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Spectroscopic Studies of Polyethylenimine with Pyrimidine or Purine Grafts

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Spectroscopic Studies of Polyethylenimine with Pyrimidine or Purine Grafts

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SYNOPSIS

Hypochromic effects of 2-(thymin-1-yl)propionyl graft polyethylenimine (P-T) and its related monomer (M-T) and dimer model (D-T), and 2-(adenin-9-yl)propionyl graft polyethylenimine (P-A) and its monomer (M-A) and dimer model (D-A) have been systematically investigated, and the base-stacked conformation in comparison with corresponding polynucleotides and dinucleotides has been discussed. The results suggest that the graft polymers and even their dimer models may have a particular preference for a stacked conformation compared with the corresponding poly- and dinucleotides. A study of solvent effect on the NMR spectra of the dimer model suggests the existence of a preferred base-stacked conformation. Base pairing effects between the complementary bases have also been studied for various combinations among the model systems. Only the combination P-T/P-A showed a considerable hypochromic effect.

INTRODUCTION

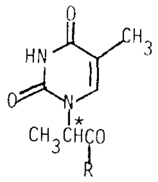
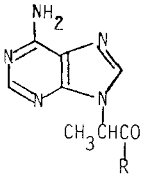
Hypochromicity (or hypochromism) of UV absorbance at the $\pi \rightarrow \pi^*$ band of nucleic acid base has been used most often for studies on the conformations of polynucleotides, oligonucleotides, and dinucleotides. Interactions between adjacent bases in a nucleic acid strand contribute significantly to the structural stability of the strand [1-4]. This strand is also well known to be stabilized by intermolecular hydrogen bonding between complementary bases [1-4]. In order to investigate these interactions, a number of model polymers of polynucleotides have been synthesized, most of which are cited in a preceding paper [5].

In general, the hypochromic effect is common to the systems which are stacked with the chromophores one on top of another, such as the stacking of the base planes in the helical structure of nucleic acids. Although the interaction between complementary bases has been of main concern for the spectroscopic investigations of the synthetic model polymers, it should be noted that base-pairing in the polynucleotide structure does not contribute directly to hypochromic effect, but that it contributes indirectly in such a manner as it stabilizes the stacked structure [1]. Therefore, it seems to be very important to study the hypochromic effect systematically on the individual polymer model using its related monomer and dimer model before one can investigate and discuss the complementary base-pairing interaction in terms of hypochromic effect of the mixture.

In the previous paper [5], the syntheses of grafts of purine and pyrimidine base derivatives onto linear polyethylenimine (PEI) and its related monomer and dimer models were presented. These can be regarded as a model system of polynucleotides, dinucleotides, and nucleosides. In the present paper we wish to report a spectroscopic investigation on the systematic set of the PEI with (-)-2-(thymin-1-yl)-propionyl grafts and its related monomer and dimer models, and the corresponding complementary series of (±)-2-(adenin-9-yl)-propionyl grafts described previously [5]. In order to obtain reliable values of UV absorbance and hypochromicity, special care was paid to the purification of these compounds.

UV spectra were recorded on a Perkin-Elmer 402 spectrometer or Beckman DU spectrophotometer. For the qualitative determination of molar extinction coefficient, the latter instrument was used. All spectra were determined against the appropriate blank using a matched set of sample and reference cells: cell length, 10 mm. The cell chamber of the spectrometer was thermostatted at 25°C. Measurements were carried out 3 days after the solutions were prepared.

TABLE 1. Structural Formulas and Abbreviations of the Polymers and Their Related Models

R		
$(CH_3CH_2)_2\overset{|}{N}$	M-(-)T	M-A
$(CH_3\overset{|}{N}CH_2\text{—})_2$	D-(-)T	D-A
$\text{—(—}CH_2CH_2\overset{|}{N}\text{—)—}$	P-(-)T[a]	P-A[b]

[a] 100 mole % graft.
[b] 56.5 mole % graft.

RESULTS AND DISCUSSION

We have previously synthesized a series of graft polyethylenimine with pendant (-)-2-(thymin-1-yl)propionyl group and its related model compounds and also a corresponding complementary series with 2-(adenin-9-yl)propionyl group [5]. The samples employed in the present study are listed in Table 1.

