
The spatial configuration of ordered polynucleotide chains.

II. The poly(rA) helix

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ABSTRACT

Approximate details of the spatial configuration of the ordered single-stranded poly(rA) molecule in dilute solution have been obtained in a combined theoretical analysis of base stacking and chain flexibility. Only those regularly repeating structures which fulfill the criterion of conformational flexibility (based upon all available experimental and theoretical evidence of preferred bond rotations) and which also exhibit the right-handed base stacking pattern observed in nmr investigations of poly(rA) are deemed suitable single-stranded helices. In addition, the helical geometry of the stacked structures is required to be consistent with the experimentally observed dimensions of both completely ordered and partially ordered poly(rA) chains. Only a single category of poly(rA) helices (very similar in all conformational details to the individual chains of the poly(rA) double-stranded X-ray structure) is thus obtained. Other conformationally feasible polynucleotide helices characterized simply by a parallel and overlapping base stacking arrangement are also discussed.

INTRODUCTION

It is well known that polyriboadenylic acid (poly(rA)) in dilute aqueous solution exhibits an ordered single-stranded structure characterized by a unique arrangement of the bases stacked one above the other in a parallel fashion along the helical or long axis of the molecule. A complete account of the spatial configuration of the entire chain, however, is not attainable using present experimental techniques. The available data derived principally from nuclear magnetic resonance (nmr),¹⁻¹⁰ optical rotation,^{11,12} and Raman studies^{13,14} furnish (as outlined below) partial details of the rotational preferences of the individual bonds comprising the poly(rA) repeating unit and also structural information describing the organization of the adenine bases within the framework of the single-stranded helix.

According to both specific deshielding effects by the phosphodiester group on the adenine H(8) magnetic resonance signal^{1-3,5} and the nuclear spin-lattice relaxation time of this proton,⁴ the bases of the poly(rA) chain are oriented in anti conformations about the glycosyl bond (χ). In this

orientation the bulky six-membered pyrimidine ring of the adenine base is directed away from the sugar-phosphate backbone of the polymer. In addition, it is known from the ring current shielding effects associated with the π electrons of the bases^{1-3,5,6} that the adenine residues attached to the ordered backbone stack in a so-called "right-handed" pattern. Corresponding atoms in adjacent parallel bases along the poly(rA) backbone are related through a right-handed transformation of cylindrical (r, θ, Z) coordinates (see below). This transformation involves a positive right-handed rotation about a vertical axis perpendicular to the planes of the bases followed by a positive displacement (of approximately 3.5 Å) along this base stacking axis.¹⁵ This right-handed anti base stacking arrangement is similar to the stacking arrangements observed in the X-ray fiber diffraction studies of polynucleotide fibers¹⁶ and is also consistent with the base-base configuration determined from theoretical analyses of the measured circular dichroism (CD) of ApA.^{11,12}

Analysis of the vicinal proton-proton coupling constants characterizing the sugars in ordered poly(rA) and model systems indicates that the ring favors a C(3')-endo-like pucker^{3,5,7-9} and that the exocyclic C(5')-C(4') bond rotation preferentially assumes the so-called gg conformation (where $\psi \sim 240^\circ$ in Figure 1)^{8,9} well characterized in X-ray crystallographic investigations¹⁶ of low molecular weight nucleic acid analogs. Even at the low temperatures characterizing the ordered poly(rA) chain, however, it is necessary to include a small proportion of C(2')-endo ring puckering⁷⁻⁹ and some tg (where $\psi \sim 0^\circ$) exocyclic conformers⁸⁻⁹ in order to account theoretically for the observed coupling constants. The rotations ϕ and ϕ' (see Figure 1) about the C-O bonds of the polynucleotide backbone, on the other hand, are each restricted to a single range of values. Furthermore, according to the three bond nmr coupling constants between the P-H(3') and P-H(5') atom pairs in low molecular weight poly(rA) analogs, both rotations are confined to trans orientations.⁸⁻¹⁰

