Biochemistry

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Volume 10, Number 25 December 7, 1971

Vinyl Analogs of Polynucleotides*

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ABSTRACT: Poly(9-vinylhypoxanthine) was prepared by three different methods and its properties studied. The mutual interactions of four neutral analogs of nucleic acids, poly(1-vinyluracil), poly(1-vinylcytosine), poly(9-vinyladenine), and poly(9-vinylhypoxanthine), and their interactions with polyribonucleotides, were investigated in dilute aqueous solutions. Poly(9-vinylhypoxanthine) shows considerably less tendency for any complexing than the other vinyl polymers, resembling thus the behavior of polyinosinic acid at high ionic strength. In other respects the formation of complexes follows the specificity pattern of polynucleotide interactions—except that the nature of the interactions is modified by two additional factors. First, because the bases of a vinyl polymer cannot completely pair with a single strand of any other polymer, some of the bases of the complexed vinyl polymer are potentially free to bind to additional strands. Secondly, because of the incomplete base pairing, the strand-strand bonding is weaker and could be offset by coulombic repulsion forces. As a result, the nature of these interactions is determined by the electric charges on the polymers. If neither component carries any charge, which is the case when both are vinyl polymers, no coulombic repulsion acts in the system and the growth of the complex continues until a high aggregate is formed. When one component is neutral (vinyl polymer) and the other negative (polynucleotide), a soluble, nonaggregated complex is formed, the repulsive forces acting against progressive joining of further negative strands. These complexes have the following characteristics: (a) broad and incompletely reversible melting and (b) filamentous appearance upon electron microscopic examination (observed for the poly(1-vinyluracil)·polyadenylic acid complex). Similarly, the complex between poly(9-vinyladenine) with intercalated negative detergent, and poly(1-vinyluracil), is nonaggregated. When both components carry an electric charge of the same sign, as in the case of two protonated vinyl polymers, or of a negative polynucleotide and a vinyl polymer with intercalated anionic detergent, then no complex is formed; apparently the incomplete pairing cannot overcome the coulombic repulsion between two strands.

In order better to understand the relation between the structure and function of nucleic acids, many analogs and model systems of nucleic acids have been devised and studied (Felsenfeld and Miles, 1967; Halford and Jones, 1968). In the present work we have studied a set of four vinyl polymers (CH·base–$CH_2)_n$, where the base is uracil, cytosine, adenine, or hypoxanthine (Scheme I). Since such polymers differ from polynucleotides in well-defined ways, their study improves our understanding of the structural determinants of the different physicochemical properties of polynucleotides. These polymers also are useful in elucidating the basis for the unique position of polynucleotides in nature. Under prebiotic conditions the formation of other types of base-containing macromolecules is equally plausible and the study of the set of vinyl polymers shows us why such molecules were eliminated in evolution. Lastly, the properties and interactions of vinyl polymers have some interest in themselves, as these macromolecules show several interesting effects. For example, poly(VU)[1] serves as a template in the chemical synthesis of oligoadenylates (Orgel and Pitha, 1971),[2] although neither poly(VU) nor poly(VA) will direct protein synthesis in *in vitro* systems (Pitha and Grunberger, 1971).[2] Furthermore, a complex of poly(VC) with poly(I) shows pronounced antiviral activity, probably because of the easy uptake of this compound by mammalian cells (Pitha and Pitha, 1971); this observation suggests that perhaps similar complexes may be able to serve as nontoxic carriers for nucleic acids across the cell wall.

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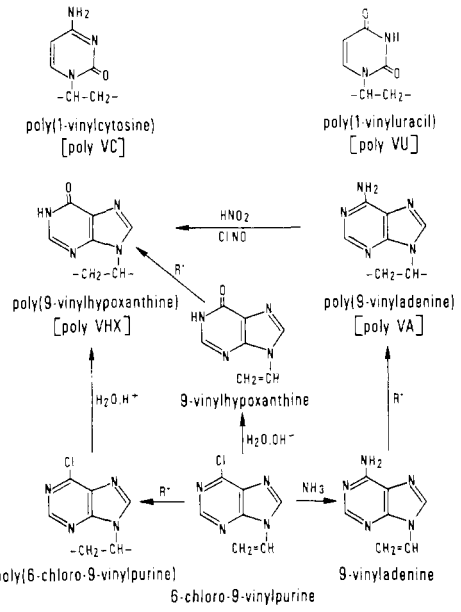
[1] For formulas of poly(VU), poly(VA), poly(VC), and poly(VHX), see Scheme I; poly(iA), polyisoadenylic acid; SDS, sodium dodecyl sulfate.

[2] This manuscript is "in preparation."

SCHEME I



The preparation of poly(9-vinylhypoxanthine) is described. Thus the fourth counterpart has been added to the previously prepared poly(1-vinyluracil) (Pitha *et al.*, 1970), poly(1-vinylcytosine) (Pitha and Michelson, 1970), and poly(9-vinyladenine) (Pitha and Pitha, 1970), and eighteen more polymer–polymer interactions studied, in addition to the eight described previously. In this way all the obtainable binary combinations can now be described thus providing an understanding of which of all the theoretically possible pairing schemes survive in this system. Base pairing, when investigated in different systems (*e.g.*, cocrystals or associative equilibria in nonpolar solvents), can give slightly different numbers of observable complexes (Felsenfeld and Miles, 1967) and thus such results are not redundant. Furthermore, the absence of interaction in certain combinations proves that the tendency to form nonspecific complexes is very low even in this system. Finally, the structure of one complex has been studied by electron microscopy (Appendix).

