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Calculation of Induced Circular Dichroism of Acridine Orange–Poly(riboadenylic acid) Complexes and Confirmation of Their Structure

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ABSTRACT: Visible circular dichroism induced in acridine orange–poly(riboadenylic acid) complexes has been calculated on the basis of exciton theory to zeroth- and first-order perturbations, assuming an appropriate helical structure and geometry of the complexes. The calculated circular dichroism has been compared with the observed value. The most favorable values of the geometrical parameters for intercalated dyes, half-intercalated dyes, and externally associated dyes have been determined as follows: for the ^1L_b transition dipole of the dye chromophore, its polar angle is 80–90° from the poly(riboadenylic acid) helix axis and its azimuthal angle is 90–130° in the plane perpendicular to a helix axis, while its radial distance from the helix axis is 3 Å for the intercalation, 6.0–7.5 Å for the half-intercalation, and 12 Å for the external association, irrespective of whether the strand is double or single. Better agreement is obtained between the calculated and observed circular dichroism when the shape of the absorption band is chosen for the corresponding circular dichroism band, instead of a Gaussian shape, and when the unperturbed band position of circular dichroism is shifted by 5–10 nm toward the blue or red.

Introduction

We have previously measured absorption spectra and induced circular dichroism of aqueous solutions of acridine orange mixed with poly(riboadenylic acid) [poly(rA)] and reported that acridine orange interacts with poly(rA) in different ways, depending on the pH of the solution and the mole ratio, [P]/[D] (nucleotide residue/added monomeric dye).[1]

At acid pH, where poly(rA) is in a double-stranded helix conformation,[2–5] four kinds of binding modes are successively manifested as the mixing ratio is decreased. Two of them are assigned to intercalation of monomeric dye molecules between base pairs, the first being of dye molecules isolated from the others, and the second being of dye molecules with mutual coupling at neighboring sites. The third is the formation of half-intercalated dimers,[6,7] which consist of a partly intercalated monomer molecule and another monomer molecule incompletely overlapped with it. The fourth is external association of dimeric dye molecules stacked in an antiparallel way.[8]

At neutral pH, where poly(rA) is single-stranded,[3–5,9] isolated intercalation of monomeric dye molecules can occur at the helical parts of poly(rA) when a small amount of dye is added. At intermediate mixing ratios, half-intercalated dimeric dye molecules are bound to adjacent sites. In the presence of excess dye, dimeric dye molecules of the antiparallel type are externally associated with poly(rA).

Two types of mechanisms have been considered to be operative for the induction of optical activity in a symmetrical dye bound to a polynucleotide.[10] The first mechanism involves electronic coupling between dye molecules bound to neighboring sites arranged in a helical manner along the polynucleotide chain, which induces a conservative pair of circular dichroic bands on both sides of the absorption band of the dye. In an alternative mechanism, a bound dye molecule undergoes a dissymmetric perturbation from the nucleotide bases in a helical conformation, inducing a nonconservative circular dichroic band at the wavelength of the absorption band of the dye.

Such induced circular dichroism can be calculated on the basis of the exciton theory of Moffitt, Fitts, and Kirkwood[11] and Tinoco.[12] We have evaluated the circular dichroism of acridine orange bound to DNA by means of the exciton theory of zeroth-order perturbation, which gives a conservative pair of circular dichroic bands associated with dipole–dipole coupling between bound dyes arranged helically.[13]

In this work, we calculate a conservative pair of circular dichroism bands induced in the acridine orange–poly(rA) complex in terms of the zeroth-order perturbation theory, assuming appropriate structures for the complex. Moreover, the present calculation is extended to the treatment of the first-order perturbation by taking account of the dipole–dipole interaction of the electronic transition of the bound dye with another transition of the dye or with

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electronic transitions of the bases, in order to explain the nonconservative nature of an induced circular dichroic band. The calculated circular dichroism for each binding mode or for the appropriate combination of two kinds of binding modes is compared with the corresponding observed spectra, which are sometimes represented by a mixed form of two binding modes.[1]

Theoretical Aspects

Let us suppose that an array of N bound dyes formed along N' successive residues on a helical strand of poly(rA) constitutes N units of chromophore, so that these dye chromophores can form N exciton levels for the excited state of a chromophore.

Let vectors μ_{k0e} and μ_{l0e} be the electric dipole moments of the kth and lth chromophores, respectively, for an electronic transition 0–e. According to the exciton theory,[12] the rotatory strength for the Kth exciton level of an electrically allowed transition 0–E of the array is described by

$$R_{EK}^{(0)} = -\frac{\pi\nu_{E0}}{2c}\sum_{k=1}^{N}\sum_{\substack{l\neq k\\ l=1}}^{N} C_{keK}C_{leK}\{\mathbf{r}_{kl}\cdot[\mu_{k0e}\times\mu_{l0e}]\} \qquad (1)$$

to the zeroth-order perturbation, where c is the light velocity, ν_{E0} is the unperturbed frequency of a dye chromophore in the static field of the rest of the array, and C_{keK} and C_{leK} are the exciton coefficients. $\mathbf{r}_{kl}$ is the displacement vector from the transition dipole of the kth chromophore to that of the lth chromophore.

The frequency for the Kth exciton level of a 0–E transition, ν_{EK}, is shifted from the unperturbed frequency, ν_{E0}, by the perturbation and is given by

$$\nu_{EK} = \nu_{E0} + \frac{1}{h}\sum_{k=1}^{N}\sum_{\substack{l\neq k\\ l=1}}^{N} C_{keK}C_{leK}V_{k0e,l0e} \qquad (2)$$

where h is Planck's constant. The exciton coefficients, C_{keK} and C_{leK}, are obtained by the orthonormal conditions and by solving a set of linear equations, and the interaction potential, $V_{k0e,l0e}$, between electric dipoles of chromophores k and l can be calculated by the dipole approximation.[13,14]

The conservative molar ellipticity per dye chromophore associated with the excited state E is written as

$$[\theta]_E^{(0)} = \frac{48\pi^2 N_A}{Nhc}\nu\sum_{K=1}^{N} R_{EK}^{(0)}f(\nu-\nu_{EK}) \qquad (3)$$

at the frequency ν, where N_A is Avogadro's number and $f(\nu - \nu_{EK})$ is the shape function of a circular dichroic band associated with the exciton level EK.

