of small chemicals to nucleic acids have been the subject of intense study due to their potential application in designing DNA-targeted drugs and probes for DNA[1]. DNA provides four distinguishable binding sites for relatively small drugs-intercalation, minor groove, major groove, and random surface binding. It is well known that many drugs recognize these binding sites. For example, simple planar aromatic cations, such as the acridines, generally intercalate between the base pair of double-stranded DNA and the triplet of triplex DNA[2-5], whereas large unfused aromatic hydrocarbons, such as Hoechst or 4',6-diamidino-2-phenylindole, bind preferentially in the minor groove of the AT-rich region of DNA[6-10]. One rare example for small major groove binding drugs is methyl green, which was shown to bind in the major groove of DNA[11]. The impetus for these bindings is believed to be a combination of stacking interaction, hydrophobic interaction, electrostatic force, and the formation of hydrogen bonds.

We have studied the basic concept in drug-DNA interaction, especially the forces which govern intercalation and minor groove binding. The drugs we designed for this work consist of an anthracene chromophore and positively charged methylamine and methylethylenediamine side chains (referred to as AMA and AME in this article, *Chart* 1); we studied their binding geometry by polarized light spectroscopy, i.e., circular and linear dichroism. Planar anthracene moiety meets the requirement for efficient intercalation into the DNA helix[12], and its lowest

energy absorption band is well separated from that of DNA. The positively charged side chains provide improved solubility in water for parent hydrocarbons and are expected to enhance the DNA binding affinity due to the increased electrostatic attraction with the phosphate group of DNA[13,14]. In this work, we specifically address the question of how the side chains, which possess different lengths and positive charges, affect the DNA-binding properties.

The binding properties of one of the drugs, AMA, was thoroughly studied by Kumar and Asuncion using fluorescence techniques[15,16]. The most interesting discovery from the DNA-AMA binding study is that the excited energy of the nucleo-base can be selectively transferred; a strong energy transfer was observed from the nucleo-base when AMA was bound to poly[d(A-T)$_2$], but no energy transfer occurred when it was bound to poly[d(G-C)$_2$]. Relative orientation and the distance between the acceptor and donor are the critical factors for energy transfer between the molecules. However, distance cannot be a factor in the different energy transfer efficiency in an AMA-DNA complex, because the intercalated drug is literally in contact with the donor nucleo-bases. The other factor, relative orientation, may play an important role in this kind of energy transfer. Relative orientation between the nucleo-base and the anthryl derivative can be understood as the binding geometry in a DNA-drug complex. Another issue we address in this work is the binding geometry-energy transfer relationship.

MATERIALS AND METHODS

Materials. Anthryl derivatives were synthesized from 9-(chloromethyl) anthracene by the method described by Bottini *et al*[17]. Synthetic polynucleotides, purchased from Pharmacia, were dissolved in a buffer containing 100 mM NaCl, 5 mM cacodylate, and 1 mM EDTA at pH 7.0 and dialyzed 5 times at 4°C for at least 5 hours against 5 mM cacodylate buffer at pH 7.0; 5 mM cacodylate buffer at pH 7.0 was used throughout this work. Concentrations of the anthryl derivatives and polynucleotides were determined spectrophotometrically using the molar extinction coefficients in water of $\varepsilon_{254\,nm}=8,400$ cm^{-1}M^{-1} for poly[d(G-C)$_2$], $\varepsilon_{262\,nm}=6,600$ cm^{-1}M^{-1} for poly[d(A-T)$_2$], $\varepsilon_{360\,nm}=3,220$ cm^{-1}M^{-1} for AMA, and $\varepsilon_{366\,nm}$

CH$_2$NHCH$_2$CH$_2$NH$_3^+$

Anthracene N-methyl ethylenediamine(AME)

CH$_2$NH$_3^+$

Anthracene methylamine(AMA)

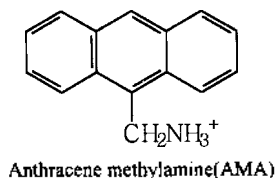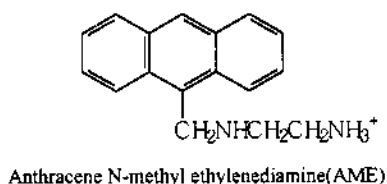*Chart* 1.