Unfortunately, experimental work to date provides no direct details of the preferred phosphodiester rotations (ω' and ω in Figure 1) in ordered single-stranded poly(rA) in solution. Crystallographic evidence from studies of model compounds¹⁷ as well as theoretical analysis¹⁸ of conformation indicate that the restrictions to rotation about the two O-P bonds are much less severe than those about the remaining bonds of the chain backbone. The phosphodiester bonds are the major source of flexibility in the poly(rA) backbone and the principal determinants of configuration-dependent properties of the chain as a whole. Evidence adduced from Raman studies of poly(rA)^{13,14}

as well as of the model trimer ApApA,¹⁴ however, points to the occurrence of a single phosphodiester conformational geometry characterizing the ordered forms of these compounds. This undetermined conformation is believed to be quite unlike the P-O backbone geometry (also undefined) found in the double-stranded protonated helix ($\text{poly}(\text{rAH}^+)_2$) in dilute solution but very similar to the phosphodiester solution conformations of a number of complementary double helical structures (including $\text{poly}(\text{rA}):\text{poly}(\text{rU})$ and RNA from a variety of sources).¹³ As we shall demonstrate, the unique geometry of the phosphodiester linkage and hence the nature of the single-stranded helix may be deduced by comparison of overall properties of the ordered $\text{poly}(\text{rA})$ chain calculated as functions of the ω' and ω angles with experimentally determined values.

Most recent low temperature hydrodynamic measurements on $\text{poly}(\text{rA})$ fractions in dilute solution indicate the single-stranded polymer structure to be a rigid rod with an approximate repeating length of $3.2 \text{ \AA}/\text{residue}$.¹⁹ This value corresponds to the mean distance spanned by a single repeating unit of the chain along the long axis of the ordered molecule. If all residues of the $\text{poly}(\text{rA})$ chain conform to identical spatial arrangements, this distance then represents the step height z (see below) of the single-stranded helix.

In this paper we utilize the structural information outlined above in conjunction with a recently developed single virtual bond treatment of polynucleotide helices¹⁵ in order to assess the more probable spatial arrangements of the $\text{poly}(\text{rA})$ single-stranded helix in dilute solution. This scheme clearly surpasses the rough approximation of single-stranded $\text{poly}(\text{rA})$ ordered structure reported previously.²⁰ The earlier estimate of a base stacked structure was based upon the helical backbone parameters determined for eight specific regularly repeating polynucleotide chains. These few structures were generated by permutations of the mean backbone rotation angles deduced from available X-ray fiber diffraction investigations of polynucleotide double helices. Here we report the helical parameters describing a much broader spectrum of regular structures which include all $\text{poly}(\text{rA})$ helices consistent with the conformational preferences of this molecule and its low molecular weight analogs in solution. In addition, we present for each structure a set of base stacking parameters which directly define the arrangement of these moieties in the helix. On the basis of these calculations we then distinguish the feasible $\text{poly}(\text{rA})$ helices as those structures which exhibit the right-handed stacking pattern observed in nmr investigations and also conform in overall dimensions to the helical step height suggested by the hydrodynamic studies. In addition, we examine the effects of those conformations which

meet the geometric criteria of stacking and step height on the unperturbed dimensions of partially ordered poly(rA) chains. Comparison of the theoretical dimensions (determined for a noncooperative helix-coil process) with the experimental behavior of the partially stacked chain permits some further estimation of the structural details of this molecule in dilute solution.

ORDERED POLY(rA) STRUCTURE

Structural Criteria

The structural parameters describing any regularly repeating or helical polynucleotide chain (z - the vertical displacement of adjacent residues along the helix axis, r - the radial distance of the phosphorus atoms from the helix axis, and θ - the cylindrical rotation angle about the helix axis between atoms of neighboring units) as well as the base stacking parameters (Z - the mean distance between adjacent base planes, Λ - the mean angle between neighboring bases, θ - the cylindrical rotation about the base stacking axis describing the angular displacement of neighboring bases, and η - the angle between the base stacking axis and the helix axis) may be calculated using methods outlined elsewhere.¹⁵ The three helical parameters (z , r , and θ) are functions of the six rotation angles of the sugar-phosphate repeating unit (see Figure 1) as well as of the fixed bond lengths and valence bond angles comprising the backbone. The stacking parameters (Z , Λ , θ , and η) depend not only upon these 18 values but also upon the glycosyl rotation χ (describing the orientation of the base with respect to the pentose) and the bond lengths and valence bond angles of the sugar-base structural moiety. The helix is described as right-handed if the ratio z/θ is positive and the base stacking as right-handed if the Z/θ ratio is positive. Left-handed helices and stacks are described by the respective negative ratios.