The first-order circular dichroism of the electrically allowed transition 0–e of the dye chromophore arises from electronic coupling with different transitions 0–f of dye or transitions 0–f' of adenine base. Its rotatory strength is given by

$$\sum_{K=1}^{N} R_{EK}^{(1)} = \frac{2\pi}{hc}\,\frac{\nu_{E0}\nu_{F0}}{\nu_{F0}^2-\nu_{E0}^2}\sum_{k=1}^{N}\sum_{\substack{l\neq K\\ l=1}}^{N}\{\mathbf{r}_{kl}\cdot[\mu_{k0e}\times\mu_{l0f}]\}V_{k0e,l0f} \qquad (4)$$

or

$$\sum_{K=1}^{N} R_{EK}^{(1)} = \frac{2\pi}{hc}\,\frac{\nu_{E0}\nu_{F'0}}{\nu_{F'0}^2-\nu_{E0}^2}\sum_{k=1}^{N}\sum_{l'=1}^{N'}\{\mathbf{r}_{kl'}\cdot[\mu_{k0e}\times\mu_{l'0f'}]\}V_{k0e,l'0f'} \qquad (5)$$

respectively, and the nonconservative molar ellipticity per dye chromophore associated with the excited state E is written as

$$[\theta]_E^{(1)} = \frac{48\pi^2 N_A}{Nhc}\nu f(\nu-\nu_{E0})\sum_{K=1}^{N} R_{EK}^{(1)} \qquad (6)$$

The interaction potentials $V_{k0e,l0f}$ and $V_{k0e,l'0f'}$ can also be calculated by the dipole approximation.[13,14]

The shape functions $f(\nu - \nu_{EK})$ and $f(\nu - \nu_{E0})$ of a circular dichroic band are approximated by the Gaussian function with a half-width Θ_E for model calculations given in the section "Calculated Results". Later the Gaussian shape is substituted by the observed band shape of the corresponding absorption spectrum, in order to reproduce the observed circular dichroism much better, as seen in the section "Comparison with Experimental Results".

Although the molar ellipticity, eq 3 or 6, is given per chromophore, either monomeric or dimeric dye bound, depending on the binding modes of the dye, the calculated values of molar ellipticity, $[\theta]$ (deg cm^2 dmol^{-1}), are presented in the unit per mole of bound dye.

We will focus our attention on the calculation of the nonconservative circular dichroism for the ^1L_b transition[14] (462, 502, or 506 nm) of acridine orange perturbed by the ^1B_b transition[14] (261 nm) of the dye and by the electronic transitions (261, 240, 207, and 187.5 nm) of adenine base in poly(rA), as well as the conservative circular dichroism for the ^1L_b transition of the dye itself. The contributions from the other transitions, ^1L_a and ^1B_a[14] (540 and 294 nm), of the dye may be disregarded; it has already been demonstrated that they are very weak, since their dipole moments are much smaller than that for the ^1B_b state.[14]

Models and Parameters

Poly(rA) adopts a parallel double-stranded helix conformation in acid solution at temperatures lower than 75 °C,[3-5] and the geometry of its molecular structure is given on the basis of X-ray analysis.[2] A parallel double-stranded helical chain has the repeat distance per residue 3.80 Å along the helix axis, the azimuthal angle of successive bases 45° about the helix axis, and eight base pairs per helical turn. The molecular plane of adenine bases tilts slightly from the direction perpendicular to the helix axis. The angle of tilt can be taken as 10°, as demonstrated by a simple geometrical analysis.[15] The distance from the helix axis to a transition dipole of an adenine base, that is, to the middle point between the C_4 and C_5 carbons of the base, is 2.95 Å.

At neutral pH, a single-stranded poly(rA) is stable, and its conformation gradually changes from helical to random coil as the temperature rises.[3-5,9,16] Since only dyes bound to helical parts are associated with induced optical activity,[1] only a helical sequence of a single-stranded poly(rA) is taken into account. The helical conformation is stabilized by the pairwise stacking interaction between adjacent bases and is proved to be close to a single-stranded B-form DNA conformation.[3-5,9,17,18] The molecular plane of the bases is perpendicular to the helix axis, the repeat distance of a residue along the helix axis is 3.40 Å, and the number of bases per helical turn is 10. The detailed geometry has been described by Arnott and Hukins.[19] According to them, the distance from the helix axis to the dipole moment of the base is 2.97 Å.

The intercalation model of Pritchard, Blake, and Peacocke,[20] which was utilized in the calculation of circular dichroism induced in acridine orange intercalated in DNA,[13] is adopted and modified for the present case of dye intercalated in poly(rA). The intercalation of dye occurs at consecutive sites along the poly(rA) helix, and bound dye molecules are arranged in a right-handed helical way along a polynucleotide chain, irrespective of whether the helix is double-stranded or single-stranded. The occupied sites and their neighbors would be more or less subject to the geometrical perturbation, especially for the intercalation of dyes into the adjacent sites. However, no such perturbation is taken into account. Instead, it is assumed that the dye intercalated between adjacent bases increases the distance between bases only by their repeat distance, without twisting the poly(rA) helix. The molecular plane of intercalated dye is parallel to the plane of the base and its longer axis is almost tangential about the helix axis. The transition dipole of the dye is approximately set to the middle point between the dipoles of two consecutive adenine bases inserting it.

In the half-intercalation model, bound dimeric dye molecules adopt a partially stacked structure of a partly intercalated monomer molecule and another monomer molecule incompletely overlapped with it.[1] In this case molecules forming dimeric species are assumed to be displaced along their longer axis from the completely stacked structure. Such half-intercalation occurs at consecutive sites along a poly(rA) helix, and bound dye molecules arrange in a helical way along the poly(rA) chain. Then the spacing between two adjacent bases increases by their repeat distance, as in the case of an intercalation model. The transition dipole of the half-intercalated dimer is put at the center of the intercalated monomer and the externally bound monomer, and it is located on the radial line passing through the middle point of the transition dipoles of two consecutive bases. The molecular plane of the dye may be parallel to the molecular plane of the base, but its longer axis may not always be tangential to the helix axis.