Experimental Section

Materials and Chromatography Methods. Polynucleotides (mol wt $>10^5$) were obtained from Miles Co., Elkhart, Ind. Polyisoadenylic acid was a gift of Dr. A. M. Michelson (Michelson *et al.*, 1966). The purity of low molecular compounds was checked by chromatography using either a descending system on Whatman No. 3MM paper or an ascending system on silica gel, Eastman Sheet 6060. The compounds were located by their absorption of ultraviolet light; water-saturated 1-butanol was used as eluent.

9-Vinylhypoxanthine. 6-Chloro-9-vinylpurine (1 g) (Pitha and Ts'o, 1968) was suspended in a solution of trimethylamine (25 g) in 500 ml of water. After stirring for 24 hr at room temperature, the homogeneous solution was evaporated *in vacuo*. The residue was repeatedly evaporated with water and recrystallized from water, yielding 654 mg of white leaflets. According to the chromatography and ir spectrum (potassium bromide pellet) the product is not contaminated with starting material. The melting point of the product is above 250°, but it may be sublimed at 0.1 mm. *Anal.* Calcd for $C_7H_6N_4O$ (162): N, 34.55. Found: N, 34.77.

Poly(VHX) by Polymerization of 9-Vinylhypoxanthine. Polymerization was performed at 100°. A solution of monomer (100 mg) in 10 ml of water was purged with nitrogen, after which 0.8 mg of potassium persulfate in water (1 ml) was added; after 30 min the reaction mixture was cooled to the room temperature. The polymer was then precipitated by addition of ethanol (160 ml) and collected by centrifugation. Drying *in vacuo* gave 5 mg of the product, having an ir spectrum (potassium bromide pellet) identical with that of poly(VHX) prepared by other procedures described below.

Alternatively, 100 mg of monomer was dissolved in dimethyl sulfoxide (0.5 ml) at 100°, after which a solution of *N,N'*-azobisisobutyronitrile (0.5 mg) in 0.02 ml of the same solvent was added. The polymer starts to precipitate after a few minutes. After 60 min at 100° the suspension was added to ethanol (60 ml) and stirred for 1 hr, then centrifuged, and the sediment dried at 100° *in vacuo* (0.1 mm), giving 89 mg of the polymer. Poly(VHX) was further dissolved in 8 ml of 0.1 N NaOH, then the solution was filtered through sintered glass, and the polymer was precipitated by neutralization to pH 7 with hydrochloric acid; the sediment was then collected by centrifugation and dried *in vacuo* at room temperature. According to the elementary analysis the preparations so obtained are hydrated and contain 80–85% poly(VHX). The molar extinction coefficient, measured after dissolving the polymer in 0.1 N NaOH, was found to be 10,050 (corrected for hydration) at 250 mμ (uv maximum). Addition of SDS to the basic solution does not change the uv absorption at the maximum.

Poly(VHX) by Hydrolysis of Poly(6-Chloro-9-vinylpurine). The starting polymer (140 mg), prepared by polymerization in dimethylacetamide (Pitha and Pitha, 1970), was dissolved in concentrated hydrochloric acid (25 ml) at room temperature, and was stirred for an additional 2 hr during which period a white precipitate was formed. The mixture was then evaporated repeatedly *in vacuo* with the addition of water. The residue was dissolved in 0.1 N NaOH, filtered through sintered glass, neutralized to pH 7, and the precipitated polymer collected by centrifugation and washed with an equal amount of water. The combined supernatants had a uv absorbance at the maximum of less than 1; drying of the sediment *in vacuo* yielded 107 mg of the polymer.

By examination of the ir spectrum, no starting material could be detected in the product; prolongation of the acid treatment to 18 hr did not change the ir spectrum of the product.

9-Vinyladenine. In a stainless steel autoclave, a suspension of 1 g of 6-chloro-9-vinylpurine in 25 ml of anhydrous methanol was saturated by ammonia at 0°, and subsequently heated at 60° for 48 hr. The reaction mixture was then evaporated *in vacuo* to dryness. The residue was extracted with 300 ml of boiling toluene, filtered hot, and the filtrate evaporated to half-volume; after cooling, 660 mg of white crystals was collected. The product was homogeneous upon chromatography, and gave the expected doublet of an amino group (3423 and 3537 cm^{-1}) in the ir spectrum (1-cm cells, carbon tetrachloride). It was eventually found that the product when recrystallized from ethanol has a different ir spectrum (potassium bromide pellet) compared to the toluene-recrystallized sample. But, again, the spectra of chloroform solutions of both samples are the same; apparently two crystalline modifications of 9-vinyladenine exist. When recrystallized from ethanol, the compound has a melting point of 199–200°, as described for

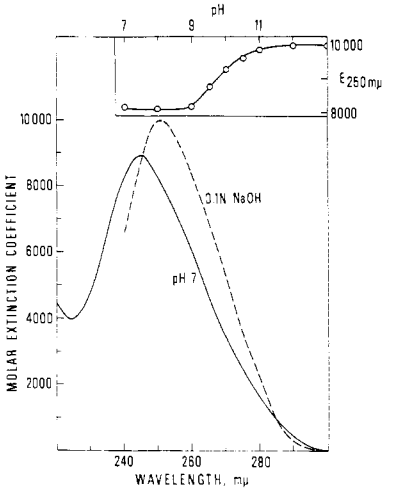


FIGURE 1: Ultraviolet spectra of poly(9-vinylhypoxanthine) in detergent-containing buffer (100 mM NaCl–10 mM sodium cacodylate–10 mM SDS, pH 7) and in detergent-containing (10 mM SDS) aqueous alkali. Inset: pH dependence of $\epsilon_{250\,m\mu}$ of poly(9-vinylhypoxanthine); buffers used had an ionic strength of approximately 0.1 and contained detergent (10 mM SDS).

