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The Properties of Structurally Homogeneous Poly(1-vinyluracils) and Their Interactions with Polynucleotides

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ABSTRACT

It has been found that highly syndiotactic PVU has a greater hypochromism and fluorescence emission intensity along with a different dimethyl sulfoxide denaturation profile than the less syndiotactic polymer in aqueous solutions. These results are probably attributable to an interrelation between the configurational arrangement of uracils, the conformation of the polymers, and base stacking between uracils. In the neutral pH range, before the onset of aggregation and precipitation, the polymers are thought to be in tightly packed coillike conformations which are stabilized by intramolecular base stacking forces. The reaction of highly syndiotactic PVU with Poly A was thought to form a triple stranded PVU:2 Poly A complex, while the less syndiotactic polymer formed a metastable double stranded 33.3% Poly A complex which on equilibration rearranged into a 25% Poly A complex probably containing PVU loops. In the presence of 20% DMSO this system formed a 13% Poly A complex indicative of partial denaturation of the base pairs with DMSO. PVU was also

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found to form a metastable 33.3% poly(9-vinyladenine) complex. It has been concluded that tacticity and solvent effects have a dramatic effect on the base stacking and base pairing interactions of these polymers. The results suggest that the polymers might be potentially powerful macromolecular therapeutic agents.

INTRODUCTION

During the last 6 years we have been involved in a study of the synthesis and properties of simple hydrocarbon polymer analogs of the nucleic acids in order to further our understanding of nucleic acids and to extend polymer chemistry into new frontiers at the polymer-biochemistry interface [1-6].

We have previously reported that poly(9-vinyladenine) (PVAd) [4] can react with polyuridylic acid to form double and triple stranded complexes, and more recently we have determined the unperturbed dimensions [7] of this polymer and the fact that it is a powerful macromolecular reagent for inhibiting the growth of rats [7]. Other groups are now also involved with the physicochemical and biochemical studies of nucleic acid analogs [8-14]. Our studies with poly(1-vinylurucil) (PVU), however, have been delayed because our early polymers contained a high percentage of cyclopolymerized units [5]. The solution properties of a PVU prepared by a different synthetic procedure has also been reported [9]. These workers have found that their PVU did not completely base pair with polyadenylic acid, but instead formed a complex containing 10% adenine bases [9].

Recently we have been able to prepare structurally homogeneous PVU's of varying tacticity [6]; we now wish to report that the properties and interactions of our polymers are different from those previously reported and that the reactions of these materials with polynucleotides are sensitive to tacticity.

RESULTS

Poly(1-vinylurucils)

The UV extinction coefficients (ϵ_{max}) of PVUI and PVUII as a function of pH are shown in Fig. 1. The solution of PVUI in the pH 7-9 range was found to be very unstable, with aggregation and precipitation eventually occurring. For this reason the reproducibility of the data was found to be poor. The solubility of PVUII was found to be better than PVUI in 0.01 M Tris buffer (pH 7-9). At pH 7 the concentration of

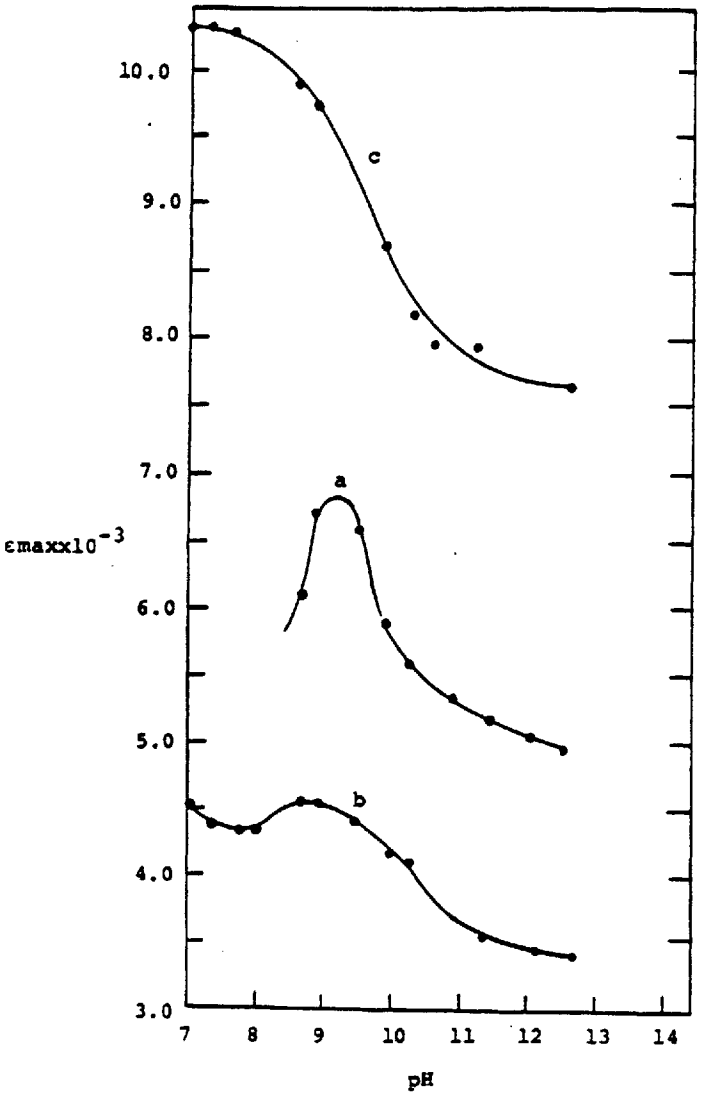

FIG. 1. Variation of ϵ_{max} with pH of freshly made PVU solutions at 25° in 0.2 M NaCl at 263 mμ: (a) PVUI, (b) PVUII, (c) 1-ethyluracil.