Externally bound dyes are constituted of dimeric units, each of which has an antiparallel stacked arrangement of two molecules.[1,8,13,14,21,22] The dimeric dye molecules interact strongly with one another, and they form a polymeric array on a poly(rA) helix, by binding electrostatically with phosphate ions, without any modification of the poly(rA) chain. The external association model, which was found to be plausible for the dye–DNA complex,[13] is adopted. The longer axis in the molecular plane of the bound dye is almost perpendicular to the helix axis, and the shorter one is in its radial direction. In such an arrangement of dimeric dye, consideration of the steric hindrance and the stacking tendency of the dye molecules would lead to a structure in which every other phosphate group on a poly(rA) chain is a binding site for a helical sequence of dye molecules. The transition dipole of the dye is located outside about 3 Å from the phosphorus atom of poly(rA). Possible intercalation of a monomeric dye which might simultaneously occur is ignored, because the amount of monomeric dye was found to be too small to be detected in the absorption spectra.[1]

The ^1L_b electronic transition of acridine orange can be assigned to a strong visible absorption band around 462–506 nm; it has a polarization along the longer axis of the dye.[14] The ^1B_b transition is at 261 nm and has the same polarization direction as the ^1L_b transition.[14] The position and direction of the transition dipole of the dye bound to a poly(rA) helix are defined by its radial distance, a, its polar angle, θ, both with respect to the helix axis, and its azimuthal angle, ϕ, in the plane perpendicular to the helix axis.[13]

The numerical values of the electronic parameters of acridine orange are given in Table I. The magnitude of the electric transition dipole moment of the visible absorption bands was graphically[13,14,21,22] calculated from the observed absorption spectra of the corresponding dye–poly(rA) mixtures[1] at which the bound dye species would be free from the electronic coupling with the other bound dyes. The numerical values of the ultraviolet transition dipole moment were estimated by the previously reported method.[13,14,22] The half-width of the visible bands was also obtained graphically by regarding its shape as Gaussian, although this is not necessarily true, especially for the monomeric band.[1] This will be discussed later. The half-width values obtained differ from those adopted previously.[13,14,21,22]

Table I
Electronic Parameters for the Visible and Ultraviolet Transitions of Acridine Orange

species	A	c/ν_{A0}, nm	μ_{0a}, D	Θ_A/c, cm^{-1}	ref[a]
intercalated monomer in acid solution	E F	506 261	5.95 6.64	1248	[P]/D] = 300 pH 4.39
intercalated monomer in neutral solution	E F	502	6.49	1208	[P]/[D] = 609 pH 6.77
half-intercalated and externally associated dimers in both acid and neutral solutions	E F	462 261	7.85 8.80	1496	[P]/[D] = 9 pH 7.15

[a] Electronic parameters were evaluated from the observed absorption band of solutions at the given conditions.

Table II
Electronic and Geometrical Parameters for the Ultraviolet Transitions of Adenine Base

		acid solution		neutral solution	
c/ν_{F0}, nm	$\mu_{0f'}$, D	θ, deg	ϕ, deg	θ, deg	ϕ, deg
261	3.59	90	−20	90	−68.5
240	1.77	80	67	90	26.5
207	4.44	80	70	90	23.5
187.5	4.27	90	−20	90	−68.5

The numerical values of the calculated electronic and geometric parameters of a poly(rA) strand are summarized in Table II. The assignment and polarization of the electric transition moments of the adenine base are derived from data from the polarized absorption spectrum of a single crystal of 9-methyladenine,[23-26] although they differ from those estimated by molecular orbital calculations.[27,28] It was shown[25] that the circular dichroism of a polynucleotide calculated on the basis of experimental data agrees with the observed value better than that calculated from transition moments based on molecular orbital calculations.[29,30] The magnitudes of the transition moments of the adenine base are obtained graphically from the observed spectra of the monomeric species.[31]

Calculated Results

Isolated Intercalation of Monomeric Dye on Poly(rA) in Acid Solution. For acridine orange sparsely intercalated on the parallel double-stranded helix of poly(rA), the electronic coupling of the visible electronic transition of the dye with the electronic transitions of each of the ten adenine base pairs on both sides of the intercalated dye is taken into account, the calculation being performed to the first-order perturbation by means of eq 6. Values of parameters for the location of the transition moment of the bound dye are changed within a sterically possible region, as shown in Table III. The calculated values of the molar ellipticity of the circular dichroic band are given in Table III and compared with the observed results.

For the change of ϕ from 80° to 120°, a negative circular dichoric band at 505 nm is obtained, having molar ellip-

Table III
Molar Ellipticities of Circular Dichroic Bands Calculated for Various Isolated and Electronically Coupled Intercalation Models in Acid Solution

			isolated		electronically coupled			
a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta] \times 10^{-4}$	c/ν, nm	$[\theta] \times 10^{-4}$	c/ν, nm	$[\theta] \times 10^{-4}$
3.00	80	80	505	−6.1	490	−6.6	545	0.8
3.00	80	90	505	−5.9	490	−6.0	545	0.8
3.00	80	100	505	−5.2	490	−5.4	540	1.3
3.00	80	110	505	−4.3	485	−4.9	535	2.4
3.00	80	120	505	−3.3	485	−4.8	530	4.1
2.00	80	110	505	2.8	480	−4.1	525	10.3
2.50	80	110	505	−0.7	480	−4.2	525	6.2
3.00	80	110	505	−4.3	485	−4.9	535	2.4
3.50	80	110	505	−7.4	495	−6.1	560	1.5
obsd[a]			501	−3.12	478	−3.39	522	

[a] Values observed at [P]/[D] = 300 and pH 4.39 for isolated intercalation and at [P]/[D] = 50 and pH 4.31 for electronically coupled intercalation.[1] The latter includes some contribution from isolated intercalation.