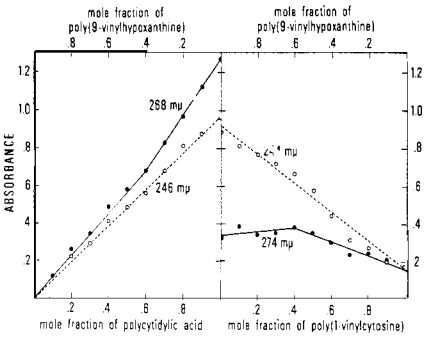


FIGURE 2: Continuous variation studies of complex formation with poly(9-vinylhypoxanthine). At the left: poly(9-vinylhypoxanthine) and polycytidylic acid (25% by volume of propylene glycol–10 mM sodium chloride–5 mM phosphate; pH 8.1). The solution of the vinyl polymer was prepared by neutralization of an alkaline solution, and the polymer is in an aggregated state. Mixtures were made from freshly prepared solutions, equilibrated for 4 days at room temperature, and then centrifuged at 10,000 rpm for 1 hr. Absorbance values of supernatants are plotted. At the right: poly(9-vinylhypoxanthine) and poly(1-vinylcytosine). Solutions (100 mM NaCl–10 mM sodium cacodylate–10 mM SDS, pH 7) were mixed, heated for 10 min at 100°, slowly cooled to room temperature, and centrifuged at 10,000 rpm for 1 hr. Absorbance values of supernatants are plotted.

9-vinyladenine prepared in a different way (Kaye, 1970; Veda *et al.*, 1968).

Poly(VA) by Polymerization of 9-Vinyladenine. The procedure described for the polymerization of 9-vinylhypoxanthine in water was followed. After the ethanol precipitation and drying, the polymer, 84 mg from 100 mg of monomer, was dissolved completely in 5 ml of water by stirring overnight, and then dialyzed exhaustively against distilled water. The solution remains homogeneous at 30°, but lowering the temperature to 2° brings about a reversible partial precipitation. The solution was cleared by centrifugation (30°, 5000 rpm, 1 hr); the polymer in the solution was precipitated by ethanol as described above. Drying *in vacuo* gave 74 mg of white powder with an ir spectrum (potassium bromide pellet) identical with that of poly(VA) which was prepared from poly(6-chloro-9-vinylpurine) (Pitha and Pitha, 1970).

Spectral Measurements. All absorbance values given were obtained in 1-cm cells. The instrumentation used was described previously (Pitha and Pitha, 1970). Continuous variation experiments—Job method (Job, 1928)—were conducted at 10^{-4} M total concentration (monomer units) in cacodylate buffer (50 mM sodium chloride–5 mM sodium cacodylate, pH 7) or detergent–buffer (100 mM sodium chloride–10 mM sodium cacodylate–10 mM sodium dodecyl sulfate, pH 7). Experiments in aqueous propylene glycol were performed, unless otherwise stated, with a solvent prepared from 75 volumes of aqueous buffer (5 mM sodium chloride–5 mM sodium cacodylate, pH 7) and 25 volumes of propylene glycol and at a concentration of 5×10^{-5} M (monomer). Routinely, 11 equidistant points were used and the reaction was followed up to 7 days at room temperature. The results of the melting experiments are corrected for water expansion.

Results and Interpretation

Preparation of Polymers. The solubility of the polymeric sample depends on the degree of cross-linking and on its molecular weight. These two parameters consequently depend on the method of preparation. Thus, in order to obtain poly(VHX) of optimal properties, different synthetic methods were tested (Scheme I). 6-Chloro-9-vinylpurine is easily hydrolyzed to 9-vinylhypoxanthine by aqueous trimethylamine. Radical polymerization of that monomer in water gives only low conversions, but in dimethyl sulfoxide the yields are good.

Other preparations of poly(VHX) start from macromolecular materials. Poly(VA) is deaminated by HNO_2, but the reaction is slow and because of precipitation it is not complete so that only poly(VA,VHX) copolymer is produced. Deamination by NOCl was studied; unfortunately, poly(VA) is not soluble in dimethylformamide, a solvent in which the deamination of adenosine derivatives is very fast (Sigel and Brintzinger, 1965), and in acetic acid also the deamination of poly(VA) is incomplete. Alternatively, poly(6-chloro-9-vinylpurine) may be used. Because treatment of chloropurines by alkalies is often accompanied by some ring opening (Montgomery and Temple, 1957; Walsh and Wolfenden, 1967; Barlin, 1967), which would lead to randomly distributed diaminopyrimidine units in the resulting polymer, the acid hydrolysis of the chloropolymer was investigated. Dilute hydrochloric acid (1 N) does not dissolve the polymer and even after 24 hr at room temperature the product contains some starting material. On the other hand, concentrated hydrochloric acid (37%) dissolves the polymer, and hydrolysis proceeds smoothly and is complete in 2 hr; the product has the same spectral characteristics as that obtained by direct polymerization.