PVUII can be kept at 8×10^{-5} M for a few days without precipitation, whereas the PVUI solution is stable for less than 1 hr. The very low solubility of these polymers has unfortunately prevented us from carrying out osmotic pressure and light-scattering studies.

The ϵ_{max} of PVUII at pH 7 has been found to change with time, whereas at pH 12 a reproducible constant value was obtained. Compared to 1-ethyluracil, it can be seen that in the pH 7-13 range the PVUI and PVUII are very hypochromic at 263 mμ. At pH 12 the hypochromism was 29% for PVUI and 51% for PVUII. In neutral solution the hypochromism was even larger. Because the polymers aggregate and eventually precipitate, no definite value can be given for the percent hypochromism at neutral pH values.

The fluorescence emission spectra of PVUI and PVUII in solution at pH 8.9 are shown in Fig. 2. After 1 day of standing, precipitation was found to occur in the solution of PVUI, suggesting that the intensity of Curve b might be changing with time. It is significant that these

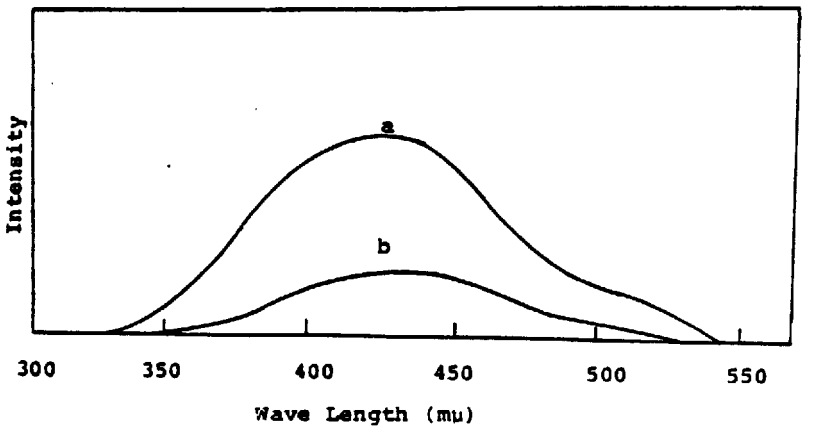

FIG. 2. Fluorescence emission spectra of freshly prepared PVUI and PVUII in 0.01 M Tris buffer pH 8.9, 0.01 M NaCl solution, C = 1.54×10^{-4} M at 25° excited at 278 mμ: (a) PVUII, (b) PVUI.

are the first reported fluorescence emission spectra of uracil derivative at ambient temperature [15].

The variation in UV extinction coefficients of the PVU solutions with percent DMSO is shown in Fig. 3. It can be seen that the extinction coefficient, ϵ_{max}, of the model compound, 1-ethyluracil, remained almost constant as the percent DMSO was varied (Curve d). For PVUI,