Hypochromism is a well known feature of UV spectra of the polynucleotides; the absorption is considerably less than the sum of the absorptions of the constituent monomer. The earliest successful theoretical explanation of this phenomenon was made by Tinoco [6] and Rhodes [7], showing induced dipole-dipole interaction in the chromophores could result in either hypochromism or hyperchromism depending on the relative geometry of stacked chromophores. Following the theory of Tinoco and Rhodes, several developments of the theory were reported [8-13]. However, the classical Tinoco-Rhodes theory is now recognized as essentially correct, in which the exchange of electrons among the chromophores is neglected. If one base has random orientation to another in the polymer arrays, then the absorption is nearly equal to that of the constituent monomer; however, if the bases are stacked parallel, there is a significant decrease in absorption. The variation of hypochromism is interpreted as a variation in the degree of base stacking [14]. Therefore the hypochromism in a given polymer could be a rough measure of the relative

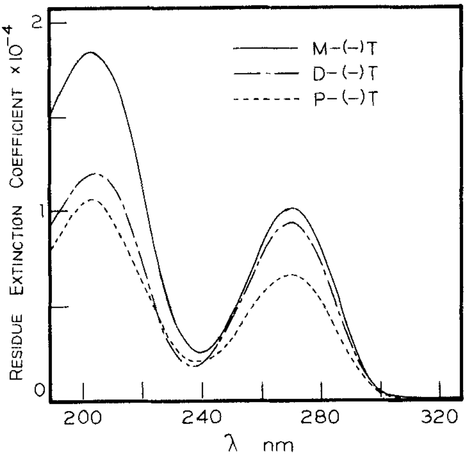


FIG. 1. UV spectra of P-(-)T and its related monomer and dimer models in neutral aqueous solutions.

orientation of the constituent bases. Figure 1 shows UV spectra of (-)-2-(thymin-1-yl)propionyl graft polyethylenimine [P-(-)T] and its related monomer model [M-(-)T] and dimer model [D-(-)T] measured in neutral water. The assignments of UV absorption of pyrimidine and purine nucleosides have been studied by comparing the CD, ORD, and UV [15-21]. The observed $\pi \rightarrow \pi^*$ transitions in the bases were classified into B_{2u}, B_{1u}, and E_{1u} in analogy with the $\pi \rightarrow \pi^*$ transitions in benzene [16]. The B_{2u} and B_{1u} bands, polarized perpendicular to each other in the plane of the base, show up in the 230-280 nm region. In the 180-220 nm region, there are the two bands of E_{1u}, doubly degenerate in benzene. Besides these $\pi \rightarrow \pi^*$ transitions, there are $n \rightarrow \pi^*$ transitions which are polarized perpendicular to the base plane. Miles et al. [19] assigned the 262 nm bands as the B_{2u}, a weak band at 240 nm as the B_{1u}, and the 216 and 196 nm bands as the E_{1u} pair for uracil nucleoside, which is taken to have identical spectra to thymine in most approximations [1]. Due to the relative weakness of the $n \rightarrow \pi^*$ band, they were unable to detect the presence of any $n \rightarrow \pi^*$ transition. In Fig. 1, the absorption peak at 270 nm is assigned as the B_{2u} band and the band at about 206 nm is the overlap of the E_{1u} pair and $\pi \rightarrow \pi^*$ of amide bond. The weak B_{1u} band

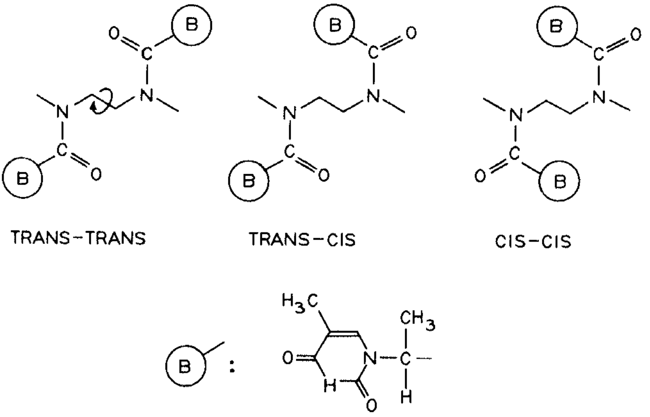
TABLE 2. Quantitative Ultraviolet Absorption Data and Hypochromicity (h) in Aqueous Solution