$=2,870 \, cm^{-1} M^{-1}$ for AME. The mixing ratio R is defined as the total number of added drug molecules per nucleotide base.

Absorption measurement. Binding of a drug to DNA generally produces hypochromism, broadening of the envelope, and red-shift in the drug absorption region. This effect is especially pronounced for intercalated drugs. The absorption spectra were measured on a Jasco V-550 or a HP8452A diode array spectrophotometer.

Energy transfer from the nucleo-bases to the drugs. The method to evaluate the amount of energy transfer from DNA to the intercalated ethidium (i.e., the "contact energy transfer") was explained in detail by Le Pecq and Paoletti[18]. The appearance of energy transfer has been generally accepted as evidence of drug intercalation; however, it was recently demonstrated that minor groove binding drugs produce a similar energy transfer[19]. The amount of energy transfer is denoted by the ratio

$$Q(\lambda) = \frac{q_b(\lambda)}{q_f(\lambda)} = \frac{I_b(\lambda)\varepsilon_f(\lambda)}{I_f(\lambda)\varepsilon_b(\lambda)} \qquad (1)$$

where q is the quantum efficiencies and I and ε are the measured fluorescence intensities and molar extinction coefficients at wavelength λ. The bound and free drugs are represented by b and f. The ratio $Q(\lambda)/Q_{310nm}$ was then plotted with respect to the wavelength. We chose the normalization factor Q_{310nm} because the absorbance of DNA at this wavelength is negligible. The energy transfer measurements were performed by the reported method[19]. The fluorescence excitation spectrum in 220–320 nm was recorded through the emission window at 420 nm with a slit width of 20 nm. The excitation slit width was 5 nm. The sample concentrations for the absorption measurement were 50 μM polynucleotide and 5 μM drug. Ten-times diluted samples were used for the fluorescence excitation spectra to avoid an inner-filter effect. We used a conventional Jasco FT-777 fluorometer to record the fluorescence measurements.

Circular dichroism and linear dichroism (CD and LD). Anthracene derivatives induce a CD spectrum when they form a complex with DNA, although they do not contain any chiral center. This induced CD (ICD) is believed to be induced by the interaction between the transition moments of achiral drugs and a chirally arranged nucleo-base. ICD is known to be sensitive to the binding mode and location of the drug and the nature of nucleo-base[20-22]. All CD spectra were recorded on a Jasco J715 spectropolarimeter.

Linear dichroism (LD) id determined by the differential absorption between plane polarized light, with the polarization parallel and perpendicular to the reference axis. The measured LD spectrum is divided by isotropic absorption to give a reduced linear dichroism (LD^r) spectrum, from which the angle between the transition dipole moment of the drug and the flow direction (DNA helix axis) is obtained[22-24]. The LD^r calculation under our conditions demands that the unbound drug, which is involved in isotropic absorption spectrum but not in the LD^r spectrum, be cautiously treated. The absorption spectrum of the unbound drug was calculated from the concentration of the unbound drug, which was obtained from equilibrium constant, and was subtracted from the absorption spectrum of the mixture to obtain the true isotropic absorption spectrum of the drug-DNA complex. The LD spectra of the drug-DNA complex were measured on a Jasco 500C spectropolarimeter equipped with an Oxley prism to convert the circularly polarized light into linearly polarized light.