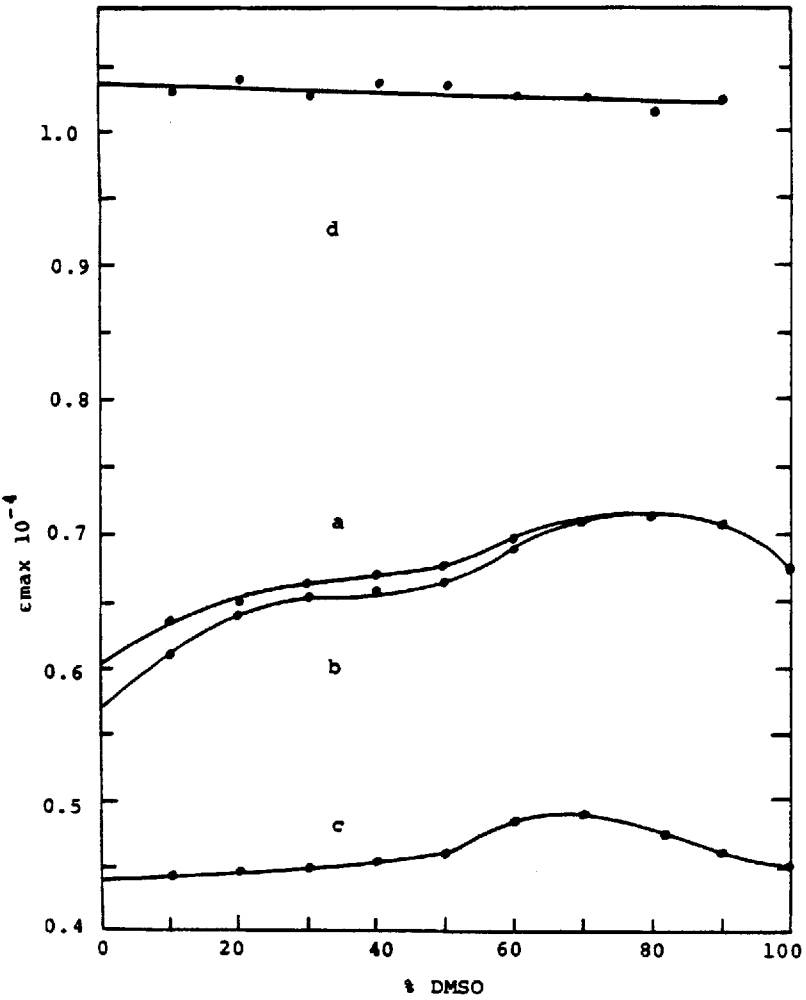

FIG. 3. Variation of ϵ_{max} with % DMSO in 0.01 M Tris buffer solutions of PVU at 263 mμ: (a) PVUI originally at pH 8.9, (b) PVUI originally at pH 8.45, (c) PVUII originally at pH 8.45, (d) 1-ethyluracil at pH 8.45 and 267 mμ.

both at pH 8.45 and at pH 8.95 (Curves a and b), diphasic denaturation curves were observed with shallow transitions at 15 and 60% DMSO suggesting that two or more conformations exist for PVUI in DMSO-H_2O

solutions. On the other hand the solid state PVUII (Curve c) showed only one melting transition at about 57% DMSO. The thermal melting profiles of these polymers are shown in Fig. 4 where it can be seen that the absorbance is relatively insensitive to temperatures.

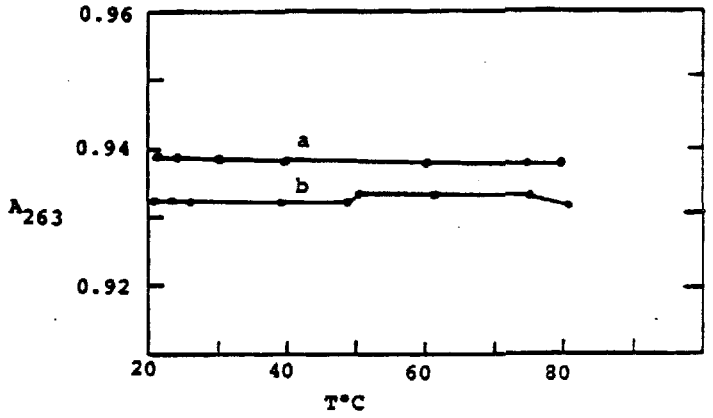

FIG. 4. Temperature dependence of the absorbance of PVU II (a) and PVU I (b) in 0.01 M Tris buffer pH 8.45, 0.01 M NaCl.

Base Pairing of Poly(1-vinyluracils)

Complexes of PVUI with Poly A

In order to study the base pairing properties of PVU with a complementary polynucleotide, synthetic Poly A was selected for our investigations. We had hoped to make this study at pH 7.3, the physiological pH. Unfortunately, because of the low solubility of PVUI at this pH, we were forced to carry out the continuous variation mixing experiment [16] at pH 8.45. After allowing these mixed solutions to stand for 3 hr at 25°, all of the solutions were neutralized to pH 7.75 by adding equal volumes of pH 6.65 buffer. The UV absorbance mixing curve is shown in Fig. 5 (Curve a) after being allowed to stand for 3 hr at 25° after neutralization. A reproducible break was observed at 33.3% Poly A, which indicated that complex formation had occurred. After standing for 15 hr the absorbance was found to fall off at about 12% Poly A. This is probably due to association or aggregation of PVUI in the very low concentration of Poly A. The 10% Poly A solution was still clear 15 hr after neutralization but with a much lower absorbance compared to the higher concentration Poly A solutions. It is apparent