	Pure H_2O			0.1 <u>N</u>HCl			0.1 <u>N</u> NaOH		
Sample	λ_{max} (nm)	$\epsilon \times 10^{-4}$	h (%)	λ_{max} (nm)	$\epsilon \times 10^{-4}$	h (%)	λ_{max} (nm)	$\epsilon \times 10^{-4}$	h (%)
M-(-)T	270	1.02	-	270	1.02	-	265	0.848	-
D-(-)T	270	0.944	7.45	270	0.877	14.0	265	0.816	3.8
P-(-)T	270	0.645	36.8	270	0.621	39.1	265	0.696	17.9
M-A	260	1.47	-	260	1.38	-	260	1.81	-
D-A	260	1.37	6.80	260	1.53	-1.1	260	1.47	18.8
P-A	260	0.803	45.4	260	1.38	0	-	-	-

TABLE 3. Quantitative Ultraviolet Absorption Data and Hypochromicity (h) in Ethanol and 2,2,2-Trifluoroethanol

	Ethanol			TFE		
Sample	λ_{max} (nm)	$\epsilon \times 10^{-4}$	h (%)	λ_{max} (nm)	$\epsilon \times 10^{-4}$	h (%)
M-(-)T	270	0.62	-	270	0.862	-
D-(-)T	270	0.58	6.5	270	0.862	0
P-(-)T	270	0.51	17.7	270	0.749	13.1
M-A	260	0.94	-	260	1.43	-
D-A	260	0.81	13.8	260	1.33	7.0
P-A	260	0.61	35.1	260	1.25	12.6

should be overlaid by the tails of these strong bands. The intensity of the B_{2u} band for the polymer is weaker than that of monomer model by about 37%. This value is within the typical hypochromism (20-40%) in polynucleotides in neutral water [1]. This hypochromic effect was also observed for the dimer model (about 7.5%), suggesting the presence of stacking even in the dimer model, although much smaller than in the case of the polymer. Tables 2 and 3 show the molar extinction coefficient and hypochromicity in various solvents. In order to estimate the extent of electronic perturbation caused by base-base interaction, hypochromicity (h) is more often used than hypochromism (H) which corresponds to an integrated hypochromicity, simply because the former is much easier to calculate. Although it is obvious that a comparison of absorption intensities at only one wavelength would not give an accurate estimate, the H and h result in quite similar values in DNA, RNA, and polynucleotides, mainly because of the similarity of the band shape of the native and denatured forms as well as the constituent mononucleosides [1]. In Tables 2 and 3, h is used instead of H for the above reason. P-(-)T shows large hypochromicity in neutral and acidic aqueous solution, while it considerably decreases in 0.1 N sodium hydroxide in which the thymine group is negatively ionized [22]. P-A also shows large hypochromicity in neutral aqueous solution, while no hypochromicity was observed in 0.1 N hydrochloric acid, in which the adenine group is protonated [22]. These facts clearly indicate that the electrostatic repulsion between charged bases substantially prevents stacking interaction. The tendency can be seen for the dimer models. It is noted that negative charge appeared to be less effective than positive charge in reducing hypochromicity, as pointed out by Leonard et al. [23] in their study on the trimethylene-spaced dinucleotide analogs. The hypochromism of poly-U is generally much smaller at room temperature than that of poly-A, indicating lesser extent of stacking in poly-U [24], although at temperatures below 10°C, it shows significant stacking features [3]. Unlike poly-U, P-(-)T shows considerable hypochromicity. Dinucleotides are also known to show significant hypochromism, ranging from 0 to 13%, at room temperature [14]. Warshaw and Tinoco [14] estimated the extent of stack-forming ability of the various bases in dimers and reported that uracil (U) prefers the unstacked arrangement, while adenine (A), cytosine (C), and guanine (G) prefer to stack. Leonard reported [23] the extensive study of the stacking interaction on the dinucleotide model compounds in which the bases are linked by a polymethylene chain. They clearly showed the order of interaction in neutral aqueous solution is purine-purine > purine-pyrimidine > pyrimidine-pyrimidine. However, as seen in Table 2, even D-(-)T dimer showed quite large hypochromicity in neutral and acid aqueous solution. In the stacked molecules, the bases are considered to situate most of the time on top of each other with their base rings parallel, whereas in the unstacked molecule they are separated by solvation [14]. It appears that in the cases of P-(-)T and D-(-)T there might be a particular preference for stacking between neighboring bases in the array. A study of a space-filling model of D-(-)T dimer reveals the following facts. The freedom of motion of 2-(thymin-1-yl)propionyl side chain is considerably restricted as a result of the partial double bond character of amide bond and the restriction of free rotation around the OC—CH bond due to steric hindrance. Therefore, the ethylenediamine backbone in the dimer seems to be the only flexible part, at the nitrogen of which virtually rigid 2-(thymin-1-yl)propionyl groups are combined. The dimer model has three possible conformers with respect to the amide bonds: trans-trans, trans-cis, and cis-cis. One would immediately realize from the space filling model that the trans-trans conformation is the most convenient conformation for the two bases either to separate in the farthest distance or to fit one above another by rotating approximately 180° around the CH_2—CH_2 bond. It should be noted that in the cis-cis conformation, base stacking is sterically impossible. In the NMR spectra the exchange between one conformer to another is so slow at room temperature that each conformer gives well resolved peaks [5]. Figure 2 shows the 100-MHz NMR spectra of the N-methyl proton in various solvents at room temperature. As reported in the preceding paper [5], the trans-trans conformation predominates over cis-cis conformation by a factor of 4 in DMSO-d_6. In chloroform the spectrum is quite similar to that in DMSO-d_6 (trans-trans conformation is a little more favorable than that in