RESULT

Absorption spectra of anthyl derivatives in the presence of polynucleotides. The absorption spectra of AMA and AME in the presence of poly[d(A-T)₂] are depicted in *Figs.* 1a (AME) and 1b (AMA). The data were collected for constant drug concentration, while the concentrations of the polynucleotides were gradually increased. The absorption spectra are shown with the pure DNA spectrum subtracted to facilitate easy comparison. Similar absorption patterns were observed in the presence of poly[d(G-C)₂] for both AMA and AME (data not shown). The lowest energy absorption peaks of free AMA and AME shift from 386 nm to 396 nm when they are bound to either poly[d(A-T)₂] or poly[d(G-C)₂], and the absorbance decreases to less than 50%. Several isosbestic points (257-258 nm, 307-308 nm, 391 nm) were observed, suggesting that these systems involve two kinds of anthracene chromophores, i.e., free and polynucleotide-bound. A polynucleotide-bound drug exhibits an homogeneous absorption pattern unless the absorp-
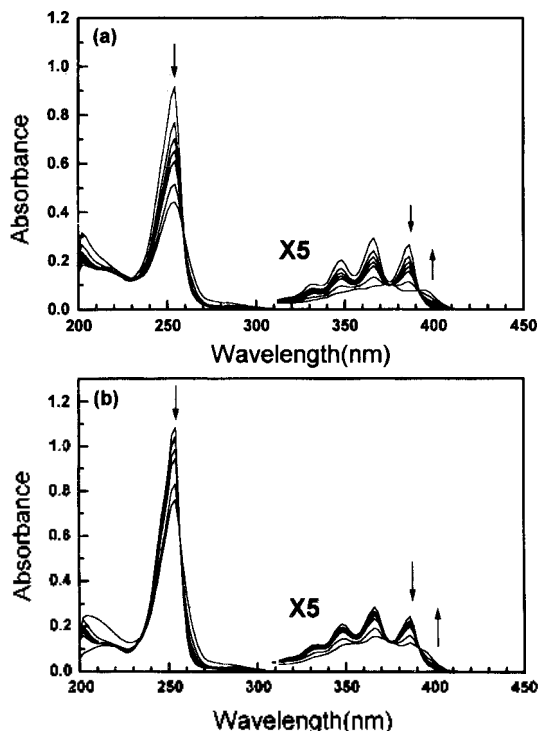
Fig. 1. Absorption spectra of (a) AME and (b) AMA complexed with poly[d(A-T)₂]. [Anthryl derivative]=20 µM. The concentration of poly[d(A-T)₂] was increased in the direction of the arrow to 0.0, 3.7, 7.3, 14.2, 27.3, and 44.7 µM. The absorption spectrum of the corresponding poly[d(A-T)₂] was subtracted from each spectrum for easy comparison.

tion spectra of anthracene at different binding site are coincidentally identical, which is very unlikely. The hypochromism of the anthracene chromophore of AME in the presence of polynucleotide is notably lower than that of AMA, suggesting either that the binding of AME to a polynucleotide is less effective, or that the interaction between AME and the nucleo-base is weaker than with AMA.

Energy transfer from nucleo-base to anthryl derivatives. A method has been established to measure the qualitative energy transfer from the nucleo-base to the bound drugs[18,19]. This method is occasionally used to investigate whether a given drug is intercalated between nucleobases[24-28]. For example, when the ratio $Q(\lambda)/Q_{310nm}$ is plotted for intercalated ethidium, the shape of the resulting curve is similar to the DNA absorption spectrum, indicating that a strong energy transfer occurs in the

DNA-ethidium complex[18]. Similar $Q(\lambda)/Q_{310nm}$ plots were recently obtained from the minor groove binding drugs 4´6-diamidino-2-phenylindole and Hoechst 33258. Therefore, the $Q(\lambda)/Q_{310nm}$ plots do not necessarily indicate intercalation of a drug. It was concluded[19] that the $Q(\lambda)/Q_{310nm}$ plot, which is similar in shape with the DNA absorption spectrum, is an indication that the drug is in contact with nucleo-bases. This contact energy transfer measurement was repeated for ethidium in this work (Fig. 2a). We noticed for the first time that the contact energy transfer is always stronger for poly[d(A-T)₂] than poly[d(G-C)₂], although ethidium intercalates to and in contact with both A-T and G-C base pairs in similar manner.