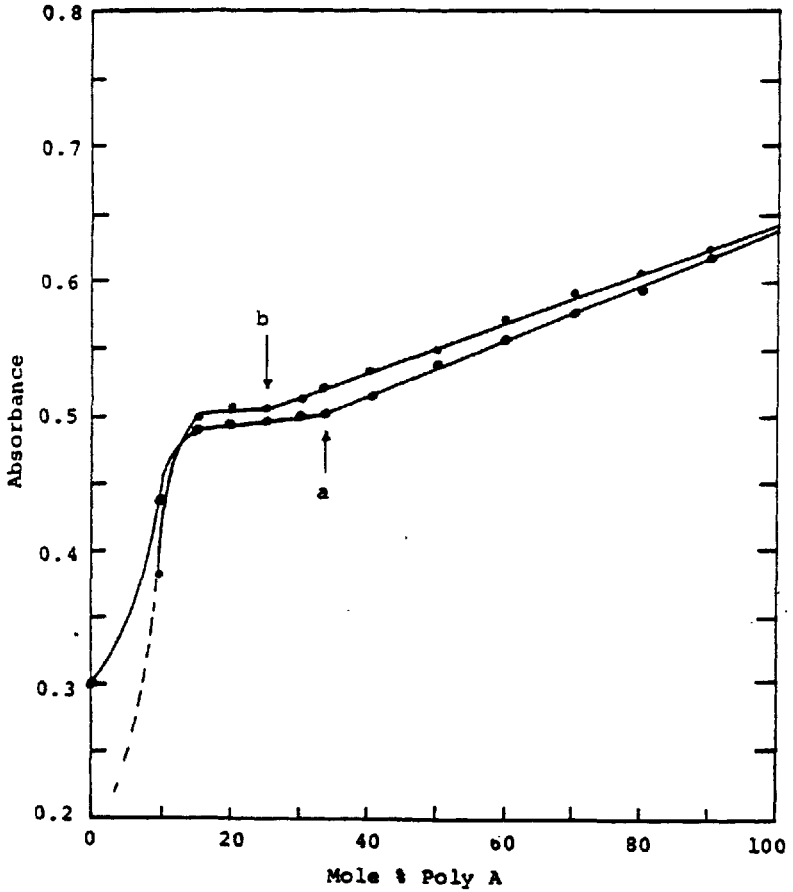

FIG. 5. Continuous variation mixing curves for PVUI and Poly A in 0.01 M Tris buffer pH 7.75, 0.01 M NaCl at 263 mμ and 25°. The total polymer concentration was 6.60×10^{-5} M. After the samples were equilibrated at pH 8.85 for 3 hr, they were neutralized to pH 7.75: (a) Measurements were then made after 3 hr at 25°, (b) after 15 hr at 25°.

from this that complex formation with Poly A prevents the PVUI from precipitating out of solution as it would have done if the Poly A was absent. In this system the 33.3% Poly A complex is unstable at 25° and it shifts to a 25% Poly A complex on equilibration. The CD spectral properties of these complexes are recorded in Table 1.

TABLE 1. Circular Dichroism Data for Complexes of PVU with Poly A

% Poly A in Complex	CD max (mμ)	$R_{ba} \times 10^{-40}$ (cgs)	CD min (mμ)	$R_{ba} \times 10^{-40}$ (cgs)	CD max (mμ)	$R_{ba} \times 10^{-40}$ (cgs)
50	262	22.5	247	-6.92	221	14.1
33	266	4.73	247	-5.2	220	4.32
25	273	16.0	254	-7.8	226	8.74

Because DMSO has been found to be a very powerful denaturation agent for PVU solutions (Fig. 3), the effect of DMSO on the stability of the PVU-Poly A complexes was also studied. The results are shown in Fig. 6. In 20% DMSO, either after 3 hr at 25° or after 4 days at 4°, the mixing curves showed only one complex at 13% Poly A.

Complexes of PVUII with Poly A

The results of the continuous variation mixing experiments between PVUII and Poly A, which were carried out at pH 6.9, are shown in Fig. 7. The measurements after equilibrating for 10, 24, and 51 hr

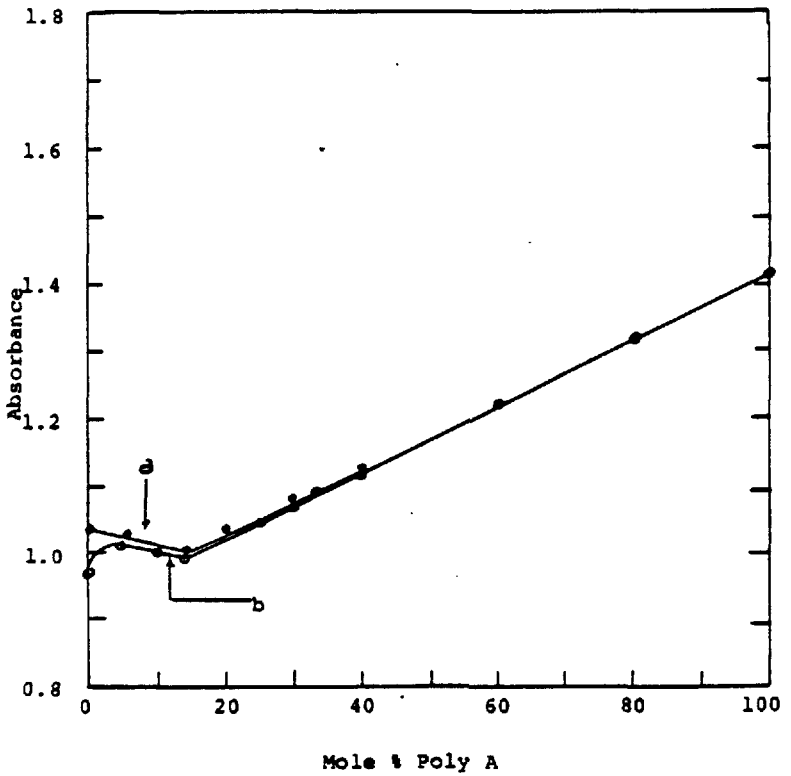

FIG. 6. Continuous variation mixing curves at 263 mμ for PVUI and Poly A in 20% (v/v) DMSO, 0.01 M Tris buffer pH 8.45, 0.01 M NaCl. The total polymer concentration was 1.38×10^{-4} M: (a) The measurements were made 3 hr after mixing at 25°, (b) after 3 days of equilibration at 4°.