In the field of adenine derivatives, we studied the reaction of 6-chloro-9-vinylpurine with ammonia and found conditions which gave good yields of 9-vinyladenine. This monomer is easily polymerized to give poly(VA) with an ir spectrum identical with that of the polymer prepared by treatment of poly(6-chloro-9-vinylpurine) with ammonia. Nevertheless, there is a difference between the two methods of preparation: the former gives a product which dissolves more easily and to a higher concentration (~150 mg ml^{-1}) in water than the latter.

Properties of Poly(VHX). The polymer is poorly soluble in simple solvents. Trifluoroacetic acid, formamide, dimethylacetamide, dimethylformamide, dimethyl sulfoxide, or triethyl

TABLE I: Complex Formation as Detected by the Continuous Variation Method (Concentration 10^{-4} M or Lower in Monomer) and Ultraviolet Spectrometry.

a. Aqueous Solutions

	Poly(U)	Poly(A)	Poly(iA)	Poly(VA)	Poly(C)	Poly(I)
Poly(VA)	Complex Pu∼Py	0	—[a]	0 (H^+)	0	Complex
Poly(VU)	0	Complex Py≫Pu	Complex Py>Pu	Aggregate Py∼Pu	0	0

b. Water–Propylene Glycol Solutions

	Poly(U)	Poly(A)	Poly(C)	Poly(VC)	Poly(I)	Poly(G)
Poly(VC)	0	0	0	0 (H^+)	Complex Py>Pu	Complex Py>Pu

c. Aqueous SDS Solutions

	Poly(U)	Poly(VU)	Poly(A)	Poly(C)	Poly(VC)	Poly(I)	Poly-(VHX)
Poly(VHX)	0	—	0	0	0 complexing only after heating	0	
Poly(VA)	0	Complex Py>Pu	—	—	—	—	0
Poly(A)	Complex 2U:1A	—	—	—	—	—	

[a] Combinations that were not investigated are indicated by a dash.

phosphate do not dissolve the polymer appreciably on stirring at room temperature for 2 days. On the other hand, poly(VHX) is readily soluble in aqueous alkali, although upon neutralization a sediment starts to form around pH 8.5. Neutral suspensions were prepared in this way from all the different poly(VHX) preparations and were dialyzed exhaustively against water; however, in no case did the uv peak absorbance of supernatant exceed 0.3 (1-cm cell), pointing to the low solubility of all preparations. An effort was made to prevent precipitation by adding different solutes to the alkaline polymer solution prior to the neutralization. The following compounds did not prevent massive precipitation: ethylene glycol (20%), propylene glycol (20%), glycerin (20%), diethanolamine (10%), and Brij 23 (∼10 mM) (a neutral detergent, $CH_3(CH_2)_{11}(OCH_2CH_2)_{20-25}OH$). On the other hand, upon the addition of an anionic detergent, sodium dodecyl sulfate (10 mM), clear, neutral solutions were easily prepared. Since such polymer solutions may be filtered through sintered glass filters, centrifuged (10,000 rpm for 30 min), or passed through Sephadex G-200 columns without any significant change in uv absorption, it may be presumed that they contain solubilized polymer molecules rather than high aggregates.

Ultraviolet spectra of neutral alkaline solutions of poly(VHX), given in Figure 1, compare favorably with those of 9-ethylhypoxanthine, for which λ_{max} is 250 mμ at pH 7 or 13 and with ϵ_{max} = 11650 at pH 7 and ϵ_{max} = 12,400 at pH 13 (Montgomery and Temple, 1957). Spectrophotometric titration of poly(VHX) in SDS solution is also depicted in Figure 1. Dissociation is apparently shifted to the more basic region, compared to 9-ethylhypoxanthine (pK_a = 9.31) (Reinert and Weiss, 1969). This shift is apparently caused by greater coulombic repulsion on the polymer level: hypoxanthine – hypoxanthine$^-$ and hypoxanthine$^-$–detergent$^-$. In this context it is interesting to note that SDS augments the protonation of poly(VA), since in this case coulombic attraction takes place: adenine H^+–detergent$^-$ (Pitha and Pitha, 1970).

Interactions of Poly(VHX). The low solubility of this polymer strongly impedes formation of any complexes. Dissolution of poly(C) in alkaline solutions of poly(VHX) followed by neutralization, either directly or by stepwise dialysis (pH 9, 8, and 7), leads to formation of a precipitate even in high excess of polynucleotide. Examination by uv spectroscopy of the supernatant after centrifugation at 10,000 rpm for 10 min and of the sediment by ir (KBr pellets), excludes an admixture of poly(VHX) in the supernatant or of poly(C) in the sediment, of greater than 5%. Thus, instead of complex formation, a nearly complete separation on the basis of solubility occurs.

Use of an organic solvent does not lead to any extensive complex formation, either. Continuous variation studies (Job, 1928) of poly(VHX)·poly(C) and of poly(VHX)·poly(A) (25% propylene glycol, pH 7 or 8.1, freshly prepared polymer solutions) could not be fitted by single lines; this deviation is due mainly to aggregation phenomena. Centrifugation (10,000 rpm, 1 hr) gives supernatants with uv spectra indicating only a very slight occlusion of polynucleotide with the sediment (Figure 2, 268 mμ), while no poly(VHX) was solubilized (Figure 2, 246 mμ). Thus, the overall extent of complexing is very small.