In the analysis that follows we require stacked conformations of the poly(rA) helix to meet the following criteria: (1) parallel alignment of bases (characterized by values of $\Lambda < 35^\circ$), (2) average vertical separation Z of base planes between 3.0 and 4.0 Å, and (3) right-handed base-base stacking. In addition, when viewed down the base stacking axis, the planes of adjacent bases are required to overlap partially. The reader is referred to reference 15 for further discussion of the basis of these criteria.

Chain Geometry

The bond lengths and valence bond angles employed in these computations have been detailed previously.²¹ The rotation angles along the backbone are taken to be 0° in the planar trans conformation and are assigned positive values for right-handed rotations. In contrast, the six backbone rotation

angles are measured relative to the cis conformation in crystallographic studies¹⁷ and hence will differ from the values reported here and in other configurational statistics studies^{15,18,20,21} by 180°. The glycosyl rotation is measured relative to the trans conformation of bonds O(1')-C(1'), C(1')-N(9), and N(9)-C(4). Values of χ described by this scheme are identical in magnitude to the angles reported by crystallographic workers who define the 0° reference state as the cis arrangement of atoms O(1')-C(1')-N(9)-C(8).¹⁷

The preferred anti conformation of the glycosyl bond in poly(rA) is here estimated by the rotational isomeric state $\chi = 30^\circ$. This single value was chosen in view of the limited range of this angle determined both in X-ray crystallographic investigations on model purine nucleosides, nucleotides, dimers, and trimers^{17,22,23} and in semiempirical energy estimates of the preferred conformation in poly(rA).²¹ The predominant C(3')-endo and the minor C(2')-endo pucker are each described by a theoretical geometry²¹ in which the atom of interest is displaced exactly 0.5 Å above a plane containing the four remaining heavy pentose atoms. The rotation angle ψ' about bond C(4')-C(3') is fixed at the values 266° and 317°, respectively, in these units. The ψ rotation about bond C(5')-C(4') is treated as twofold with rotational isomeric states at 0° and 240° corresponding respectively to the so-called tg and gg conformations referenced by crystallographers.¹⁷ The gt conformation centered in the range $\psi \sim 120^\circ$ is omitted from the calculations in view of the severe steric conflicts known^{20,21} to arise between adjacent phosphates along the polynucleotide backbone. The preferred trans conformations of the C-O bonds ϕ' and ϕ are fixed at the values 35° and 0°, respectively. Minor variations in any of these rotational angles have negligible influence on the computed values of the helix and base stacking parameters.

The rotations ω' and ω about the phosphodiester bonds are assumed to be threefold corresponding to the usual trans (t), gauche⁺ (g⁺) and gauche⁻ (g⁻) conformations. The t range is chosen here to extend between -20° and +20°, the g⁺ range from 90° to 120°, and the g⁻ range between 220° and 260°. These boundaries correspond to the usual outer limits of the P-O rotations ranges observed in X-ray crystallographic studies of low molecular weight phosphodiester analogs^{17,22} and also predicted by conformational energy estimates.^{20,24,25}

Helical Structures

Listed in Tables I and II are the helical and base stacking parameters compiled for all conformationally feasible (based upon the rotational preferences outlined above) single-stranded poly(rA) helices. The C(3')-endo hel-

ices are described in Table I and the C(2')-endo helices in Table II. In both instances computations were performed at 10° intervals within the specified ranges of ω' and ω . For simplicity only the average values of the parameters associated with each region of $\omega'\omega$ conformation space (i.e., tt, tg⁺, etc.) are reported in the tables. In order to differentiate these values which do not refer to real structures from the values of z , r , θ , Z , Λ , Θ , and η associated with a given helix, the parameters are enclosed by angle brackets. In the case of tt helices, for example, the bracketed values in the table represent the mean parameters of the 5² helices which fall at 10° increments within the area bounded by the $\pm 20^\circ$ ω' axes and the $\pm 20^\circ$ ω axes. These average data in all cases are reasonably representative of the helical and base stacking parameters describing each helix within a particular region of $\omega'\omega$ conformation space (i.e., the helices within a certain conformational domain are generally described by very similar sets of structural parameters). The plus or minus signs within the last columns of the tables indicate the existence or non-existence, respectively, of partial base-base overlaps in a given helix. The g⁺g⁻ and g⁻g⁺ helices were not examined in view of the known close-range steric contacts in these structures.²⁰ Those helices described by other combinations of the $\omega'\omega$ rotational domains which engender additional steric overlaps between atom pairs in the sugar-phosphate backbone, are denoted by an asterisk in the second column of each table.