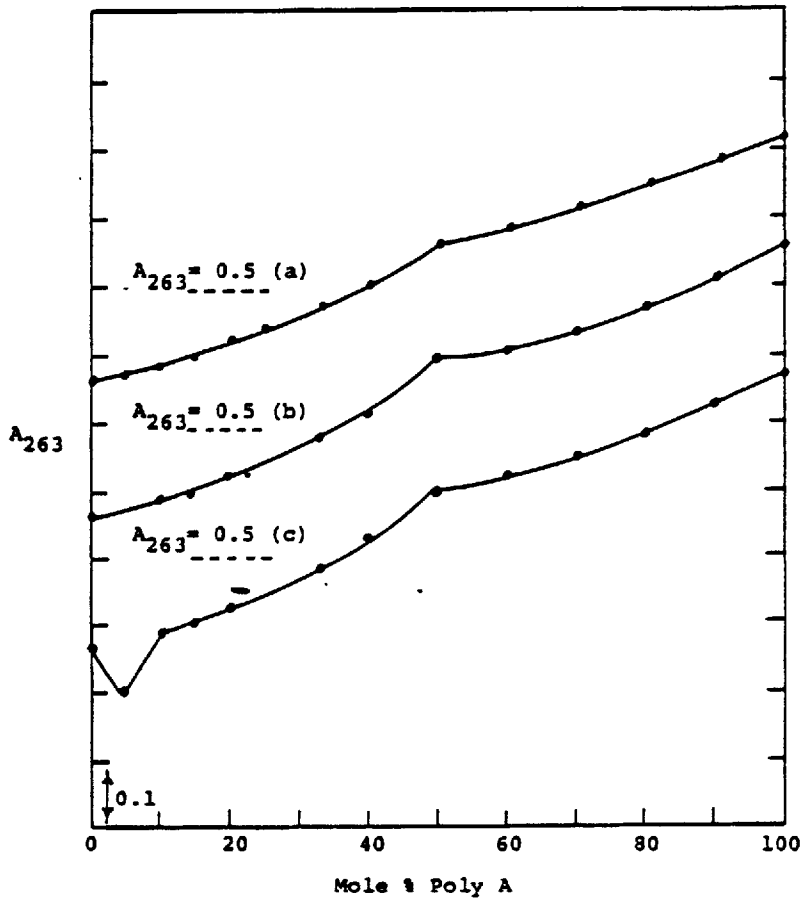

FIG. 7. Continuous variation mixing curves at 263 mμ for PVUII and Poly A in 0.01 M sodium cacodylate, pH 6.9, 0.01 M NaCl at 25°. The total polymer concentration was 8.16×10^{-5} M: (a) after 10 hr at 25°, (b) after 24 hr at 25°, (c) after 51 hr at 25°.

at 25° are plotted in Curves a, b, and c, respectively. It can be seen that the absorbance at 50% Poly A is hyperchromic and increases with time. This effect is not surprising because of the very large hypochromism observed in PVUII. The breakage of uracil-uracil base stacks and pairs with formation of uracil-adenine base pairs simply indicates that the uracil-adenine pairs are less hypochromic. An

analogous situation has been observed in guanosine-cytidine pairing [17]. The CD spectrum of this complex is summarized in Table 1.

Complexes of PVUII with PVAd

PVUII has been found to form a complex with poly(9-vinyladenine) (PVAd) with about 33.3% stoichiometry in PVAd after standing for 3 hr at 25° (Fig. 8). The system was found to precipitate after standing for 5 days. A summary of the base pairing properties of the PVU's are shown in Table 2.

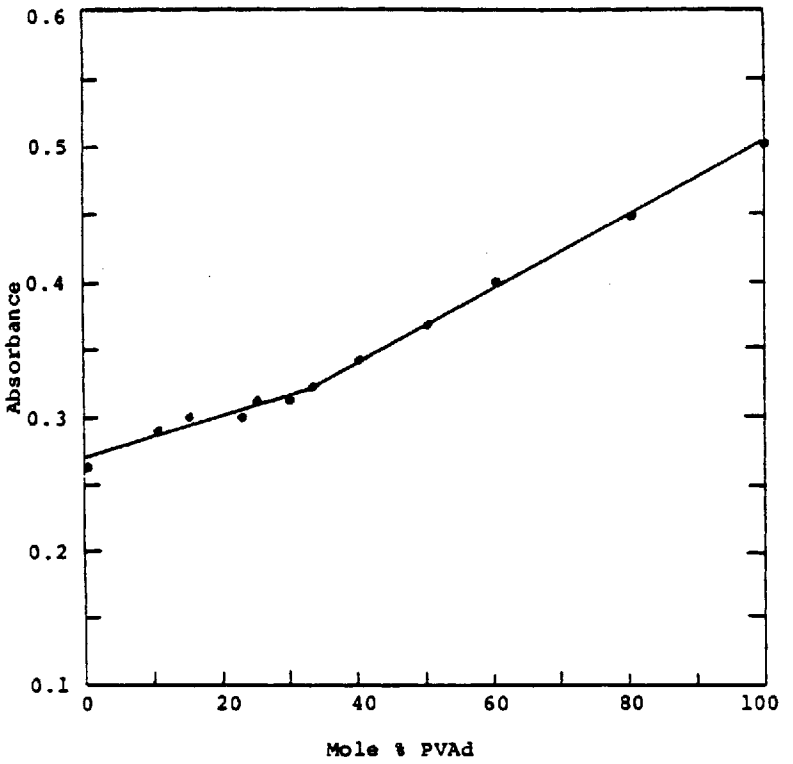

FIG. 8. Continuous variation mixing curve at 263 mμ for PVUII and PVAd in 0.01 M sodium cacodylate pH 7, 0.01 M NaCl at 25°. The total polymer concentration was 7.77×10^{-5} M. The measurements were made 3 hr after mixing.