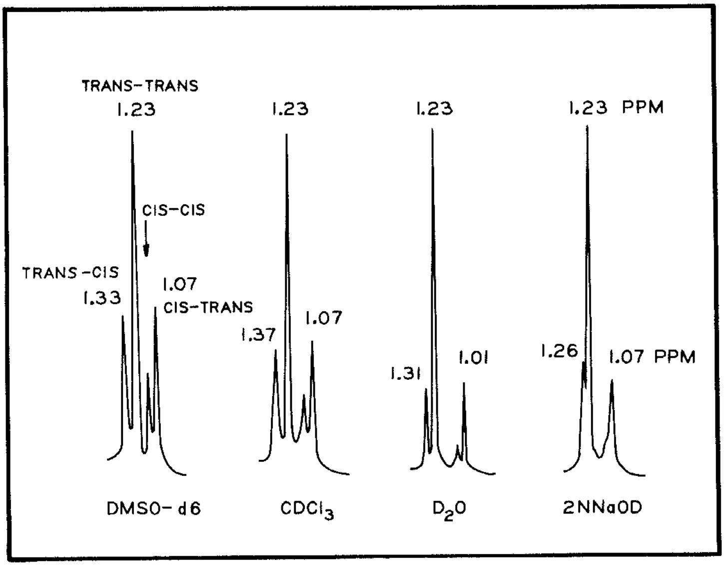


FIG. 2. 100 MHz spectra of the N-methyl proton in the dimer model compound in various solvents. Chemical shifts from thymine C_3-CH_3 are presented in ppm.

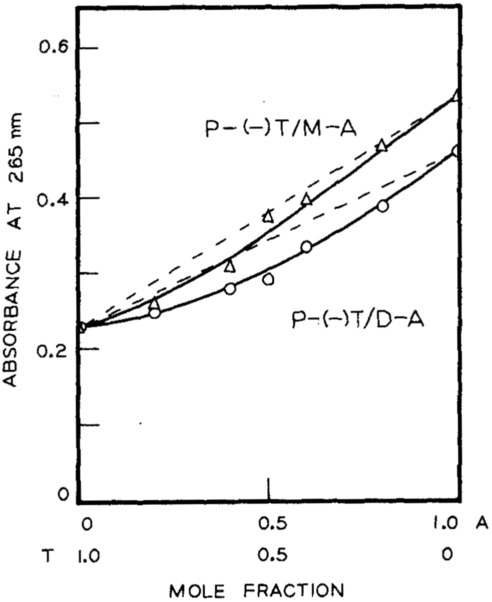


FIG. 3. Continuous variation mixing curves for P-(-)T/M-A and P-(-)T/D-A in pure water at 25°C: Concentration of residue, [A + T], 3.6×10^{-5} M.