According to the data recorded in Tables I and II there are a wide variety of conformationally feasible ordered structures of single stranded poly(rA). Only seven of the 28 helical categories examined may be ruled out by the longer-range backbone conflicts which arise in the regularly repeating polymeric structures. As evident from the tables all but one of these seven structures are characterized by gauche⁻ conformations of the ω' rotation. Such orientations bring about close steric contacts between the atoms in the two pentose-phosphate units located on either side of the O(3')-P bond (whose rotation is measured by the ω' angle). The remaining helix (C(2')-endo, $\psi = \text{gg}$, $\omega'\omega = \text{g}^+\text{g}^+$) is conformationally infeasible principally because of nonbonded contacts that occur between the 2'-hydroxyl group and the pentose of the following residue in the chain.

The helices described in Tables I and II are divided approximately equally between left-handed ($z/\theta < 0$) and right-handed ($z/\theta > 0$) structures. It is particularly interesting to note that the handedness of the helical backbone does not necessarily correlate with the handedness of the base stacking in each structure. When the base stacks are approximately perpendicular to the helix

TABLE I
Mean Helix and Base Stacking Parameters
of Ordered C(3')-endo Poly(rA) Chains

Conformation		Helix Parameters			Base Stacking Parameters				Overlaps
ψ (range)	ω, ω' (domain)	$\langle z \rangle$, Å	$\langle r \rangle$, Å	$\langle \theta \rangle$, deg	$\langle z \rangle$, Å	$\langle \Lambda \rangle$, deg	$\langle \theta \rangle$, deg	$\langle \eta \rangle$, deg	
tg	tt	4.38	3.91	-81.3	8.44	35.1	-77.9	28.6	-
tg	tg ⁺	2.40	3.57	122.4	8.71	59.0	-117.3	88.1	-
tg	tg ⁻	6.05	1.54	-138.2	1.81	77.2	-108.6	42.0	-
tg	g ⁺ t	4.96	7.40	32.2	5.66	14.6	29.9	29.5	+
tg	g ⁺ g ⁺	5.66	2.13	117.6	9.98	85.0	44.0	59.7	+
tg	g ⁻ t	5.10	2.24	161.6	4.29	34.6	165.0	17.7	-
tg	g ⁻ g ^{-*}	2.82	3.08	158.9	2.17	81.4	-137.3	50.7	+
gg	tt	5.58	0.53	-127.5	2.12	83.4	-96.5	48.0	-
gg	tg ⁺	3.00	3.98	-75.6	6.23	29.0	-72.6	25.3	+
gg	tg ⁻	4.04	2.11	139.8	7.63	43.7	-105.7	84.5	-
gg	g ⁺ t	2.94	2.84	-117.2	2.70	64.2	12.1	89.1	-
gg	g ⁺ g ⁺	3.06	5.97	46.5	3.77	26.2	40.0	37.6	+
gg	g ⁻ t*	3.07	2.41	178.1	1.15	80.5	-145.5	50.0	-
gg	g ⁻ g ^{-*}	0.66	2.82	175.6	5.46	38.6	146.3	81.0	+

*Sterically infeasible helix.

TABLE II
Mean Helix and Base Stacking Parameters
of Ordered C(2')-endo Poly(rA) Chains

Conformation		Helix Parameters			Base Stacking Parameters				Overlaps
ψ (range)	ω, ω' (domain)	$\langle z \rangle$, Å	$\langle r \rangle$, Å	$\langle \theta \rangle$, deg	$\langle z \rangle$, Å	$\langle \Lambda \rangle$, deg	$\langle \theta \rangle$, deg	$\langle \eta \rangle$, deg	
tg	tt	2.33	9.64	-44.9	1.65	14.8	-41.6	21.2	+
tg	tg ⁺	5.51	2.72	123.9	3.26	67.7	-56.5	71.0	-
tg	tg ⁻	7.00	1.28	-111.3	4.61	70.1	-43.0	81.2	-
tg	g ⁺ t	5.74	3.75	74.2	8.42	43.5	57.7	38.5	+
tg	g ⁺ g ⁺ *	6.00	2.05	142.4	7.12	61.3	39.7	65.7	+
tg	g ⁻ t	4.34	3.03	-156.0	1.28	36.2	-150.8	18.6	-
tg	g ⁻ g ^{-*}	4.17	3.01	-176.9	1.58	30.3	-162.9	75.6	+
gg	tt	6.20	1.29	-99.4	3.66	84.1	23.7	78.1	-
gg	tg ⁺	2.60	13.43	-8.7	-0.09	12.3	-28.0	26.1	+
gg	tg ⁻	5.65	1.84	146.5	2.91	65.5	-69.8	62.2	-
gg	g ⁺ t	1.26	4.13	-104.9	1.49	83.7	68.0	70.5	-
gg	g ⁺ g ⁺	4.87	3.42	83.7	6.27	60.0	55.1	49.0	+
gg	g ⁻ t*	4.64	2.38	-170.4	2.44	20.0	-160.4	84.3	-
gg	g ⁻ g ^{-*}	1.36	3.33	158.4	7.43	83.6	-175.3	43.3	+