Poly(VHX) in neutral SDS-containing solutions also did not form any detectable complexes. A study by the Job method at room temperature did not detect any complexing in a number of systems given in Table Ic. When samples containing poly(VHX)·poly(VC) mixtures were heated briefly (10 min, 100°) and then slowly cooled to room temperature, precipita-

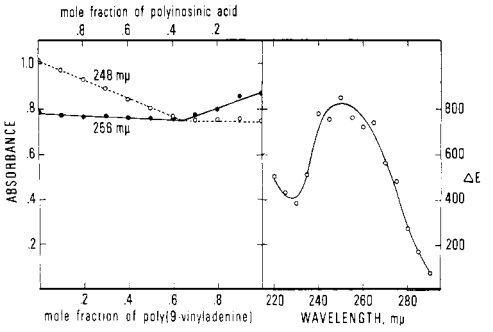


FIGURE 3: Complex formation between poly(9-vinyladenine) and polyinosinic acid (50 mM NaCl–5 mM sodium cacodylate, pH 7). At the left is the continuous variation curve. At the right is the decrease in the absorbance due to complex formation at the maximally hypochromic composition.

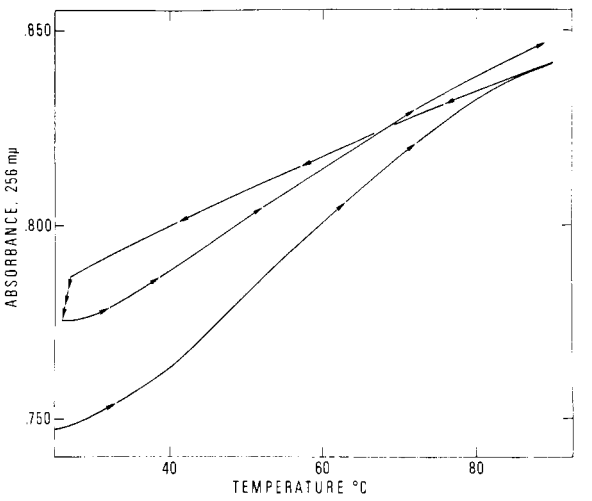


FIGURE 4: Temperature dependence of the absorbance at 256 mμ of the complex of poly(9-vinyladenine) with polyinosinic acid. Conditions as in Figure 3. The solid lines represent the response to varying temperature at approximately 0.5°/min; the broken line at the left represents the change on overnight standing.

tion occurred in samples with high pyrimidine concentration. After centrifugation (10,000 rpm, 1 hr) the uv spectrum of the supernatant clearly shows that complex formation occurred in the process (Figure 2). Heating and cooling are apparently necessary steps, since after a simple centrifugation no complex formation was detected. The heating and centrifugation sequence did not lead to any detectable complex formation in the poly(VHX)·poly(C) system, however.

Preparation of complexes with poly(VHX) by dialysis of SDS–buffer solutions against the buffer alone was also attempted. In the case of the poly(VHX)·poly(VC) system (0.1 mM polymer concentration, Job method) the removal of SDS consistently led to the formation of aggregates which sedimented easily (10,000 rpm, 1 hr), and no soluble complex was detected. In the case of the poly(VHX)·poly(C) system, also, a similar experiment showed no extensive complexing.

Thus, the complex-forming abilities of poly(VHX) are distinctly lower than those of the other three vinyl polymers. Some kinetic factors may be involved, such as fast precipitation of poly(VHX), but the failure of both gradual addition and dialysis to generate complexes indicates that kinetic factors alone are not responsible.

Interactions of Polymers in Simple Aqueous Solutions. The following systems have been previously investigated: poly(VU)·poly(A), poly(VU)·poly(U) (Pitha *et al.*, 1970), poly(VA)·poly(U), poly(VA)·poly(VU), and poly(VA) (protonated)·poly(VA) (protonated) (Pitha and Pitha, 1970). Some of our results on poly(VA)·poly(U) were also independently confirmed elsewhere (Kaye, 1970). To complete the picture, the remaining water-soluble systems were studied by the Job technique. In a number of systems no interaction was detected (Table Ia); complexes were formed only with poly(VA)·poly(I) and poly(VU)·poly(iA). The corresponding graphs are given in Figures 3–5. The poly(VA)·poly(I) complex is similar to the other poly(VA) complexes in that both components are represented about equally. In the poly(VU)·poly(iA) complex, the pyrimidine component predominates, a situation analogous to that of the poly(VU)·poly(A) complex or to the complexes of poly(VC). The spectral changes due to the formation of the poly(VA)·poly(I) complex are also shown in Figure 3. The thermal dissociation curves of both newly studied complexes (Figures 4 and 5) are similar to those previously studied complexes (references given earlier): a broad melting profile with a final absorbance equal to that present before complexing, subsequent cooling producing only a partial hypochromic effect. The complex of poly(VU) with poly(iA) has a higher thermal stability than that of poly(VU) and poly(A) (Pitha *et al.*, 1970), similar to the corresponding polynucleotide·polynucleotide complexes (Michelson *et al.*, 1966).