TABLE 2. Summary of Complex Formation between PVUI and PVUII with Poly A and PVAd

Complex	Buffer, 0.01 M; NaCl 0.01 M	pH	Equilibration times and temperatures	% Poly A complex	% Poly A at break off	Fig. citation
PVUI with Poly A	Tris	7.75	At pH 8.45 for 3 hr, then pH 7.75 at 25° for 3 hr and for	33.3	12	5
			15 hr	25	12	5
PVUI with Poly A	Tris[a] 20% DMSO		After 3 hr at 25° and 4 more days at 4°	13		6
				13	4	6
PVUII with Poly A	Na cacocylate	6.9	At 25° after 24 hr	50		7
			After 51 hr	50		7
PVUII with PVAd	Tris	7	After 3 hr at 25°	33.3% PVAd		8

[a]Tris buffer component was at pH 8.45.

The results of the thermal melting of the complexes of PVUI and PVUII with Poly A and PVAd are shown in Fig. 9. All these melting curves show a broad melting range. The melting range of the 25% Poly A complex is roughly 41°, while the melting range of the 50%

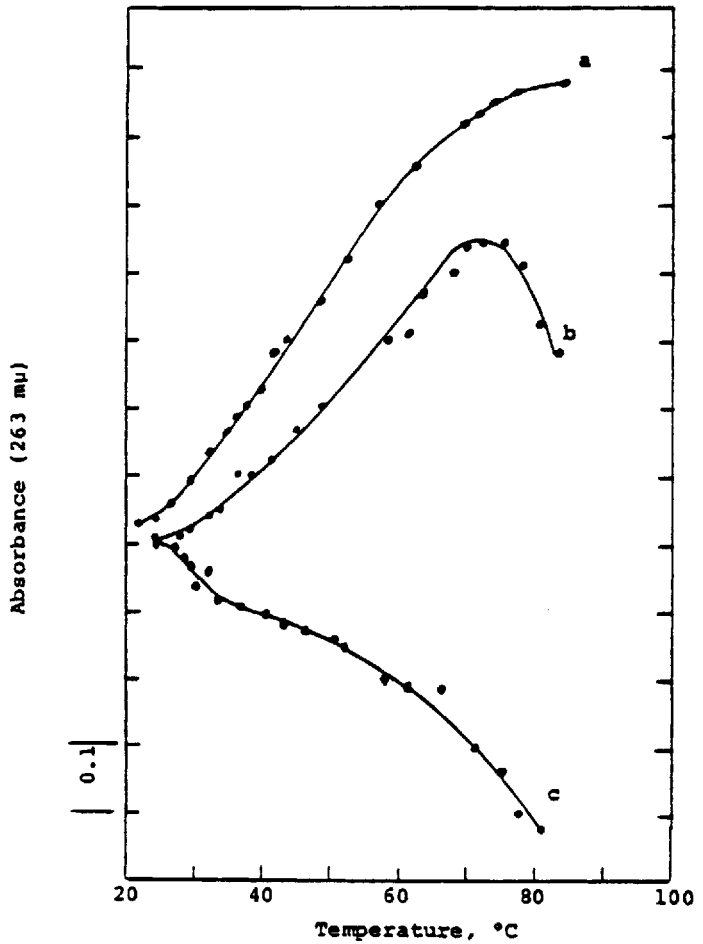

FIG. 9. (a): UV absorbance melting profile of the 25% Poly A, PVUI: Poly A complex in 0.01 M Tris buffer, pH 7.75, 0.01 M NaCl. (b): UV absorbance melting profile of the 50% Poly A, PVUII:2 Poly A complex in 0.01 M sodium cacodylate, pH 6.9, 0.01 M NaCl. (c): UV absorbance melting profile of the 33.3% PVAd, 2PVUII:PVAd complex in 0.01 M sodium cacodylate, pH 7.0, 0.01 M NaCl.

complex is about 46°. The melting curve of the complex of PVUII and PVAd shows an unusual effect where the absorbancy decreases with temperature.

DISCUSSION

Poly(1-vinyluracils)

UV studies of Poly U solutions have been reported to show only a few percent hypochromism at neutral pH and ambient temperature. At lower temperatures, however, 30% hypochromism was observed, and this was attributed to the formation of a stacked helical polynucleotide structure [18]. 1,1-Trimethylenebisuracil (Ur-C_3-Ur), a model system for PVUI and PVUII, has recently been found to have only 1.2% hypochromism at neutral pH and 0.7% hyperchromism in 0.01 m sodium hydroxide [19]. It therefore seems likely that the high percentage of hypochromism observed in PVU solutions may be attributed to 1) the configurational arrangement of uracils in proximity to each other along the chain, 2) the conformation of the polymer as influenced by the configuration of uracils, and 3) the degree of base stacking and base-pairing between these groups. The different pH-absorbance profiles for PVUI and PVUII shown in Fig. 1 indicate a different stereochemistry and/or degree of association for these polymers. The humps in Curves a and b were not observed for the PVU prepared by Pitha et al. [9], indicating that our polymers are considerably different from their material. A pronounced change in the apparent pK_a values is also observed. The pK_a value of 1-ethyluracil is about 9.4 and those of PVUI and PVUII are close to 10.3, suggesting the existence of base stacking in these two polymers [20]. In alkaline solution the N—H protons of the uracil groups in the PVU's were removed, thereby decreasing the probability for base pairing and stacking between uracils due to electrostatic repulsion forces. Regardless of the stereoregularity in PVU, the conformation of these polymers in basic solution will be highly extended coils or rods because of the polyelectrolyte effect. Even though hydrophobic base stacking must be absent, the proximity of uracils still seems to be close enough for electronic interactions to be important. The large difference in hypochromism between PVUI and PVUII in the extended conformation is most likely attributed to their differences in tacticity, with the more highly syndiotactic PVUII having the greater hypochromism.

