HYDROPHILIC POLYMERS OF ORDERED CONFORMATION— NUCLEIC ACID MODELS

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Abstract: Nucleic acids contain several characteristic structural features: a chiral center adjacent to the base, a stereoregular, hydrophilic poly(phosphate-ribose) backbone which is negatively charged at neutral pH, and a helical secondary structure arising from conformational preferences enforced by base-stacking. We have prepared and studied several synthetic polynucleotide analogs having linear poly(ethylenimine), poly(vinylamine), and poly(dehydroalanine) backbones containing chiral nucleic acid base pendant groups attached through amide bonds. This amide bond increases structural order through its partial double bond character, resulting in restricted rotation of the pendant. Through study of model compounds and from UV, CD, and NMR spectroscopy, we have obtained considerable evidence for polynucleotide-like ordered conformations in these polymers. In addition, several of the polymers have shown antiviral activity in cell culture tests.
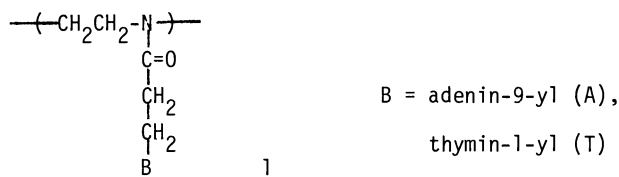
INTRODUCTION

Macromolecules have demonstrated a wide range of biological activity.(1,2) Antitumor, antiviral, interferon-inducing, immunoadjuvant, and other properties are characteristic of some macromolecules. Because of their high molecular weight, they have several potential advantages over conventional low molecular weight agents: sustained activity, greater specificity of action, lower toxicity, reduced undesirable side effects, and more direct use of the polymer-associated active agent.(3) Some of these advantages have been realized, but much more work is necessary if this great potential is to be fulfilled. Of primary importance in this regard are detailed studies of the effects of polymer structure on activity. While structure-activity studies are common in developing low molecular weight drugs, the comparatively new field of polymeric drugs needs many more such investigations. This becomes particularly significant since a polymeric agent possesses more potential structural variables than a conventional small molecule. Systematic study of closely related compounds can provide both an explanation for observed activity and a clear rationale for further synthetic design.

Our synthetic program turned towards the study of polynucleotide analogs, both for their value as models of nucleic acids and for their biological properties. Considerable attention has been given to these analogs; this includes both the homopolymers with polyphosphate backbones, most closely resembling natural nucleic acids, as well as nucleic acid models where the backbone is other than polyphosphate and/or the pendant groups have been modified.(4-6) There have only been a limited number of studies on the biological activity of the nucleic acid models with backbone and/or pendant group modification.(7) As an example of one of the more well-studied systems, poly(9-vinyladenine) has been found to inhibit the replication of Friend leukemia virus in mice. It does not inhibit the replication of Semliki forest virus (a non-leukemia virus). A similar result was found in a cell culture test studied prior to the experiments with mice. The mechanism of action of this electroneutral polymer has been shown not to be through interferon induction or through enhancement of the host immune response system. Instead, the inhibition has been deduced to be caused by direct inhibition of some important, early step in the viral replication mechanism. Work which has shown that poly(9-vinyladenine) inhibits the reverse transcriptase of murine leukemia virus lends credence to this rationale. Additionally, poly(9-vinyladenine) has been shown not to inhibit the latter processes of viral replication.(7,8)

The potential of forming double stranded systems with these polynucleotide analogs has been explored. Hence a complex of poly I and poly(vinylcytidylic acid) has shown promise in in vitro experiments. Although it is not as good an interferon inducer as poly I:C, the fact that it induces interferon, while none of the single stranded polyvinylnucleic acid analogs do is of interest. As Hoffmann(9) has implied, the importance of these analogs in aiding us to gain understanding in the mechanisms of action of naturally occurring poly-

nucleotides is profound.