Figure 6 shows the optical rotatory dispersion spectra of two vinyl polymer–polynucleotide systems. Our various optical rotatory dispersion results show that the complexes of the pyrimidine-containing vinyl polymers have lower rotatory strength than the polynucleotide component alone. The behavior of the poly(VU)·poly(A) system is shown in Figure 6; the poly(VU)·poly(iA) system behaves similarly, as does poly(VC)·poly(G), which was previously studied in aqueous propylene glycol (Pitha and Michelson, 1970). The complexes of poly(VA), on the other hand, have an opposite behavior: their rotatory strength is higher than that of the polynucleotide component; the change is quite pronounced for the poly(VA)·poly(U) system (Figure 6) but only slight for the poly(VA)·poly(I) combination. The increase does not imply that this vinyl polymer is stereoregular or, thus, that its complexes are. The irregularly spaced sequences of short stereoregular sgements, which could be present even in the mainly atactic polymer, after

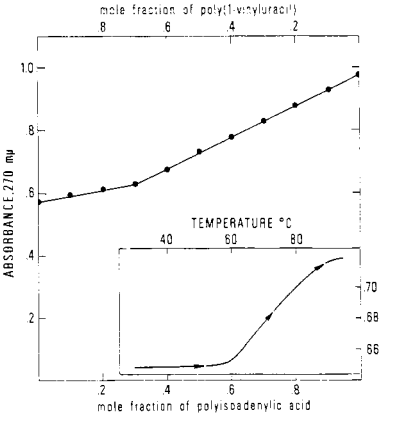


FIGURE 5: Continuous variation of poly(1-vinyluracil) with polyisoadenylic acid (50 mM NaCl–10 mM sodium cacodylate–1 mM $MgCl_2$, pH 7). Inset: temperature dependence of absorbance of the complex having maximal hypochromicity.

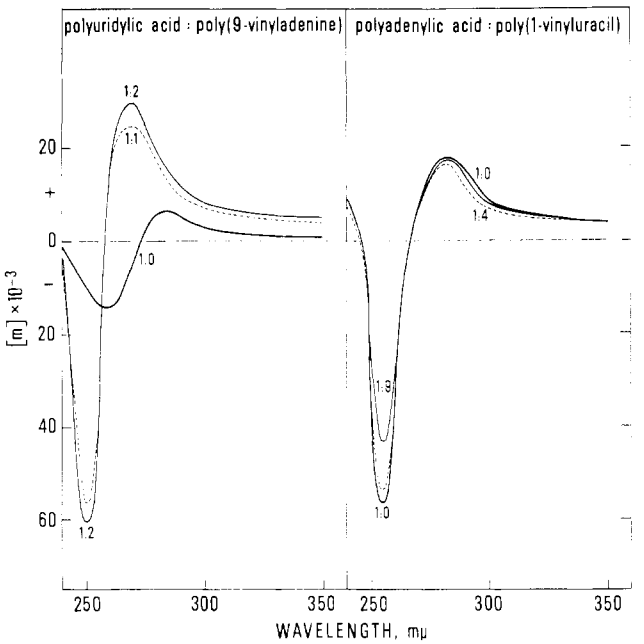


FIGURE 6: Optical rotatory dispersion spectra of polynucleotides and their complexes with vinyl polymers. Spectra were measured in buffer (50 mM NaCl–5 mM sodium cacodylate, pH 7) in a 1-cm cell (ambient temperature) using the Cary 60 spectropolarimeter. The concentration of polynucleotides was kept constant: poly(U), 5.8×10^{-5} M; poly(A), 2×10^{-5} M. Residue rotation (m) is expressed per phosphate unit.

complexing with the stereoregular polynucleotide may be the cause of the observed effect.

Interactions of Polymers in Aqueous Propylene Glycol Solutions. Aqueous propylene glycol is a particularly suitable solvent for studying interactions of the water-insoluble polymer, poly(VC). In addition to the poly(VC)·poly(I) and poly(VC)·poly(G) systems studied previously (Pitha and Michelson, 1970), the remaining combinations (Table Ib) were investigated; no complex formation was detected.

Interactions of Polymers in Aqueous SDS Solutions. The observation of the lack of complexing of poly(VHX) in the detergent solutions led to a more general investigation of SDS effects. This agent solubilizes both water-insoluble vinyl polymers, poly(VHX) and poly(VC), the former to a higher extent. Results of the interaction study are summarized in Table Ic. Interaction between polynucleotides (poly(U)·poly(A)) is clearly not hampered; there is however no detectable complex formation between poly(VA) and poly(U), polymers which in the absence of SDS complex easily. Furthermore, the interaction between poly(VU) and poly(VA) is also changed drastically in the presence of SDS. Without detergent, easily sedimenting aggregates are formed; while in SDS solution, a complex is formed which cannot be sedimented under the same conditions (10,000 rpm, 1 hr). Properties of this complex will be dealt with in the description of antiviral protection by poly(VA)·poly(VU) systems (P. M. Pitha *et al.*, 1971).[2] Other results from Table Ic are discussed in the section: Interactions of Poly(VHX).

Gel Filtration of the Vinyl Polymers and Their Complexes. The macromolecular character of all four vinyl polymers has been established by their sedimentation characteristics: all have molecular weights over 10^5. Their behavior on columns of Sephadex G-200 has now been investigated. The column was extensively tested using cacodylate buffer and SDS–buffer (see Spectral Measurements). High molecular weight polynucleotides (poly(U), poly(A), and poly(I)) were eluted at the void volume of the column (50 ml); low molecular weight compounds (pU and $(Ap)_6A$) were eluted at 140 ml. Poly(VU) (in aqueous buffer) behaves similarly to polynucleotides; poly(VHX) (in SDS–buffer solutions) is eluted in a broader band, indicating some chemical binding. The binding is so strong for poly(VA) that this polymer cannot be eluted by either cacodylate buffer or SDS–buffer solutions. The complexes poly(VU)·poly(A) or poly(VA)·poly(U) are, however, again eluted in narrow bands near the void volume of the column. All the observed phemomena can be explained by the strong binding of adenine residues to the Sephadex matrix. The binding is the predominant operating force when the polymer is neutral, and furthermore, must be stronger than poly(VA)·detergent binding. If the polymer carries negative charges as do poly(A) or the poly(VA)·poly(U) complex, then coulombic repulsion with CO_2^- groups present to a small extent in Sephadex (manufacturer's information) overcomes the binding and the sample is sieved according to its molecular weight.