DMSO-d_6); however, in neutral aqueous solution the dimer model was shown to exist overwhelmingly in the trans-trans form. From the peak ratios in the NMR spectra, the trans-trans conformation is about 2.5 times preferable to the trans-cis and more than 20 times preferable to the cis-cis conformation. In 2 N sodium deuteroxide aqueous solution the peak ratios seem to be approximately the same as in neutral aqueous solution, although the cis-cis form gave no well resolved peak. These facts imply that the dimer model compound exists in base-stacked conformation most of the time in neutral aqueous solution and base-separated form in sodium hydroxide aqueous solution due to electrostatic repulsion [22].

Table 3 shows hypochromicity in ethanol and 2,2,2-trifluoroethanol (TFE). DNA is well known to be denatured by many organic solvents including ethanol [23, 25, 26]. Leonard et al. [23] investigated the effect of ethanol on the stacking interaction of Ad-$(CH_2)_3$-Ad and Th-$(CH_2)_3$-Th and found that virtually no interaction was detectable in either case, showing clearly that the reason for the denaturation is not only the increased electrostatic repulsion of phosphates [27]

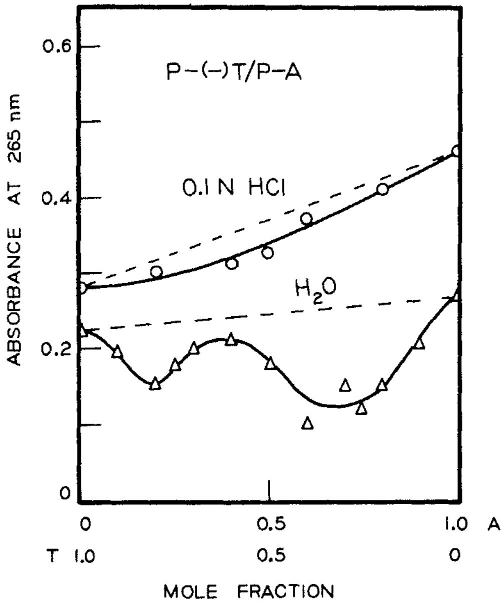


FIG. 4. Continuous variation mixing curves for P-(-)T/P-A in pure water and 0.1 N HCl at 25°C. Concentration of residue, [T + A], 3.6×10^{-5} M.

and increased intramolecular hydrogen bonding in the organic solvents, but also the direct decrease in stacking interaction between the bases. It is noticeable that our dimer models and polymers, either in the case of thymine or adenine, show considerable hypochromic effects in ethanol, although they are relatively smaller than those in neutral aqueous solutions. Even in TFE solution, noticeable hypochromic effects are still observed. These facts may imply the particular preference for a stacking conformation for these compounds arisen from the structural convenience.

The formation of complementary base pairs was observed by continuous variation mixing curve (Job plots [28]) of UV absorption for various combinations of complementary bases. Figure 3 shows the Job plots for the combination of P-(-)T/M-A and P-(-)T/D-A. In both cases, only a slight deviation from Beer's law was seen, indicating very little base pair formation. On the other hand, the polymer-polymer combination P-(-)T/P-A resulted in marked hypochromic effects (Fig. 4) at certain mixing ratios in neutral aqueous solution. In acidic aqueous solution, however, only