Our interest in synthetic nucleic acid models began with our experience in making compounds 1. These linear PEI's containing nucleic acid base grafts showed the ability to interact by complementary hydrogen bonding both with each other and with synthetic polynucleotides such as poly U.(10) The polymers also gave evidence of significant base stacking. In view

$$-(-CH_2CH_2-N-)-$$
$$\begin{array}{c} C=O \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ B \end{array} \qquad \underline{1}$$

B = adenin-9-yl (A),

thymin-1-yl (T)

of these results and the promising biological effects observed in polyvinyl nucleic acid analogs, we decided to further develop this strategy. Our goals were to increase structural order in the polymers, maximize water solubility, and elucidate the relationship between macromolecular structure and biological activity. Our overall approach combines several advantages:
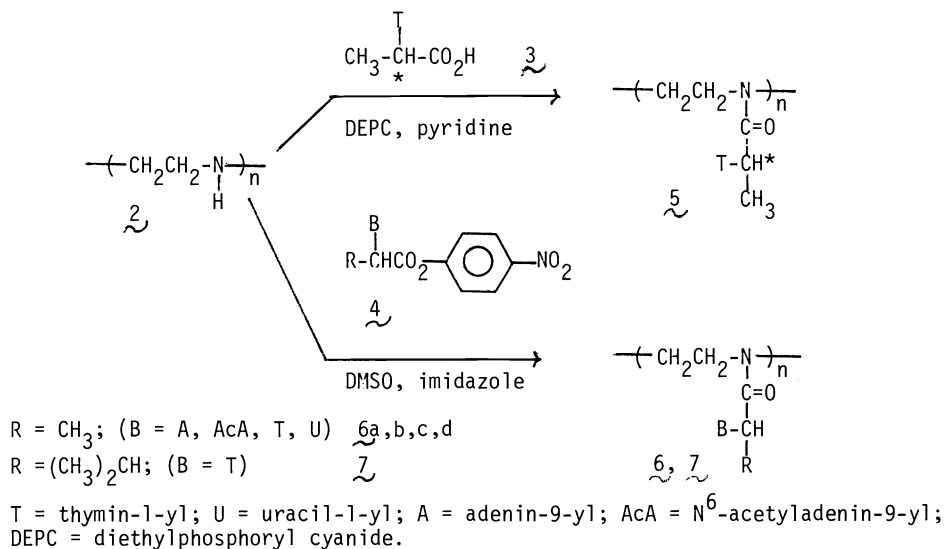
· Carboxylic acid derivatives with α-nucleic acid base substituents were synthesized. These derivatives can be resolved by conventional methods, allowing only one enantiomer (chiral center) to be attached to the polymer backbone. In addition, these derivatives are amenable to the methods used in preparing polypeptide amide bonds.

· Hindered rotation of the nucleic acid base pendant about the amide bond formed during grafting promotes an ordered, base-stacked structure of the type found in such synthetic polymers as polyadenylic acid (poly-A) and polyuridylic acid (poly-U). The trans conformation of the amide bond is greatly favored; this was demonstrated by NMR studies of model compounds.

· If a suitable amine-containing vinyl monomer or its precursor is available, the amide bond formation described above can produce optically active nucleic acid base monomers amenable to the many methods of vinyl polymerization.

· Many backbone and pendant modifications are possible while retaining most of the above advantages to the retention of order. Macromolecular structure can thus be tailored in response to the results of biological testing, if such information is available.

RESULTS AND DISCUSSION

(a) Polyethylenimine (PEI) Derivatives.

Several approaches are being examined to incorporate nucleic acid bases such as adenine, thymine, cytosine, etc., onto a hydrophilic polymeric structure. To date, our most studied system uses linear polyethylenimine (PEI) (2) as the backbone. Linear PEI's of several molecular weights have been used for grafting nucleic acid base derivatives as summarized in Scheme 1. The PEI's were obtained either by cationic polymerization of 2-oxazoline, or by hydrolysis of linear poly(N-propanoylethylenimine) obtained from the Dow Chemical Co. Resolution of 3 prior to grafting and retention of configuration during grafting has given us an optically active system with a chiral center adjacent to the nucleic acid base. This has allowed us to examine the system not only using ultraviolet (UV) spectroscopy (hypochromicity and Job plot studies) but also using circular dichroism (CD). The CD spectra of 5 in water show marked exciton splitting. The UV spectra of this system in water indicate significant hypochromicity (36.8%). Hence from the CD and UV studies we are able to rationalize that macromolecules 5-7 have some conformational order with some significant base stacking. This highly ordered conformation gradually becomes disorganized upon heating 5 to 90°C. Upon cooling, the macromolecule returns to its original ordered conformation.