Chromatographic methods have been used repeatedly to demonstrate specific base pairing: nucleoside derivatives have been chromatographed on columns of base-containing resins, and the results discussed on the molecular level. The behavior of poly(VA) on Sephadex indicates that base–matrix interactions may complicate such studies, which should, thus, be interpreted with caution.

Discussion

The vinyl polymers, exhibit three obvious differences from polynucleotides: (a) they are electroneutral; (b) in complexes with any other polymer strand the steric strains exclude complete base pairing; (c) the base-containing region is more hydrophilic than the backbone.

When complexes of vinyl polymers are formed, factors a and b partially cancel each other: lower strand-strand coulombic repulsion should increase interstrand stability, while less base pairing should decrease that stability. These two factors then, exert a predictable influence on the complexing of all four vinyl polymers, which probably does not change drastically from one to another. The last factor is the balance between hydrophobic and hydrophilic regions; its change during complex formation is difficult to assess fully and also varies from one polymer to another. The poor ability of poly(VHX) to form complexes probably can be caused by the last factor and by very strong hypoxanthine–hypoxanthine hydrogen bonding. In this context it is interesting to note that poly(I) in high salt concentration similarly prefers self association to complexing with poly(C) or poly(A) (Rich, 1958).

Generally, the results of the interaction studies can be rationalized by assuming that the interaction scheme known from polynucleotides also holds here, and, in addition, that complex formation stops at the stage where there is one charged strand per complex molecule. Water and aqueous propylene glycol solutions do not supply any additional charges to polymers and so the interaction scheme is a simple one. With complementary neutral polymers, interaction proceeds to the polystranded, aggregated form (poly(VU)·poly(VA)). On the other hand, two strands with charges of the same sign do not approach each other, and so no stabilized acidic forms were found for poly(VA) or poly(VC). The complexes between negative polynucleotide and neutral vinyl polymer, then, are formed with the expected specificity. The assumption of one charged strand per complex is confirmed by the electron micro-

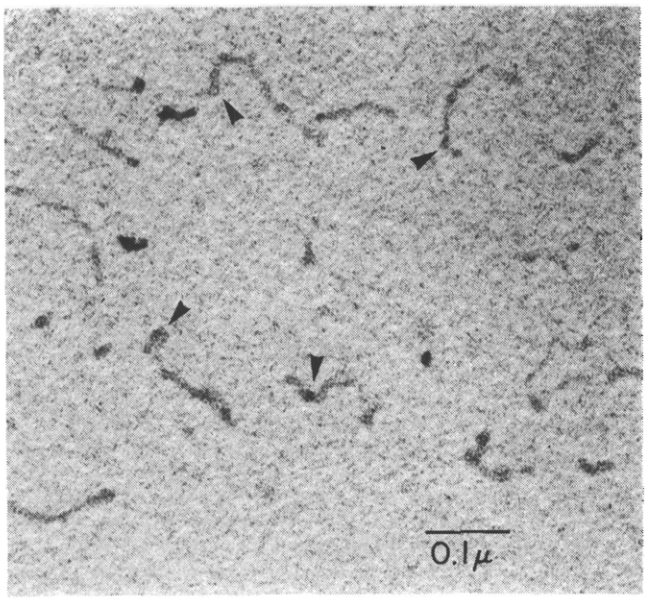


PLATE 1: Electron micrograph of 9 poly(VU)·poly(A), uranyl acetate stained. The mark represents 0.1 μm. The appearance of filaments in small bead-like chains and with larger or smaller "bushes" (arrows) is typical. Contour length and segmental length are given in Figure 7a,b.

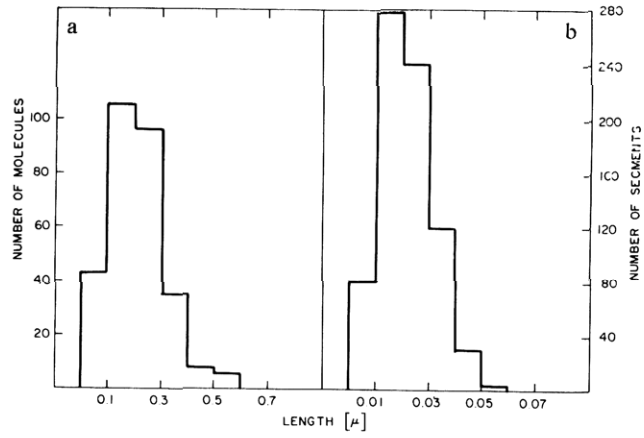


FIGURE 7: Histograms of the electron microscopic measurements on the poly(1-vinyluracil)·polyadenylic acid complex. At the left is the total length of individual ribbons. At the right is the length between knots on the ribbons as counted on 50 ribbons; on the average there were 8 knots/ribbon.

scopic results on the 9 poly(VU)·poly(A) complex. In that case it is also apparent that this assumption is not a trivial one: enough of the uracil residues are unpaired and available to bind another poly(A), which in turn could bind additional poly(VU), and so on—but this does not take place and the complex molecule contains only a few strands.