A strong energy transfer was observed from poly[d(A-T)₂] to both AMA and AME: the center of the $Q(\lambda)/Q_{310nm}$ plot was located approximately at 270 nm (AME,
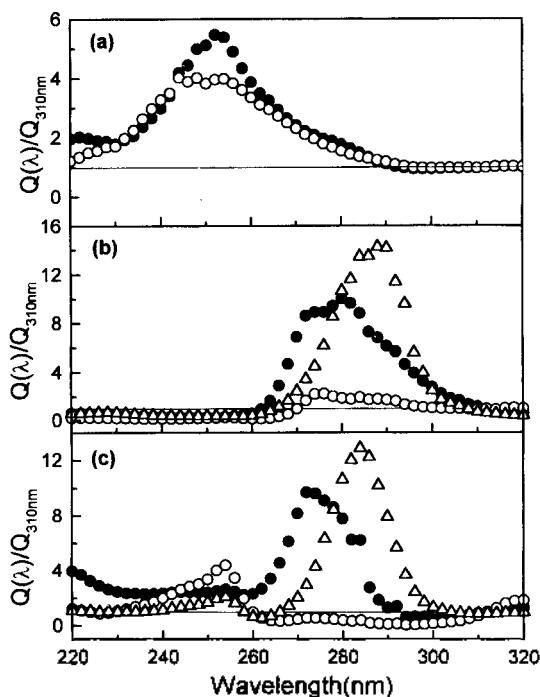

Fig. 2. The $Q(\lambda)/Q_{310nm}$ with respect to the wavelength for (a) EB (b) AME and (c) AMA complexed with poly[d(G-C)₂] (opened circle), poly[d(A-T)₂] (closed circle), and poly[d(I-C)₂] (opened triangle). The emission spectrum was recorded with 420 nm for AME and AMA, 620nm for EB emission. Slit widths were 20/5 nm for emission and excitation. The signal was averaged over ten measurements.

Fig. 2b) and 280 nm (AMA, *Fig.* 2c), while a very weak energy transfer occurred from poly[d(G-C)₂]., The center was observed at 250 nm in the ethidium (*Fig.* 2a). This observation agrees somewhat with that of Kumar and Asuncion[15]. In their work, a strong energy transfer occurred from poly[d(A-T)₂] to intercalated AMA. The energy transfer from poly[d(I-C)₂] was also measured and, surprisingly, large energy transfer were observed to both AME (*Fig.* 2b) and AMA (*Fig.* 2c). Since the only difference of inosine from guanine base is that it lacks the amine group that protrude to the minor groove of the DNA double helix, the energy transfer from a nucleobase to intercalated anthryl moiety is inhibited by the presence of amine group.

Induced Circular dichroism (ICD). The mixing ratiodependent CD spectra of the polynucleotide-anthryl derivative complexes are shown in *Fig.* 3. The data were collected for a constant polynucleotide concentration

(100 μM) and for mixing ratios of 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3, and are provided with the pure DNA spectrum subtracted. The CD spectra of AMA and AME initially appear similar. A strong negative band around 250 nm, a positive band near 270 nm, and very weak positive band in the drug absorption region are apparent (depending on the polynucleotide) from the short wavelength. Since the induced CD spectrum is very sensitive to drug's environment[20-22], this observation suggests that the environment of AMA and AME bound to poly[d(A-T)₂] and poly[d(G-C)₂] may be similar. The isosbestic points observed for all complexes suggest that the binding is homogeneous, i.e., there is only one binding mode for AMA and AME. In general, minor groove binding drugs exhibit a strong positive band in the drug absorption region[6]. A small but not negligible positive band at the lowest absorption region (350-400 nm) can also be seen in the AMA complexes. However, the magnitude of this
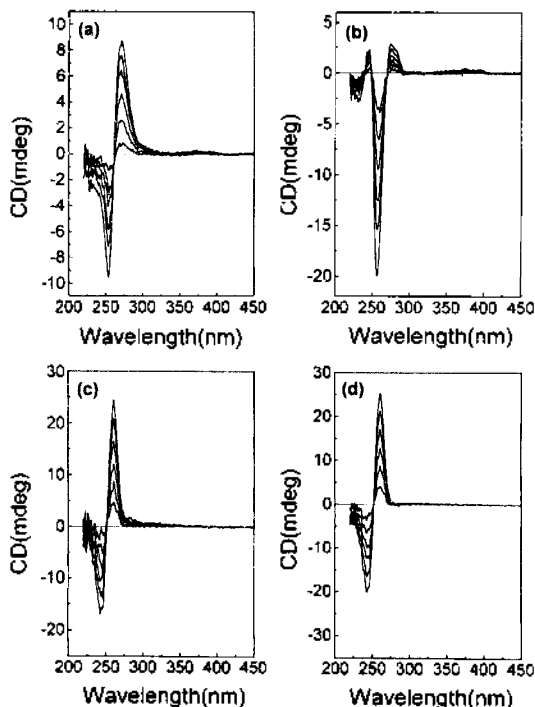