*Sterically infeasible helix.

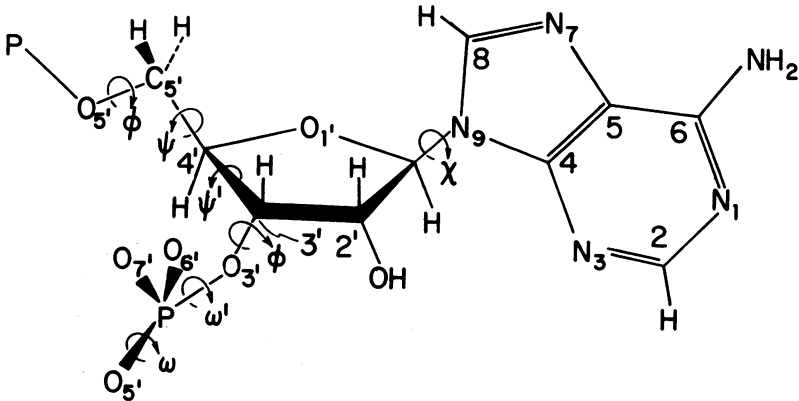


Figure 1. Section of the poly(rA) chain backbone showing chain atoms and rotation angles. The angles are measured by right-handed rotations with respect to the trans orientation.

axis (i.e., when the base stacking axis approximately parallels the helix axis and $\eta \rightarrow 0^\circ$), the handedness of the helix and stack are similar in sign and magnitude. When the base planes parallel the helix axis (i.e., $\eta \rightarrow 90^\circ$), however, the stacking may be of opposite handedness and of very different magnitude from the turn of the helix.

We also note from Tables I and II that the majority of the helices are tightly wound structures characterized by axis ratios $z/r > 1$ and by very few residues per turn of the helix (i.e., $360/\theta < 8$ or $|\theta| > 45^\circ$). Only four of the helices (the C(3')-endo $\psi = \underline{tg}$, $\omega'\omega = \underline{g^+t}$ and $\psi = \underline{gg}$, $\omega'\omega = \underline{g^+g^+}$ structures in Table I and the C(2')-endo $\psi = \underline{tg}$, $\omega'\omega = \underline{tt}$ and $\psi = \underline{gg}$, $\omega'\omega = \underline{g^+t}$ structures in Table II) may actually be categorized as loosely wound helices with several residues per turn of the helix. Such helices can usually accommodate the base pairing interactions associated with multi-stranded polynucleotide structures.

Base Stacking

Close examination of the structural data in Tables I and II reveals that only one helical structure (the C(3')-endo, $\psi = \underline{gg}$, $\omega'\omega = \underline{g^+g^+}$ helix in Table I) fulfills all the geometric criteria associated with the right-handed stacking exhibited by ordered poly(rA) molecules in solution. This conformational category is the same as that which describes the backbone geometry in the double-stranded poly(rA) fiber,²⁶ the RNA double helices,²² and also the majority of dinucleoside monophosphate solid state structures.^{22,24} We note also that the residue repeating length ($\langle z \rangle = 3.1 \text{ \AA}$) is consistent with the recently reported hydrodynamic measurement.¹⁹

Several of the conformationally feasible poly(rA) helices meet the loose classification of "stacked" as defined by a simple parallel overlapping arrangement of the bases. For convenience, the extent of base stacking (as defined by the rigorous criteria listed earlier in the text) in these structures has been tallied in a rough fashion in Table III. The plus or minus signs in the table represent the fulfillment or nonfulfillment, respectively, of a given structural criterion by each helix. We note here that all but one of the "stacked" structures in Table III fits the classification of a loosely wound helix (see above).