The interaction of polymers in aqueous SDS solutions is more complex: the detergent, intercalating into the vinyl polymer, brings into it a variable negative charge, which is not the same for different polymers and is presumably higher for the purine than for the pyrimidine polymers. This additional charge is sufficient to dissociate the polynucleotide·vinyl polymer complex poly(U)·poly(VA), and to transform the aggregated complex poly(VU)·poly(VA) into a soluble, molecular complex. But the situation is not uniform: the combinations involving poly(VHX) and poly(VC) or poly(VA) in comparable conditions do not form any complexes, detergent–polymer bonds apparently being preferred.

The necessity to use all possible base pairs to compensate for coulombic repulsions apparently holds for complexes of other analogs as well. Thus if the structure of a polynucleotide is changed enough to exclude complete base pairing, then coulombic repulsions prevent complex formation with the complementary polynucleotide. For example, poly(8-bromoguanylic) acid, where the syn conformation prevents complete base pairing, does not complex with poly(C) (Michelson *et al.*, 1970); also the polymer of L-adenylic acid does not complex with the natural D antipode of poly(U) (Fric *et al.*, 1970). This lack of complexing is by no means a trivial phenomenon, as by use of loops it is apparently possible to achieve base pairing between any two polymers; and the possibility of loop formation on an otherwise regular double helix was firmly established—*e.g.*, on the complex between copolymer poly(A,U) and poly(A) or poly(U) (Fresco and Alberts, 1960). Any apparent lack of complexing should be viewed cautiously, however, since phosphate charge neutralization achieved by high cation concentration (*e.g.*, Mg^{2+}) may eventually allow some interaction to occur.

The reversible formation of colinear complexes is a necessary condition for any self-replicative system, and the present results demonstrate the clear advantage of polynucleotides for that purpose. The present results on vinyl polymers as noncharged, base-containing macromolecules, suggest that any similar polymers which could have been formed under prebiotic conditions would have the disadvantage of containing no destabilizing factors to counterbalance the strength of the base pairing. Reversible dissociation would then be difficult or impossible.

Appendix:[3] Electron Microscopy of the Poly(1-vinyluracil)·Polyadenylic Acid Complex

By A. K. Kleinschmidt and Paula M. Pitha

The system poly(VU)·poly(A) was investigated by electron microscopy.

The polyadenylic acid used had an s_{20} value of 7.9 S (in 0.015 M cacodylate buffer, pH 7.1–0.015 M NaCl) which corresponds to a molecular weight of approximately 2.5×10^5, and in fully stacked conformation (3.5 Å/monomer unit) to an average length of around 245 mμ. Poly(1-vinyluracil) had an s_{20} value of 4.7 S; due to the preparative method used this polymer is highly linear, without branching, but has a wide distribution of molecular weight (Pitha *et al.*, 1970).

The preparation of poly(VU)·poly(A) for electron microscopy was as follows (Kleinschmidt, 1968). The diffusion procedure was applied; DFP chymotrypsin film on top of a 10^{-5} M polymer solution (9 poly(VU)-1 poly(A) (Pitha *et al.*, 1970) in buffer containing 10 mM sodium cacodylate, 1 mM $MgCl_2$, and 50 mM NaCl. After 30 min, the polymer–protein film which arose to the surface was transferred to grids and positively stained with uranyl acetate (Gordon and Kleinschmidt, 1968). Micrographs of the mounted complex, taken at 23,000× magnifications (Elmiskop 1A, Siemens electron microscope, 40 kV, 50-μm objective aperture) showed ribbon-like structures with a number of knots and small bushes as illustrated in Plate 1. The contour length of ribbons (Figure 7) is spread

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between 50 and 600 mμ with an average length of 267 mμ, and there are roughly eight bushes per complex molecule.

Poly(VU) and poly(A) when prepared separately under the above conditions do not have a filamentous appearance; these molecules form only globular particles.

If it is realized that the backbone of the vinyl polymer and the polynucleotide have vast steric differences, then it is apparent that mutual binding between strands leads to the steric strains which must be compensated for by the formation of loops. The overall stoichiometry (9U:1A) indicates that the majority of loops is formed by the poly(VU) component of the complex alone. The loop formed by a single strand of the neutral vinyl polymer cannot be expected to stand out clearly above the film background. Consequently the bushes observed by electron microscopy must be formed by the collapsed strands of unpaired vinyl polymer or of the polynucleotide component. The ribbon-like structures, then, correspond to the strand of polyadenylic acid stiffened by the binding of one or several strands of the neutral polymer. Accordingly, the average length of the ribbons (267 mμ) corresponds roughly to the molecular size of the polyadenylic acid used, if this length is calculated on the assumption of the fully stacked conformation.

Thus, the complex between poly(1-vinyluracil) and polyadenylic acid (9U:1A) has a gross appearance somewhat analogous to that of the polynucleotide complexes and the structure of the complex must then be envisaged as having a straight side by side or a twisted side by side arrangment of the components.

Acknowledgment

We thank Drs. K. I. Berns, G. L. Eichhorn, A. M. Michelson, and R. S. Umans for comments on the manuscript.

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