The corresponding racemic adenine graft 6a has also been synthesized. Significant hypochromicity (45.4%) has been observed for this compound in aqueous solution. The conclusion from the hypochromicity studies indicate that polymers 5-7 have more conformational order than the single strand polynucleotides poly-A and poly-U. A combination of 5 and 6a in neutral aqueous solution resulted in marked hypochromic effects at certain mixing ratios (mole fraction 6a equals 0.8 and 0.3). In acidic aqueous solution, only a small deviation from Beer's law was seen. Mixing 6a (racemic) and 5 in chloroform/ethanol (1/1, v/v) also resulted in a hypochromic effect, which was maximum when there were equal molar amounts of 5 and 6a. The same combination in trifluoroethanol resulted in no hypochromic effect. Mixtures of 5 with the corresponding monomer and dimer models of 6a in neutral aqueous solution gave only a slight deviation from Beer's law. In the chloroform/ethanol solvent system, a combination of the monomer models of 6c (racemic) and 6a gave no significant deviation from Beer's law. This data indicates that base pairing has occurred between 5 and 6a or 6c and 6a so as to enhance base stacking. Observations in the CD spectra of

$$CH_3\text{-}\overset{|}{\underset{*}{C}}H\text{-}CO_2H \quad \underset{\sim}{3}$$

$$\xrightarrow[\text{DEPC, pyridine}]{}$$

$$\overset{}{+}CH_2CH_2\text{-}\overset{|}{\underset{\overset{|}{C=O}}{N}}\overset{}{)}_n$$
$$T\text{-}\overset{|}{C}H*$$
$$\underset{\sim}{5} \quad \overset{|}{C}H_3$$

$$\overset{}{+}CH_2CH_2\text{-}\overset{|}{\underset{H}{N}}\overset{}{)}_n$$
$$\underset{\sim}{2}$$

$$R\text{-}\overset{\overset{B}{|}}{C}HCO_2\!\!-\!\!\bigcirc\!\!-\!\!NO_2$$
$$\underset{\sim}{4}$$

$$\xrightarrow[\text{DMSO, imidazole}]{}$$

$$\overset{}{+}CH_2CH_2\text{-}\overset{|}{\underset{\overset{|}{C=O}}{N}}\overset{}{)}_n$$
$$B\text{-}\overset{|}{C}H$$
$$\underset{\sim}{6,\ 7} \quad \overset{|}{R}$$

R = CH₃; (B = A, AcA, T, U) 6a,b,c,d
R = (CH₃)₂CH; (B = T)   7

T = thymin-1-yl; U = uracil-1-yl; A = adenin-9-yl; AcA = N[6]-acetyladenin-9-yl;
DEPC = diethylphosphoryl cyanide.

Scheme 1. Nucleic Acid Analogs Derived from PEI.

aqueous solutions of 5 and 6a support this conclusion.(11)
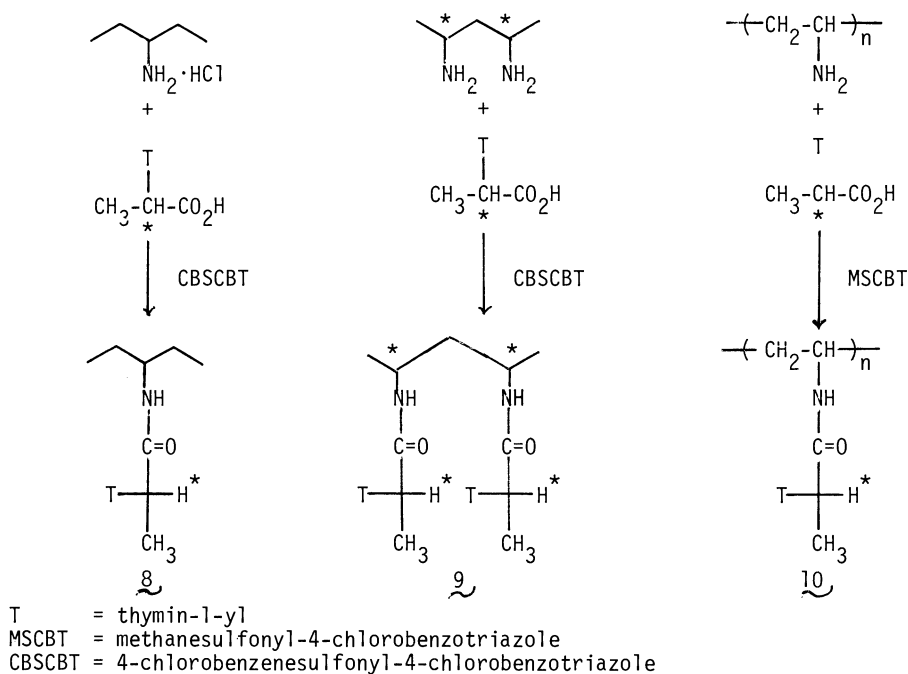
Uracilyl polymer 6d and polymer 7, a derivative of 3-methyl butyric acid, have been synthesized and examined spectroscopically.(12) The bulky R group of the latter favors trans amide bonds but may also sterically impede base stacking. NMR shows similar conformational populations, whether R = CH₃ or (CH₃)₂CH, and hypochromicities are comparable. Hypochromicities decrease as pH increases due to electrostatic repulsion between the charged rings, but increasing the ionic strength even at high pH's increased the hypochromicity. Studies in DMSO/H₂O solutions showed increased base-stacking in the more aqueous solvents; for polymer 6d with n = 100, a marked increase was noted. Polymer 6d and its monomer model also interact with poly A in TRIS buffer solutions.