Helix I (described by four plus signs) in Table III is the right-handed C(3')-endo stacked structure described in the previous paragraphs. Cross reference between Tables I and III reveals that the right-handed stacked bases of C(3')-endo helix II (in Table III) are very widely separated in space. In

TABLE III
Analysis of Stacking in Selected Poly(rA) Helices

Helix*	Pentose (pucker)	ψ (range)	ω' ω (domain)	Z (3.0-4.0 Å)	Λ (0-35°)	θ/Z_0 (>0°/Å)	Over- laps
I	C(3')-endo	gg	$\overline{g^+g^+}$	+	+	+	+
II	C(3')-endo	\overline{tg}	$\overline{g^+t}$	-	+	+	+
III	C(3')-endo	\overline{gg}	$\overline{tg^+}$	-	+	-	+
IV	C(2')-endo	\overline{gg}	$\overline{tg^+}$	+ [†]	+	-	+
V	C(2')-endo	\overline{tg}	\overline{tt}	+ [†]	+	-	+

*See Tables I and II for structural details.

[†]Fulfills criteria for some structures in region but not average structure.

fact, the distance between the bases is possibly large enough for a planar molecule to intercalate between them. The rotational parameters deduced in the original Watson-Crick X-ray DNA structure describe a helix similar to helix II but characterized by the normal base stacking distance.²⁷ We note, however, that the Watson-Crick structure was also characterized by an unusual glycosyl rotation χ near 0° which would permit the appropriate base-base separation.²⁸ The stacked bases of the C(3')-endo helix III are not only widely separated in space but also ordered in a left-handed arrangement. This helix is another example of a structure which could accommodate the intercalation of planar molecules.

It is not immediately apparent from the data in Table II that the C(2')-endo helix of type IV (in Table III) can accommodate base stacking. Strict interpretation of the geometric data in Table II indicates the average structure IV to be a left-handed helix with bases attached laterally to the outside of the helix and also at distances of separation in violation of normal van der Waals contacts. Several type IV helices in the region where $\omega' \sim 10-20^\circ$, however, are right-handed structures with bases located inside the helix framework and at the appropriate 3.0-4.0 Å separation. The observed stacking pattern in all structures of the type IV category, however, is left-handed. We also note here that the type IV helix is very similar in conformational characteristics to the C-DNA X-ray structure.²⁹ The occurrence in solution of the type V C(2')-endo helix of Table III appears to be quite improbable. This chain is a left-handed structure characterized by left-handed base stacking on the outside of the molecular framework. In addition, such configurations of the polynucleotide backbone (where the $\omega'\omega$ rotation is tt) have never been observed in the X-ray crystallographic literature.²²

PARTIALLY ORDERED POLY(rA) CHAINS

According to the above analysis of base stacking, the ordered single-stranded structure of poly(rA) in dilute aqueous solution must be very similar to the helical configuration deduced from the X-ray fiber diffraction analysis²⁶ of the double helical acid form $(\text{poly}(\text{rAH}^+))_2$ of the molecule. The viability of such a helix in solution may be further ascertained from its ability to predict the observed increase in unperturbed dimensions of the poly(rA) chain with decreasing temperature.

This increase in chain dimensions may be attributed to the formation of a partially stacked structure.³⁰ Such an arrangement of repeating units can be achieved by treating the polynucleotide chain as a copolymer of unstacked or random-coil and stacked or helical subunits.

The thermal denaturation of poly(rA), as manifested by hypochromicity, optical rotatory dispersion, and circular dichroism, occurs gradually over the temperature range 0-90°. ³¹⁻³³ This evidence, in conjunction with the fact that the apparent enthalpy of denaturation is nearly independent of chain length,³⁴ demonstrates that the ordering of the poly(rA) chain brought about by base stacking is largely a noncooperative process. Hence, stacked and unstacked residues should occur in more or less random sequence. The average dimensions of the poly(rA) chain containing stacked residues can therefore be treated using computational methods detailed previously for random copolymers.²⁰