Fluorescence emission studies of model systems such as 1-propylthymine, 1,1'trimethylenebisthymine [19], and Poly U [21] have shown that emission is not observed at ambient temperatures. At

low temperatures, however, the fluorescence emission spectra reported for DNA, polynucleotides, and some dinucleotides has been found to be broad, red shifted, and lacking vibrational fine structure [15]. This effect has been attributed to excimer formation. The configurational proximity of uracils in the PVU samples coupled with the possible existence of special structures in the polymers due to base stacking explain why broad, featureless fluorescence emission (Fig. 2) was observed for PVUI and PVUII at room temperature. The large difference in fluorescence emission intensity for the two polymers also reflects their different stereochemistries, with the highly ordered PVUII having the larger emission intensity.

Further support for the existence of base stacking interactions in PVUI and PVUII was obtained from DMSO denaturation [22] experiments (Fig. 3). A clear separation between denaturation of base stacks or uracil-uracil base pairs was, of course, not obtained here. It is reasonable to assume that the PVU polymers exist as tightly packed coils in aqueous solution because of long range intramolecular base stacking, in analogy to the behavior of the more soluble PVAd [7]. As DMSO is an excellent solvent for PVU because it can hydrogen bond with the N—H of the uracils, the hydrodynamic volume of the coils probably increased considerably with the increase in DMSO concentration. During coil expansion, the base stacks which were disrupted resulted in an increase in absorbance, as experimentally observed. The differences in the denaturation profiles of PVUI and PVUII, although small, may reflect different stacking arrangements in the coils due to their tacticities or they may also be due to the different manner in which the bases reorient themselves as the coil expands. The latter effect is thought to be responsible for the optical behavior of isotactic and syndiotactic polystyrene on coil expansion [23]. Although the possibility of association in the PVU solutions as an alternative explanation of these results has not been demonstrated here, the absence of such effects has been found in the hydrodynamic studies of PVAd [7].

The absorbance temperature profiles shown in Fig. 4 for the PVU's suggest that no conformational change has occurred if it is assumed that the UV absorbance is sensitive to such changes. It is important for us to point out here that no conclusions can be drawn from Fig. 4 in light of the fact that PVAd has been shown to undergo a conformational change that could not be detected by UV absorbance measurements [7].

Base Pairing of Poly(1-vinyluracils)

The results obtained concerning the interaction of PVUI and PVUII with Poly A and the interaction of PVUII with PVAd is somewhat in

contrast to the results of Pitha et al. [8] with their polymer. This might be attributed to structural inhomogeneity in their material due to the presence of cyclopolymerized units and or to the differences in tacticity between our polymers.

Our results with PVUI indicated that a metastable 33.3% Poly A complex was formed at 25° at pH 7.75 which eventually changed into a 25% Poly A complex. The 33.3% stoichiometry is consistent with a double-stranded structure in which every other uracil ring in mainly syndiotactic PVUI base pairs with all of the adenines in Poly A. The melting profile of the 25% complex shown in Fig. 9 is rather broad and mainly indicated the melting curve for Poly A. A schematic

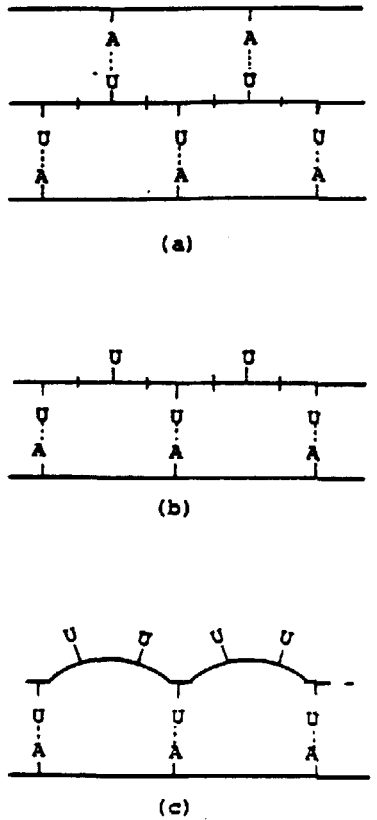

FIG. 10. Schematic representations of (a) PVUII:2 Poly A triple-stranded complex, (b) PUVI:Poly A double-stranded 33.3% adenine complex, and the (c) double-stranded 25% adenine complex.

representation of this structure is shown in Fig. 10. The detection of this complex is interesting in light of the fact that a 33.3% Poly U complex was not formed in the interaction of PVAd with Poly U [4]. In analogy with this other work it seems likely that the 25% Poly A complex is a double-stranded structure containing PVUI loops (Fig. 10). The reason for a stable double-stranded structure with loops for both the PVAd and PVUI systems has not as yet been explained, but it may be due to an entropy effect whereby the loops would allow the complex to have a greater degree of flexibility.