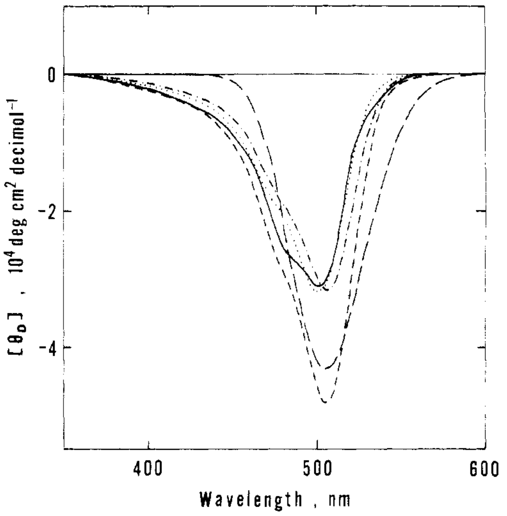

Figure 1. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 300 and pH 4.39 (isolated intercalation). (a) a = 3.00 Å, θ = 80°, ϕ = 110°: (– – –) Gaussian shape; (- - -) absorption band shape. (b) a = 3.00 Å, θ = 83°, ϕ = 115°: (-·-) absorption band shape; (···) absorption band shape and blue shift of ν_{E0} for monomeric species by 5 nm ((—) observed[1]).

ticities of −61 000 to −33 000, which is independent of the number of base pairs if more than three pairs of bases on each side in a sequence are included in the calculation. On the other hand, the molar ellipticity dramatically decreases upon diminishing the distance a, and even its sign is reversed at distances less than 2.5 Å.

Electronically Coupled Intercalation of Monomeric Dyes on Poly(rA) in Acid Solution. We assume that only two monomeric dye molecules are intercalated into consecutive sites on double-stranded poly(rA) and they are electronically coupled, so that the dye chromophores are subject to both zeroth- and first-order perturbations. Furthermore, base pairing between this pair of intercalated dyes and each of the ten base pairs on both sides is taken into account for the first-order perturbation. The molar ellipticities from the zeroth- and first-order perturbations, eq 3 and 6, are summed.

The calculated circular dichroism for the ϕ values between 80° and 120° has a stronger negative band on the shorter wavelength side and a weaker positive band on the longer wavelength side, as shown in Table III. As the ϕ value increases, the negative band shifts to the blue and decreases in magnitude, while the positive band also shifts to the blue but increases in magnitude. The molar ellipticities strongly change with a change in the distance a, as in the case of isolated intercalation. Even if the dye is located just above the 5-membered or 7-membered ring of one of neighboring bases in the strand, this feature of the calculated circular dichroism is not greatly altered.

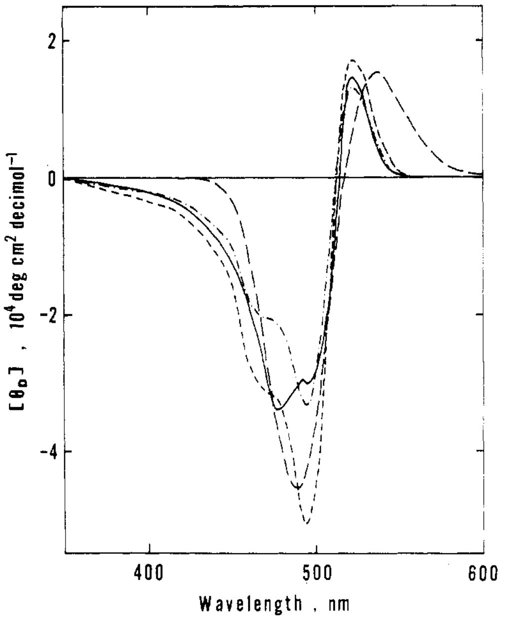

Figure 2. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 50 and pH 4.31 (isolated intercalation:electronically coupled intercalation = A:B per monomeric dye). (a) a = 3.00 Å, θ = 80°, ϕ = 110°: (– – –) Gaussian shape (A:B = 1:4); (- - -) absorption band shape (A:B = 1:1). (b) (-·-) a = 3.00 Å, θ = 83°, ϕ = 115°, absorption band shape (A:B = 2:1) ((—) observed[1]).

When compared with the experimental results,[1] the values a = 3.00 Å, θ = 80°, and ϕ = 110° are found to be more suitable for the isolated and coupled intercalation models of the dye, indicating the suitability of the Pritchard–Blake–Peacocke model, as in the case of the dye–DNA complex.[13] Figures 1 and 2 illustrate the calculated circular dichroism for the sparsely intercalated dye and for a pair of intercalated dyes coexisting with the sparsely intercalated dye, respectively, on double-stranded poly(rA), using the above values of the parameters and assuming a Gaussian band shape function.

Armstrong et al.[6] claimed that two dye molecules cannot be intercalated into consecutive sites of DNA, so that they must be inserted into alternate sites. Their binding experiments indicated that the dye could bind less than 1/4 of the number of available intercalation sites. However, if the second dye is intercalated in the second nearest

Table IV
Molar Ellipticity of the Circular Dichroic Band Calculated for Various Isolated Half-Intercalation Models in Acid Solution

a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta] \times 10^{-4}$
6.00	80	90	460	-9.0
6.00	80	130	460	-3.6
6.00	80	140	460	-1.4
6.00	80	150	460	0.7
5.50	85	130	460	-2.4
6.00	85	130	460	-2.3
6.50	85	130	460	-2.1
obsd[a]			470	-2.69

[a] Observed value at [P]/[D] = 10 and pH 4.32,[1] including some contribution from electronically coupled intercalation.

neighbor site, it can be demonstrated from a numerical calculation that its circular dichroism has a weaker positive band on the shorter wavelength side and a stronger negative band on the longer wavelength side. This result is obtained for values of parameters as follows: the direction of the transition moment of the dye is taken as $\phi = 80°$, 90°, and 110°, and the distance a is changed as 2.50, 3.00, and 3.61 Å. Dye molecules are located on the 5-membered or 7-membered ring of the bases in a strand or on the hydrogen bond of a base pair. The disagreement of the signs of the circular dichroic bands with the experimental observation[1] disproves the model of Armstrong et al. for the dye–poly(rA) complex in acid solutions. Our previous calculation for the dye–DNA complex also suggested that the intercalation into consecutive sites was more probable.[13]

Isolated Half-Intercalation of Dimeric Dye on Poly(rA) in Acid Solution. For a dimeric dye that consists of a partly intercalated monomeric molecule on parallel double-stranded poly(rA) and another monomeric molecule incompletely overlapped with it, the circular dichroism is evaluated to the first-order perturbation of the visible electronic transition of the dye, by taking account of its coupling with the electronic transitions of each of the ten adenine base pairs on both sides of the binding site.