Fig. 3. CD spectrum of (a) AME-poly[d(A-T)₂], (b) AMA-poly[d(A-T)₂], (c) AME-poly[d(G-C)₂], and (d) AMA-poly [d(G-C)₂] complexes. [polynucleotide]=100 μM in the base; the mixing ratios were 0.05, 0.1, 0.15, 0.20, 0.25, and 0.3. The CD spectrum of the corresponding polynucleotide was subtracted.
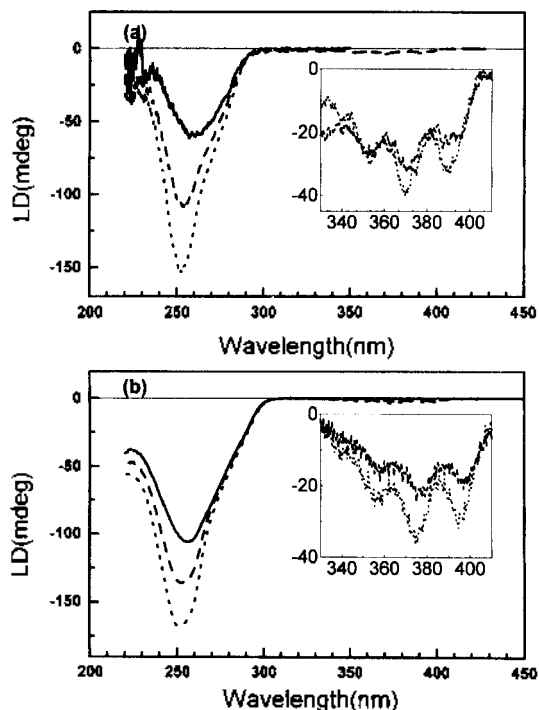
Fig. 4. (a) LD spectrum of AME (dashed curve) and AMA (dotted curve) complexed with poly[d(A-T)₂]. That of drug-free poly[d(A-T)₂] is shown as a solid curve. [polynucleotide]=100 μM and [drug]=20 μM. (b) LD spectra of the drugs complexed with poly[d(G-C)₂]. Conditions and curve assignments are the same as (a).

CD band is much smaller than that of the minor groove binding drugs.

Linear dichroism and reduced linear dichroism (LD and LD[r]). Flow linear dichroism has been proven to be a powerful tool to investigate the orientation of drug with respect to the DNA helix axis[22-24]. The linear dichroism spectra of AMA and AME complexed with poly[d(A-T)₂] and drug-free poly[d(A-T)₂] are depicted in *Fig.* 4a, and those with poly[d(G-C)₂] in *Fig.* 4b. All these complexes exhibit a strong negative LD signal in the drug absorption region, which directly excludes the possibility of groove binding. The LD[r] spectrum was calculated by dividing the isotropic absorption spectrum of each complex by the corresponding measured LD spectrum, which is depicted in *Figs.* 5a and 5b. The magnitude of LD[r] in the drug absorption region (340-400 nm) is comparable to that of the DNA absorption region, which is an indication of drug intercalation. That in the DNA absorption region (around 260 nm) was increased in the presence of the drug in all cases, indicating that the orientability of

Fig. 5. LD[r] spectra of anthryl derivatives complexed with (a) poly[d(A-T)₂] and (b) poly[d(G-C)₂]. Conditions and curve assignments are the same as in *Fig.* 4.

the DNA helix is increased due to a stiffening and elongation of the DNA upon drug binding. This result also supports drug intercalation.