The effect of 20% DMSO on complex formation of PVUI with Poly A resulted in a complex with 13% Poly A stoichiometry. This result suggests that the DMSO has denaturated some of the uracil-adenine base pairs, resulting in an incomplete base paired situation similar to the complex reported by Pitha [9]. The resulting complex observed here is probably double stranded with large PVU loops.

The interaction of PVUII with Poly A was conveniently carried out at pH 6.9 because PVUII does not precipitate out at this pH. Under these conditions the formation of a 50% Poly A complex was observed (Fig. 7), and this result is consistent with a triple-stranded complex whereby the adenines of two Poly A chains are base-paired to every other uracil on the vinyl polymer. A schematic representation of this structure is shown in Fig. 10. This, of course, is directly analogous to the triple-stranded structure proposed for the PVAd Poly U complex [4].

The melting profile of this complex shown in Fig. 9 is not particularly meaningful as it is the net result of a decrease in absorbance as the PVUII goes back into a coiled conformation and the increase of absorbance of Poly A with increasing temperature. The Poly A melting profile dominates the melting profile of the complex. It is important to note here that a 25% complex was not detected for the PVUII system. This information, in connection with the fact that PVUII can form a 50% Poly A complex whereas PVUI forms a 33.3% and a 25% complex, suggests that the structure of the complexes are quite sensitive to the tacticity of the vinyl polymers. The high degree of steric order in PVUII is consistent with the polymer's ability to form a triple-stranded completely base-paired structure whereas in PVUI the uracils are less regularly ordered and are therefore less accessible for complete base pairing with Poly A.

The 33.3% PVAd complex observed with PVUII (Fig. 8), which eventually resulted in precipitation, can be explained by a variety of structually different complexes. One suggested possibility is the triple-stranded structure shown in Fig. 11 as its cross section. In this structure half of the adenines are based-paired through both the 1,6 and 6,7 positions as is the case for the 2 Poly U:Poly A complex [24]. The instability of the 33.3% complex solution is understandable on the basis of the suggested initial triple-stranded complex, because

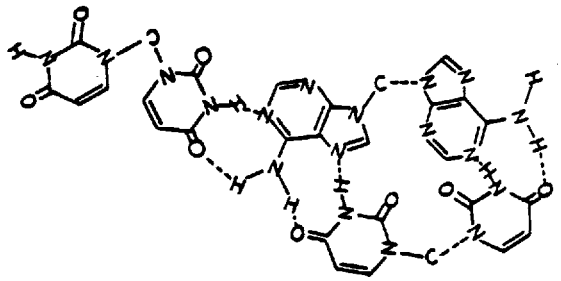

FIG. 11. A cross section of a possible 2PVUII: PVAd triple-stranded complex.

the free uracils should be able to base pair with the vacant 6,7 positions of the adenine rings, resulting in polymerization and aggregation. The apparent melting profile of the 33.3% complex shown in Fig. 9, in fact, is probably not a melting profile at all, but rather the increase in temperature may have kinetically assisted the rate of aggregation.

Because PVUI and PVUII interact with nucleic acids, it seems likely that these polymers might also interfere with replication, transcription, and translation in living cells. This suggests that these polymers may be useful probes for studying these biochemical mechanisms and that they may also have important therapeutic value.

EXPERIMENTAL

Materials

PVU was prepared by the methods previously reported [6]. The material obtained by polymerization at -78° in liquid ammonia (Expt 3) is designated PVUI, and the more highly syndiotactic material obtained by solid state polymerization (Expt 9) is designated PVUII. Poly A was purchased from Miles Laboratories, Kankakee, Illinois.

Solutions

The PVU stock solutions were made up by dissolving dry amounts of the polymers in basic buffer and then back titrating with dilute hydrochloric acid to the desired pH. The Poly A solutions were prepared using the μmoles of phosphorus per milligram of Poly A as a criterion for adjusting the concentration. Polymer solutions were prepared in

the following buffers: 0.2 m KCl, 0.2 m NaOH, pH 12-13; 0.05 $NaHCO_3$, 0.1 m NaOH, pH 9.6-11; 0.01 m trishydroxymethylaminomethane hydrochloride, pH 7-9; and 0.01 m sodium cacodylate, pH 6.9-7.0.

Instrumentation

UV spectra were recorded on a Cary UV spectrophotometer equipped with variable temperature circulating cell holders and 1-cm quartz cells containing an iron constantan thermocouple inserted through an adopter in the cell stopper. Fluorescence emission spectroscopy was carried out with a Baird-Atomic Fluorispec model SF-100 instrument in a 1-cm quartz cell. A Durrum Jasco J-20 ORD-CD spectropolarimeter was used for circular dichroism measurements operating at room temperature. A Corning Model 10 pH meter was used for pH determinations.

Methods

All solutions were degassed with helium gas before spectral measurements were made. Continuous variation [16] mixing experiments were carried out in 10 ml volumetric flasks. No corrections were made for the thermal expansion of the solutions on heating.

Circular dichroism rotational strengths were calculated from [25]:

$$R = 0.696 \times 10^{-42} \frac{\text{area}}{\lambda_{\text{max or min}}} \times \frac{\text{MW}}{(100)(1)(c)}$$

The calculation of the molecular weights, MW, of the repeat units was based on the triple-stranded structure for the 50% Poly A complex and the double-stranded structures for the 33.3% and 25% complexes. The cell path length was 1 cm.

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