(b) Poly(vinylamine) (PVam) Derivations.

We have synthesized atactic and highly syndiotactic polyvinylamine containing optically active (+) or (-)-2-(thymin-1-yl)-propionyl groups as grafted pendants (PT) and the related monomer (MT) and dimer (DT) models, shown in Scheme 2.(13) Two polymers of different tacticities were prepared by hydrolysis of poly(N-vinylacetamide) or poly(N-vinyl-t-butyl carbamate), which were obtained by free-radical polymerization of the corresponding monomers.(14,15) These compounds have been studied in detail by UV, CD, and 360 MHz-NMR spectroscopy. Although these models are even more sterically constrained than the PEI analogs, exciton coupling is not observed in the polymers; hypochromicity in H₂O is 27.9% and 22.4%, for the 31% and 81% syndiotactic polymers, respectively. The CD spectra of only one of the dimer models 9 displayed extremely solvent dependent exciton coupling, however, which could be correlated with changes in UV hypochromicity and NMR spectra, indicating a gradual conformational change from an extended to a stacked conformation as the solvent dielectric constant increased.(13) These results suggest that polymer tacticity certainly is a crucial determinant of conformation in solution and thus may be closely related to biological activity.

(c) Poly(dehydroalanine) (PDA) Derivatives.

Useful polymeric therapeutic agents are usually water soluble. As the molecular weight of the PEI and PVam derivatives described above is increased, water solubility decreases. Therefore, one direction of modification of these compounds is the attachment of hydrophilic groups to the polymer backbone. We have effectively prepared a carboxylated version of the PVam polymers by free-radical polymerization of dehydroalanines 11-14.(16,17) These monomers are made by coupling the 2-nucleopropionic acids with β-chloroalanine methyl ester hydrochloride using N,N'-dicyclohexylcarbodiimide in DMF, with the addition of N-hydroxybenzotriazole as a racemization-suppressing agent if the pendant is optically active. Dehydrochlorination of the product is effected with triethylamine; both dehydrochlorination and hydrolysis to the acid salt occur in aqueous NaOH. Polymerization of these monomers gives very water soluble products with inherent viscosities of up to 0.87 dl/g (DMF, 0.5 g/dl). The hypochromicities of the pyrimidine polymers are on the order of 10% versus the monomer models, while the purine polymers give slightly higher values. The optically active polymer 18(R=H) exhibits a CD spectrum which is very dependent on salt concentration, but much less dependent on the degree of neutralization. This behavior is paralleled in

T   = thymin-1-yl
MSCBT = methanesulfonyl-4-chlorobenzotriazole
CBSCBT = 4-chlorobenzenesulfonyl-4-chlorobenzotriazole

Scheme 2. Nucleic Acid Analogs Derived from PVam.

the viscometric titrations of the polymers.