DISCUSSION

Binding mode of anthryl derivatives: the side chain effect. Binding modes of drugs to DNA can be classified into four categories: minor and major groove binding, intercalation, and random surface binding. Intercalation of the drug may be the most classic and well known binding mode. The intercalated drug may be stabilized by a hydrophobic interaction, stacking of the π electrons between the nucleo-bases and the intercalated drug, or electrostatic interaction between the positive charge of the drug and negative charge of the phosphate groups. In general, this binding mode manifests itself by a large hypochromism and red-shift in the absorption band, a weak negative or positive induced CD spectrum, and a negative LD signal in the drug absorption band. In contrast, minor groove binding drugs are stabilized by hydrophobic interaction, electrostatic interaction, or hydrogen bonds. In this case, changes in the spectroscopic properties can be summarized as a relatively small hypochromism and red-shift, a strong positive CD band in the drug absorption region, and a positive LD signal. The spectroscopic properties of major groove binding drugs and surface binding drugs cannot be clearly determined because drugs with these binding modes are relatively free to move. In particular, the LD value of a drug that is bound to the phosphate group of DNA is zero, because the drug is complete free to rotate and therefore they cannot be oriented.

The spectroscopic properties of complexes formed between anthryl derivatives and a synthetic polynucleotide coincide with those of intercalated drugs, with a strong hypochromism and red-shift in the drug absorption region and a weak or nil CD in the drug absorption region. The LD[r] spectrum of the complexes also indicates that the drugs are intercalated between the nucleobases. Furthermore, the similarity of the spectroscopic characters of AMA and AME complexed with both poly[d(G-C)₂] and poly[d(A-T)₂] leads us to conclude that the binding mode of the complexes are similar. The similarity of the induced patterns suggests that the ori-
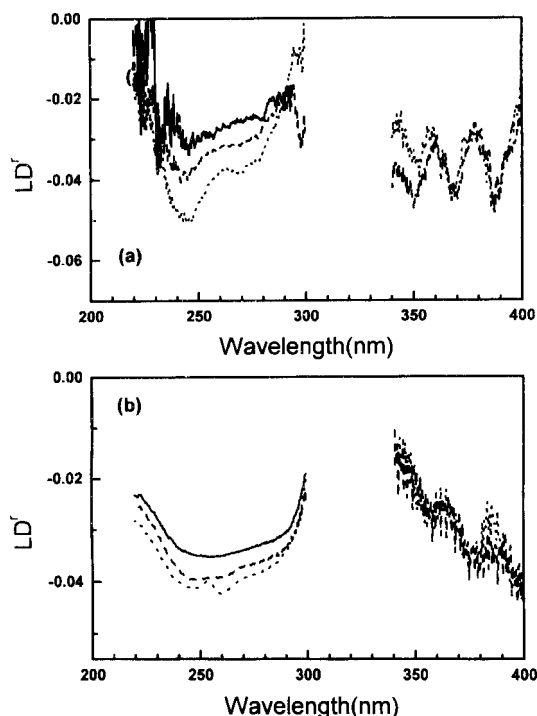
entation directions of the drugs in the intercalation pockets are also similar. Therefore, the side chains do not alter the binding mode of anthryl derivatives.

Energy transfer. We concluded in the previous section that the orientations and binding modes of AMA and AME are similar in poly[d(G-C)₂] and poly[d(A-T)₂], and that the drugs are in contact with the nueleobases, in the intercalation pockets, with no distance between them. Therefore, neither of the two important factors in energy transfer, i.e., the relative orientations of the donor and the acceptor and the distance between them, can be the reason for the different energy transfers that were observed both in this study (Fig. 2) and in that by Kumar et al. [15,16] Consequently, the different energy transfer efficiencies can be attributed to the different molecular structures of the nucleo-bases. The primary differences in the molecular structures of adenine and guanine are (1) the amine group at position C_6 of the adenine base is replaced by the carbonyl group in guanine and (2) the amine group of the guanine at the C_2 position. The latter protrudes into the minor group of the DNA. The strong energy transfer observed for both AMA and AME when complexed with poly[d(I-C)₂] suggests that the amine group in the minor groove plays an important role in the energy transfer, because the only difference between an inosine base and guanine is that the amine group of the guanine base is absent in inosine. Replacing the amine group at the C_6 position by a carbonyl group results in a red-shifted maximum of the energy transfer diagram. The differences in the tymine and cytosine do not contribute significantly since both I-C and A-T can transfer their excited energy to intercalated AMA and AME.

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