The angle ϕ of the transition moment of the bound dye should be larger than 90° in the model of half-intercalation, because only in these arrangements can the intercalation of the dye on the polynucleotide backbone occur without steric hindrance. In agreement with the observation,[1] a negative band is obtained for the angle ϕ from 90° to 140°, as seen in Table IV. The angle θ must be close to 90°, being similar to the tilt of an adenine base. With $\theta = 85°$ and $\phi = 130°$, the calculated molar ellipticities agree well with the observation. A change in the distance a from 5.50 to 6.50 Å does not seriously affect the sign and the magnitude of the circular dichroic band. Figure 3 illustrates the calculated profile of the circular dichroism induced by the isolated half-intercalation of a dimeric dye having parameters $a = 6.00$ Å, $\theta = 85°$, and $\phi = 130°$ and assuming a Gaussian band shape function. In Figure 3 the contribution from the electronically coupled intercalation of a monomeric dye is added.

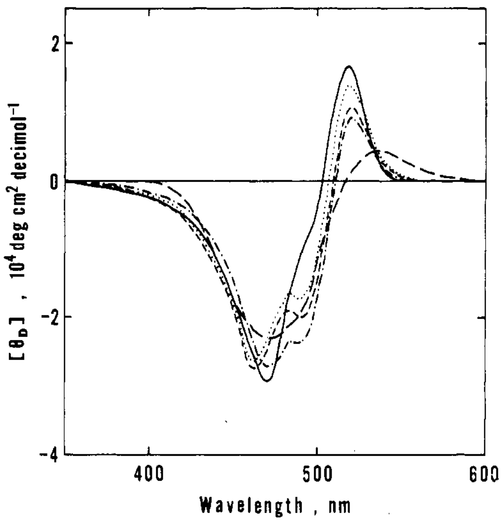

Figure 3. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 10 and pH 4.32 (electronically coupled intercalation:isolated half-intercalation = 1:4 per monomeric dye). (a) $a = 3.00$ Å, $\theta = 80°$, and $\phi = 110°$ for monomeric species and $a = 6.00$ Å, $\theta = 85°$, and $\phi = 130°$ for dimeric species: (– – –) Gaussian shape; (- - -) absorption band shape; (- · ·) absorption band shape and red shift of ν_{E0} of dimeric species by 10 nm. (b) (···) $a = 3.00$ Å, $\theta = 83°$, and $\phi = 115°$ for monomeric species and $a = 6.00$ Å, $\theta = 85°$, and $\phi = 130°$ for dimeric species, absorption band shape. ((—) observed,[1] calibrated for the bound amount of 92.6%).

External Association of Dimeric Dyes on Poly(rA) in Acid Solution. For a sequence of dimeric dyes each of which is constituted of the antiparallel stacking of monomeric dyes and is externally associated with each of the alternate phosphate groups on one chain of double-stranded poly(rA), the circular dichroism is calculated to the zeroth- and first-order perturbations of the bound dyes. The number of dimeric dye molecules in a sequence was chosen as ten, being comparable with that in the case of a dye–DNA complex.[13]

The choice of the value $\theta = 70°$ does not reproduce the characteristics of the observed circular dichroism, which shows a positive band on the shorter wavelength side and a negative band on the longer wavelength side.[1] However, the circular dichroism calculated for $\theta = 80°$ and 90° has characteristics comparable with the observed results. The numerical values are summarized in Table V. As in the acridine orange–DNA complex,[13] the external binding model with $\theta = 80°$ and $\phi = 90°$ may be preferable. However, as seen in Figure 4, the molar ellipticities calculated for $a = 8.95$ Å are too large to fit the observation.[1] An increase in the distance a reduces the molar ellipticities,

Table V
Molar Ellipticities of Circular Dichroic Bands Calculated for Various External Association Models in Acid Solution

a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta] \times 10^{-4}$	c/ν, nm	$[\theta] \times 10^{-4}$	c/ν, nm	$[\theta] \times 10^{-4}$
8.95	70	90	425	1.7	465	-3.5	505	1.4
8.95	80	90	435	6.4	480	-5.9		
8.95	90	90	440	9.4	485	-8.6		
10.0	80	90	435	4.3	480	-4.0		
11.0	80	90	435	2.8	480	-2.6		
12.0	80	90	435	1.7	480	-1.6		
obsd[a]			435	0.07	471	-0.32		

[a] Values observed at [P]/[D] = 0.6 and pH 4.07.[1]

Table VI
Molar Ellipticity of the Circular Dichroic Band Calculated for Various Isolated Intercalation Models in Neutral Solution

a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta] \times 10^{-4}$
3.00	90	80	500	-1.5
3.00	90	90	500	-0.7
3.00	90	100	500	0.3
3.00	90	110	500	1.1
3.00	90	120	500	1.7
2.00	90	115	500	6.2
2.50	90	115	500	4.1
3.00	90	115	500	1.4
3.50	90	115	500	-1.2
obsd[a]			513	1.41

[a] Values observed at [P]/[D] = 609 and pH 6.77.[1]

but values of the distance beyond 12.0 Å will be geometrically unattainable.

Isolated Intercalation of Monomeric Dye on Poly(rA) in Neutral Solution. An intercalated dye molecule must be stabilized on single-stranded poly(rA) by the dye–base stacking interaction.[1] For a dye molecule intercalated on single-stranded poly(rA) having B-form DNA geometry, the circular dichroism is calculated to the first-order perturbation caused by each of the ten bases on both sides.

The molar ellipticity becomes independent of the number of bases if more than four bases are taken on each side. When the angle ϕ is changed from 80° to 120°, the calculated circular dichroism inverts in sign at a ϕ value between 90° and 100°, and the positive sign derived at larger angles agrees with the observed one,[1] as seen in Table VI. In this case, the location of acridine orange, a, seriously affects the magnitude and sign of the circular dichroic band. When a dye molecule is closer to the helix axis, the angle ϕ must be taken smaller. The values of a = 3.00 Å, θ = 90°, and ϕ = 115° are most suitable for the isolated intercalation model in neutral solution. Figure 5 shows the calculated circular dichroism with these values as parameters.

Electronically Coupled Half-Intercalation of Dimeric Dyes on Poly(rA) in Neutral Solution. The circular dichroism observed at [P]/[D] values from 41 to 2 in neutral solution is attributed to dimeric dye molecules partly intercalated in consecutive sites of single-stranded poly(rA), and it can be concluded that the sequence of half-intercalated dimers would not be very long;[1] it would consist of two dimeric dye molecules. The circular dichroism is calculated to the zeroth- and first-order perturbations. The latter includes contributions from the electronic coupling with the ultraviolet electronic transitions of the dye and adenine base. An adenine base stacked between two intercalated dyes and each of the ten bases on both sides of them are assumed to be coupled electronically.