Monomer	B	R	Polymer
11	T, U	H, CH_3	15
12	AcA	CH_3	16
13	A	H	17
14	(-)T	H, CH_3	18
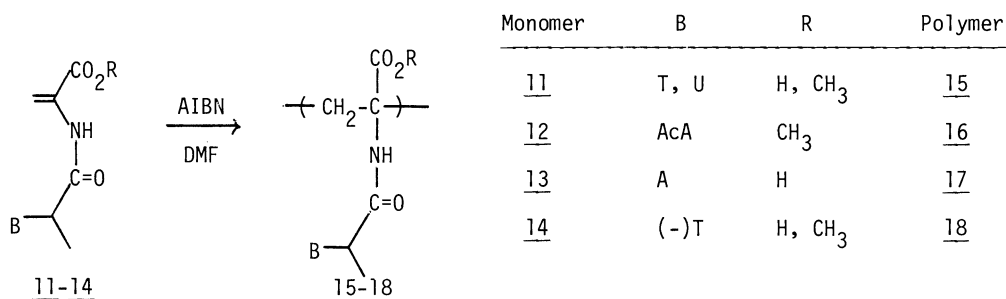
Scheme 3. Nucleic Acid Analogs Derived from PDA.

Further information on the conformational behavior of these types of polymers might be obtained by examining copolymers. A 360 MHz ^{1}H-NMR examination of several copolymers of optically active monomers 14 with 12 in DMSO at 60° revealed upfield shifts of the thymine ring methyl resonance as well as the resonances of the methyl ester and methyl group adjacent to the chiral center. Such effects of the adenine ring current are also seen in homopolymer 16, while the spectra of 15 resemble their monomer models. These results suggest that thymine units do interact with adjacent adenine units. Such interactions were difficult to detect by circular dichroism; the spectra of the copolymers were similar to homopolymers 15.

REFERENCES

1. a) Biological Activities of Polymers, ACS Symp. Ser. #186, C. E. Carraher and C. G.
    Gebelein, Eds., ACS, Washington, D.C., 1982; b) E. M. Hodnett, Polymer News, 8, 162
    (1982); 8, 323 (1983).
2. Polymeric Drugs, L. G. Donaruma and O. Vogl, Eds., Academic Press, 1978.
3. L. Gros, H. Ringsdorf, and H. Schupp, Angew. Chem. Int. Ed. Engl., 20, 305-325 (1981).
4. A. S. Jones, Int. J. Biol. Macromol., 1, 194 (1979).
5. S. Hoffmann, Z. Chem., 19. 241-249 (1979).
6. K. Takemoto and Y. Inaki, Adv. Polym. Sci., 41, 1 (1981).
7. J. Pitha, Adv. Polym. Sci., 50, 7 (1983).
8. J. Pitha, in Biomedical Dental Applications of Polymers, Polymer Science and Tech-
    nology, V. 14, C. G. Gebelein and F. F. Koblitz, Eds., Plenum Press, 1981, p. 203.
9. S. Hoffmann and W. Witkowski, ACS Symp. Ser., 74, 178-236 (1978).
10. C. G. Overberger and Y. Inaki, J. Polym. Sci., Polym. Chem. Ed., 17, 1739-1758 (1979),
    and C. G. Overberger, Y. Inaki, and Y. Nambu, ibid., 1759-1769.
11. Y. Morishima and C. G. Overberger, J. Macromol. Sci.-Chem., A13(5), 573 (1979);
    C. G. Overberger and Y. Morishima, J. Polym. Sci., Polym. Chem. Ed., 18, 1247, 1267,
    1433 (1980).
12. A. G. Ludwick and C. G. Overberger, J. Polym. Sci., Polym. Chem. Ed., 2123, 2139
    (1982).
13. C. G. Overberger and S. Kikyotani, J. Polym. Sci., Polym. Chem. Ed., 21, 525, 541
    (1983).
14. D. J. Dawson, R. D. Gless, and R. E. Wingard, Jr., J. Am. Chem. Soc., 98, 5996 (1976).
15. H. Hart, Makromol. Chem., 32, 51 (1959); A. R. Hughes and T. St. Pierre, Macromol.
    Syn., 6, 31 (1977).
16. K. A. Brandt and C. G. Overberger, Nouv. J. Chim., 6, 673 (1982).
17. C. G. Overberger, K. A. Brandt, S. Kikyotani, and Adriane G. Ludwick, Polym. Bull.,
    5, 481 (1981).
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