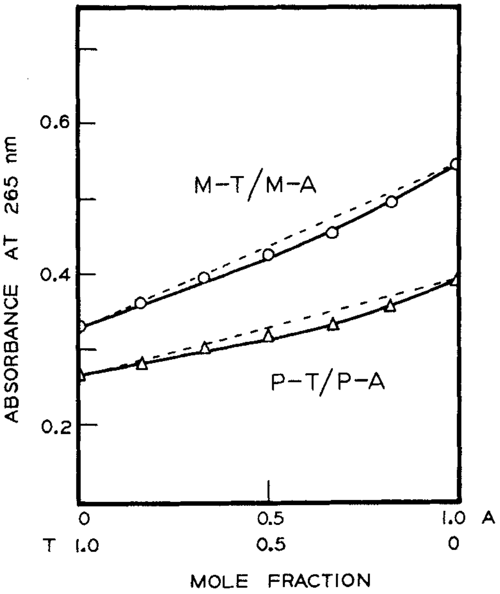


FIG. 5. Continuous variation mixing curves for M-T/M-A and P-T/P-A in TFE at 25°C. Concentration of residue, [T + A], 4×10^{-5} M.

a small deviation from Beer's law was seen. These facts strongly suggest that this polymer-polymer mixture forms base-paired complexes to stabilize the stacked conformation. The Job plot in neutral aqueous solution in Fig. 4 shows two minimum peaks, one in the P-(-)T rich-side (mole fraction T = 0.8) and the other in P-A rich region (mole fraction A = 0.7). The solutions with P-A mole fraction of about 0.7 were very slightly opalescent, suggesting the presence of partial aggregation. The scattering of the plots in the vicinity of this mixing ratio is presumably due to this partial aggregation. The large deviation from Beer's law at these mixing ratios implies that a large stabilization effect resulted from the base pairing. At the present stage, however, neither the structure nor the stoichiometry of the base-paired complex can be interpreted. Figures 5 and 6 show the mixing curves measured in TFE and chloroform-ethanol (1:1), respectively. From the nature of the solvents, the formation of an interbase hydrogen bond is expected to be highly preferable in the latter solvent, while rather unfavorable in the TFE. A complementary polymer mixture P-T/P-A showed no virtual complex formation in TFE. For this experiment, (±)-2-(thymin-1-yl)propionyl grafted

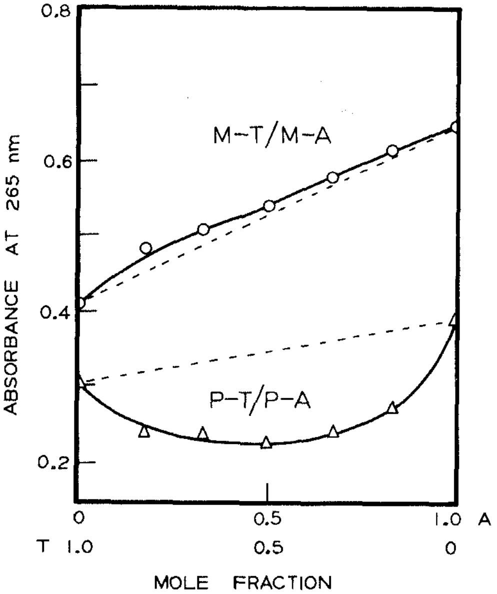


FIG. 6. Continuous variation mixing curves for M-T/M-A and P-T/P-A in chloroform/ethanol mixture (1/1, v/v) at 25°C. Concentration of residue, [T + A], 4×10^{-5} M.

PEI was used instead of optically active P-(-)T. No difference in the absorption spectra between the two had been confirmed before hand. On the other hand, in chloroform-ethanol solution, P-T/P-A showed considerable degree of base pairing. In this case, the largest deviation from Beer's law occurred in equimolar solution mixtures. These facts can reasonably be associated with the hydrogen bond forming ability of the solvent. In general, the stacking of the bases is substantial cause for the hypochromism, and base-pairing itself has no direct contribution to the hypochromism [1, 23]. However, horizontally hydrogen-bonded base pairs can result in the stabilization of the stacked array, which is likely to result in the enhancement of hypochromism. The negative deviation from Beer's law in the Job plot substantially means the stabilization effect of stacking resulted from base pairing. This understanding is supported by the fact that a set of complementary monomers, M-T/M-A, showed no hypochromicity in the whole range of composition even in chloroform-ethanol, in which hydrogen-bonded base pairing is expected to take place to some extent, even for the complementary monomer mixture.

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