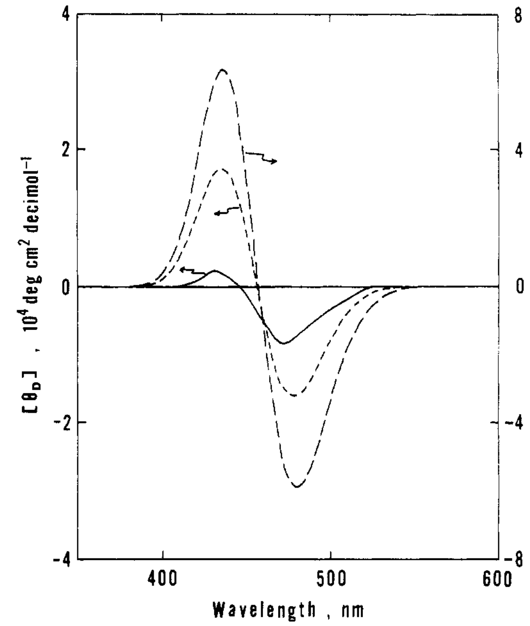

Figure 4. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 0.6 and pH 4.07 (external association). (a) (– – –) a = 8.95 Å, θ = 80°, ϕ = 90°, Gaussian shape. (b) (- - -) a = 12.00 Å, θ = 80°, ϕ = 90°, Gaussian shape. ((—) observed,[1] calibrated for the bound amount of 38.5%).

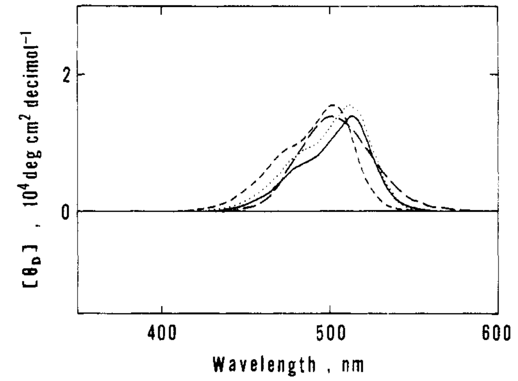

Figure 5. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 609 and pH 6.77 (isolated intercalation). a = 3.00 Å, θ = 90°, ϕ = 115°: (– – –) Gaussian shape; (- - -) absorption band shape; (···) absorption band shape and red shift of ν_{E0} by 10 nm ((—) observed[1]).

In view of the steric hindrance of the acridine dye with the polynucleotide backbone as stated for the half-intercalation model in acid solution, angles ϕ larger than 90° are plausible for the model of half-intercalation in neutral solution. For angles except for ϕ = 90°, a negative band

Table VII
Molar Ellipticities of Circular Dichroic Bands Calculated for Various Electronically Coupled Half-Intercalation Models in Neutral Solution

a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta] \times 10^{-4}$	c/ν, nm	$[\theta] \times 10^{-4}$
7.50	90	90	470	-3.7		
7.50	90	110	450	-2.4	505	0.2
7.50	90	130	435	-4.3	480	6.2
7.50	90	150	435	-7.0	480	13.6
7.00	90	120	440	-4.2	485	3.9
7.50	90	120	440	-3.1	485	2.7
8.00	90	120	440	-2.2	485	1.6
obsd[a]			456	-3.20	477	3.09

[a] Values observed at [P]/[D] = 9 and pH 7.15,[1] including a small contribution from isolated intercalation.

Table VIII
Molar Ellipticities of Circular Dichroic Bands Calculated for Various External Association Models in Neutral Solution

a, Å	θ, deg	ϕ, deg	c/ν, nm	$[\theta]\times10^{-4}$	c/ν, nm	$[\theta]\times10^{-4}$	c/ν, nm	$[\theta]\times10^{-4}$
11.91	70	90	420	0.5	460	−2.4	500	1.5
11.91	80	90	440	5.2	480	−4.8		
11.91	90	90	440	9.1	485	−8.3		
13.00	80	90	440	3.7	480	−3.4		
14.00	80	90	440	2.6	480	−2.4		
15.00	80	90	435	1.8	480	−1.7		
obsd[a]			442	0.26	475	−0.26		

[a] Value observed at [P]/[D] = 0.6 and pH 7.30.[1]

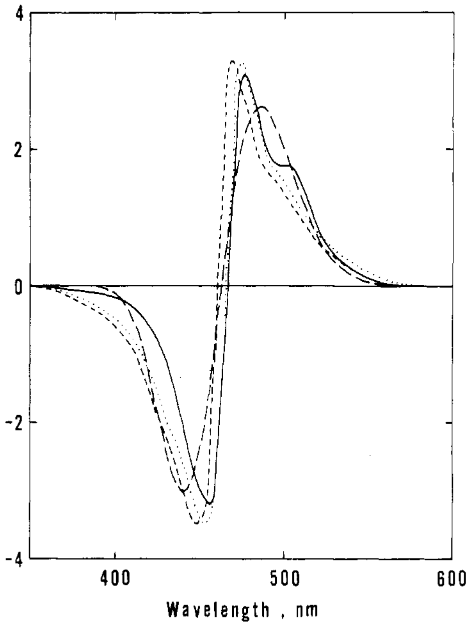

Figure 6. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 9 and pH 7.15 (isolated intercalation:electronically coupled half-intercalation = 1:24 per monomeric dye). a = 3.00 Å, θ = 90°, and ϕ = 115° for monomeric species and a = 7.50 Å, θ = 90°, and ϕ = 120° for dimeric species; (— — —) Gaussian shape; (- - -) absorption band shape; (···) absorption band shape and red shift of ν_{E0} for monomeric species by 10 nm and for dimeric species by 5 nm ((—) observed[1]).

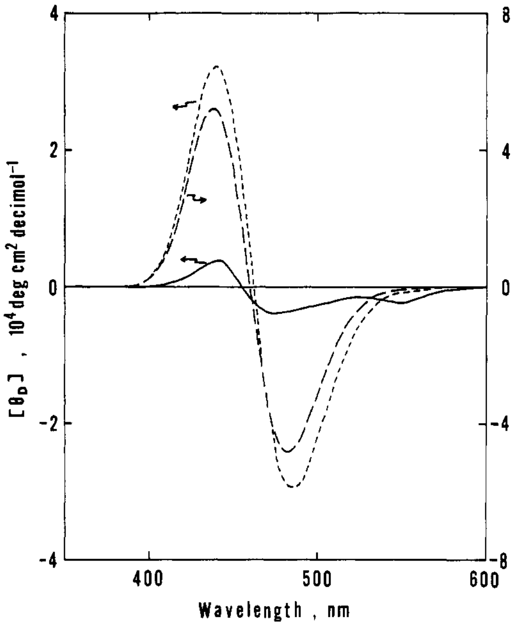

Figure 7. Circular dichroism calculated for the acridine orange–poly(rA) complex with [P]/[D] = 0.6 and pH 7.30 (external association). a = 11.91 Å, θ = 80°, ϕ = 90°; (— — —) Gaussian shape and N = 10; (- - -) Gaussian shape and N = 2 ((—) observed,[1] calibrated for the bound amount of 65.4%).

on the shorter wavelength side and a positive band on the longer wavelength side are expected, as shown in Table VII. The angles from 110° to 130° are found to be probable when the calculated molar ellipticities are compared with the observed value.[1] The molar ellipticities of the circular dichroic bands for ϕ = 120° decrease slightly with an increase in the distance a. The circular dichroism is calculated with a = 7.50 Å, θ = 90°, and ϕ = 120° and for a Gaussian band shape and is shown in Figure 6. It includes a small contribution of isolated intercalation.

External Association of Dimeric Dyes on Poly(rA) in Neutral Solution. It is assumed that ten antiparallel dimeric dyes externally bound to alternate phosphates form a sequence on the helical part of single-stranded poly(rA). Then the zeroth- and first-order perturbations from the electronic transitions of bound dyes should contribute to the circular dichroism, but the first-order perturbation from the electronic transitions of the base is not taken into account.

The numerical values for some models are summarized in Table VIII. At θ = 70°, the circular dichroism disagrees in its characteristics with the observation.[1] For θ = 80° and 90°, a positive band on the shorter wavelength side and a negative band on the longer wavelength side are obtained. Similarly to the external binding of a dye–DNA complex[13] or of a dye–poly(rA) complex in acid solution, the values of a = 11.91 Å, θ = 80°, and ϕ = 90° may be preferable but the calculated molar ellipticities of the circular dichroic bands are much larger than the observed ones,[1] as shown in Figure 7. An increase in the distance a up to 15.00 Å can reduce the calculated molar ellipticities.

Comparison with Experimental Results

Figures 1–7 show the circular dichroic spectra calculated by using favorable values for the parameters and a Gaussian band shape function. Circular dichroic spectra for acid solution with [P]/[D] = 10 (Figure 3) and for neutral solution with [P]/[D] = 9 (Figure 6) have contributions from two kinds of binding modes, and the molar ellipticities from each mode are summed with the given fraction.[1] The mole fraction of monomer species, f_m, was estimated from the corresponding absorption spectra and was taken to be equal to 0.33 and 0.07, respectively. The fraction of binding modes for acid solution of [P]/[D] = 50 (Figure 2) was somewhat arbitrarily chosen. The figures include the observed circular dichroism. The observed circular dichroic spectra for acid solutions with [P]/[D] = 10 (Figure 3) and 0.6 (Figure 4) and for neutral solution with [P]/[D] = 0.6 (Figure 7) were corrected for the proportion of bound dye obtained by equilibrium dialysis.[1]

The circular dichroism induced on the bound monomeric species does not have a Gaussian band shape but has a band shape similar to its absorption band. Therefore, the choice of the band shape function is very important. As can be seen in Figures 1–3, 5 and 6, the choice of the absorption band shape rather than the Gaussian shape gives better reproduction of the circular dichroic spectra. The absorption band shape was taken from the absorption spectra observed under the conditions given in Table I. Often the unperturbed band position of the circular dichroism must be slightly shifted toward either the blue or the red in order to get a much better fit with the observation, as in Figures 1, 3, 5, and 6. This might suggest that the unperturbed frequency in the circular dichroism spectrum differs from that in the absorption spectrum.

The calculated circular dichroism for acid solution with $[P]/[D] = 300$ gives a magnitude comparable with observation when the absorption band shape function and values of the parameters $a = 3.00$ Å, $\theta = 83°$, and $\phi = 115°$, instead of $\theta = 80°$ and $\phi = 110°$, are adopted, and if the unperturbed frequency is shifted to the blue by 5 nm. This is shown in Figure 1. In the circular dichroism calculated for acid solution with $[P]/[D] = 50$ (Figure 2), a positive band agrees fairly well with the observed one when the band shape is taken from the corresponding absorption spectra and the content of the singly intercalated monomer is increased. However, two strong negative bands still disagree in their intensities. A further increase in the content of isolated intercalation and a change of parameters from $\theta = 80°$ to $\theta = 83°$ and from $\phi = 110°$ to $\phi = 115°$ make the negative band at the longer wavelength closer to the observed one but weaken the negative band at the shorter wavelength. The circular dichroic spectrum for acid solution with $[P]/[D] = 10$ (Figure 3) is favorably reproduced by the adoption of the absorption band shape function. A better fit with the observation is obtained by a red shift of the unperturbed frequency of the dimeric dye by 10 nm or a change of parameters for the electronically coupled intercalation as above.

For neutral solutions of $[P]/[D] = 609$ and 9 (Figures 5 and 6), the circular dichroism calculated by the absorption band shape agrees remarkably well with the observed one, if the unperturbed frequencies are shifted to the red by 10 nm for the monomeric species and 5 nm for the dimeric species.

We can find that the most favorable values of the geometric parameters for the intercalation and half-intercalation models are the angles ϕ in the range 115–130°, differing from the value $\phi = 90°$ used for the dye–DNA complex.[13] This value is suitable for reducing the steric hindrance of intercalated and half-intercalated dyes with the sugar–phosphate backbone of poly(rA).

The band shape of the calculated circular dichroism for acid and neutral solutions with $[P]/[D] = 0.6$ (Figures 4 and 7) is very similar to the observed value, even if the Gaussian band shape function is taken. However, calculated molar ellipticities are rather large when an external association model similar to the corresponding model of the dye–DNA complex[13] is adopted. Since the circular dichroism observed for the dye–poly(rA) complex with $[P]/[D] = 0.6$[1] is smaller than that of the dye–DNA complex with $[P]/[D] = 0.6$,[7] the binding mode cannot be common for both complexes. On the dye–poly(rA) complex in acid solution the bound dye may be located far away from the helix axis, more than 8.95 Å, because a charged dye would be prevented from interacting with the phosphate ion by the electrostatic repulsion with the cationic adenine base. Thus a weaker circular dichroism is developed, as shown in Figure 4. On the other hand, bound dye molecules would not form a long sequence outside the flexible single strand of poly(rA) in neutral solution, again leading to a weak circular dichroism (Figure 7).

Discussion

Although the binding of dye on polynucleotide has been known since 1959,[32] the structure of the complex has not necessarily been clearly elucidated. Some workers[33–38] investigated the interaction between planar molecules of drugs and polynucleotide in order to elucidate the structure of the intercalation complex.

Bradley et al.[33] concluded from measurements of electric birefringence and dichroism that dye molecules in an acridine orange–poly(rA) complex with $[P]/[D] = 3.0$ and pH 8.0 were oriented with their long axes parallel to the electric field, indicating that dye molecules were oriented parallel to the helix axis. However, their result is inconsistent with our experimental results that half-intercalated dimeric dyes exist in solution under these conditions and that the long axes of the bound dyes are arranged perpendicular to the helical axis of poly(rA).[1]

Kamiya[34] studied the induced circular dichroism of proflavin sparsely intercalated on DNA and found it to be strongly dependent on the ionic strength. He concluded that the intercalation model of Pritchard, Blake, and Peacocke, which was associated with the electrostatic interaction between the acridine nitrogen atom and the DNA phosphate group, must be modified under relatively high ionic strengths. Any modification of helix deformation is unnecessary for our case at low ionic strengths.[1,13]

Hogan et al.[35] reported electric dichroism, fluorescence electric dichroism, and equilibrium binding studies for the complex between DNA and intercalated drugs. They showed that the expansion of the separation between two DNA base pairs resulting from intercalation is not always equal to 3.4 Å per bound drug and that the molecular planes of the intercalated drugs are not perpendicular to the helix axis. According to our calculation of the circular dichroism induced for the acridine orange–poly(rA) complex, the sign and magnitude of the evaluated circular dichroism are very sensitive to the geometrical parameters, if the spacing between two base pairs intercalating acridine orange therein is less than 6.8 Å and the long axis of the bound acridine orange is tilted by ±10° from the perpendicular. Therefore, a more accurate geometry for the complex needs to be determined experimentally.

Circular dichroism is a useful method for examining the detailed dissymmetric structure of the dye–polyelectrolyte complex,[13,14,21,22] since the circular dichroic spectra are very sensitive to the optically active stereochemical configuration of the chromophores. In our previous work[13] on the structure of the acridine orange–DNA complex, the calculated circular dichroism did not always agree well with the observation. Although many uncertain parameters had to be used for calculation, one of factors causing seriously inconsistency with observation is the assumed Gaussian shape function. The disagreement was especially remarkable in binding modes of monomeric species,[13] since the band shapes of absorption spectra and circular dichroism from monomeric species were non-Gaussian.[1]

There has been some controversy on the exciton theory of optical activity, especially in the choice of the band shape of circular dichroism.[39] This problem has been eliminated in time-dependent Hartree theory,[40–43] which is a more complete quantum mechanical approach to the optical activity. DeVoe's classical coupling oscillator theory[44,45] is equivalent to this Hartree theory but is more straightforward in formalism and easier to use.[41] Cech et al. concluded that the exciton theory can lead to a circular dichroism similar to those calculated by DeVoe's classical theory if the correct band shape and width are chosen. In this paper, we have demonstrated that good agreement can be obtained between the calculated and observed circular dichroism, even under the application of exciton theory, if the absorption band shape function is utilized.

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Photophysics of a Naphthalene Diisocyanate Based Polyurethane

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ABSTRACT: A naphthalene diisocyanate based polyurethane in dilute solution is found to form intramolecular excimers between chromophores in the polymer backbone. The excimer kinetics is described by a triple exponential decay function. On the basis of comparison with model compounds, the photophysics of the polymer system is accounted for by the "isolated monomer" scheme. The relative intensity of the excimer emission depends on the solvent system employed and correlates directly with the intrinsic viscosity of the solution.

Excimers are excited-state complexes comprised of two equivalent molecules. They are formed by the interaction between an excited-state molecule (or chromophore) with a ground-state molecule (or chromophore) of the same species. Excimers are characterized by red-shifted emission spectra resulting from a stabilized excited state.

For the past two decades, excimers formed between adjacent species attached as pendant groups to polymer chains have received widespread attention. An excellent recent review of this subject is given in ref 1. The most common chromophores studied to date have been phenyl and naphthyl groups. In only a few cases have investigations been conducted on polymers in which the excimer-forming groups are located in the polymer backbone.[2-5] Incorporation of excimer-forming species in the main chain affords the opportunity to investigate the interaction of chromophores in polymer systems that are well separated as opposed to nearest neighbors in typical systems such as poly(vinylnaphthalene) or polystyrene. In this paper, we report the photophysical behavior of a 1,5-naphthalene diisocyanate based polyurethane with naphthalene chromophores periodically spaced in the polymer backbone. Investigation of the dependence of the excimer emission intensity on concentration indicates that excimer formation occurs at concentrations far below those expected for intermolecular interaction. It is demonstrated that even in quite dilute solutions excimer formation depends directly on the nature of the solvent system employed. By including lifetime results based on emission decay curves on the target polymer system, this paper constitutes one of the first detailed photophysical accounts of excimer formation involving main-chain chromophoric units.

Experimental Section

The 1,5-naphthalene diisocyanate (NDI) (mp 126–128 °C) was synthesized by reacting 1,5-diaminonaphthalene (Fluka) and trichloromethyl chloroformate (Fluka) in *p*-dioxane at the reflux temperature. The formed product was purified by sublimation. The propyl *N*-naphthalene-1-carbamate (mp 73 °C) and dipropyl *N*,*N*′-naphthalene-1,5-diylbiscarbamate (mp 203 °C) were prepared from 1-propanol and the corresponding isocyanates in ethyl

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