Limiting values of the characteristic ratio $C_n = \langle r^2 \rangle_0 / n\bar{l}^2$ of the unperturbed mean-square end-to-end distance $\langle r^2 \rangle_0$ to the number of bonds in the

chain n times the mean-square bond length $\overline{r^2}$ are plotted in Figure 2 as a function of F_S , the fraction of stacked residues in each of four partially ordered poly(rA) chains. The unstacked residues in all four copolymers were apportioned equally and at random between the C(3')-endo and the C(2')-endo randomly coiling units previously described in semiempirical potential energy calculations.²⁰ Such chains have been found to reproduce the observed experimental unperturbed dimensions³⁰ of completely unstacked polyribonucleotide chains. The stacked residues in the copolymers are described by the respective helical and base stacking parameters listed in Table IV. Each of these helices is a C(3')-endo, $\psi = \underline{g}g$, $\omega'\omega = \underline{g}^+\underline{g}^+$ type structure which fulfills approximately the geometric criteria of right-handed base stacking.

TABLE IV
Stacked Residues of Partially Ordered Poly(rA) Chains*

Helix	ω' ,deg	ω ,deg	z ,Å	r ,Å	θ ,deg	Z ,Å	θ ,deg
1	100	90	1.32	7.15	45.4	3.15	31.5
2	100	100	2.49	7.14	41.9	3.67	33.9
3	100	110	3.65	6.29	40.4	4.24	36.0
4	100	120	4.50	4.72	41.4	4.86	37.7

*See Figure 2

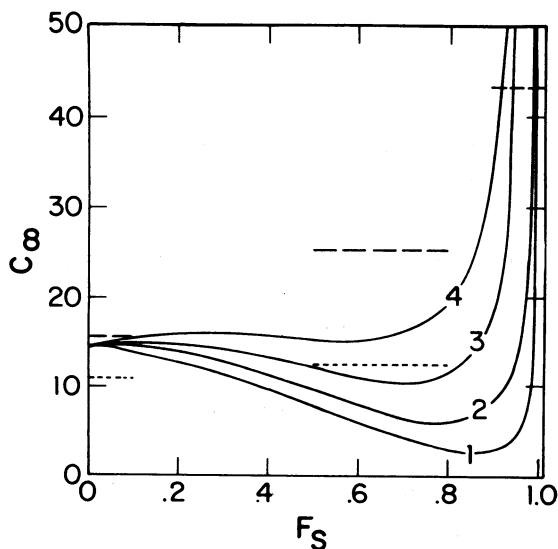


Figure 2. The characteristic ratio calculated for random copolymers of random-coil and stacked repeating units as a function of the fraction of helical content F_S . See text for further details of the curve notations and dashed and dotted lines.

The computed characteristic ratio for each of the copolymers in Figure 2 passes through two points of inflection (corresponding to a local maximum followed by a minimum) as F_s increases from zero (random coil) to unity (stacked helix). The initial appearance of helicity is marked by a small increase in C_∞ . The curve next passes through a minimum, then rises. The rise is steep for long chains inasmuch as the extension of the helical long chain greatly exceeds the mean extension of the random coil. The minimum in Figure 2 reflects the fact that helical sections of short sequences have smaller end-to-end distances than the mean for random coils of the same number of units.

The calculations indicate the rate of change in chain dimensions with F_s to depend strongly upon the type of helix incorporated into the polymer. As is evident from Table IV, the four helical arrangements considered here are described by a similar base stacking pattern (compare Z and θ) but by very different helical parameters (z , r , and θ). While the number of residues per helix turn ($360/\theta$) remains approximately constant, the helical axis ratio (z/r) varies between .18 and .95 in the four structures. According to Figure 2, incorporation of the more extended helix 4 into the chain produces a sharp increase in C_∞ at smaller values of F_s (-0.8). The characteristic ratio does not increase until $F_s > 0.99$ for the copolymer containing the compact structure, helix 1. Curves 3 and 4 fall more nearly in the range of experimentally determined values of C_∞ than do curves 1 and 2. The experimental values obtained from viscosity and light scattering studies³⁰ in poly(rA) are denoted in Figure 2 by the dotted and dashed lines, respectively. These data were obtained at three temperatures (60, 25, and 15°C) where the degree of stacking in the poly(rA) chain is estimated to lie in the indicated ranges (0-0.1, 0.5-0.8, and 0.9-1.0, respectively).

The observed sensitivity of C_∞ to slight variations in helical geometry poses a major limitation in further estimation of the single-stranded structure of poly(rA) from an analysis of dimensions in partially ordered chains. The fact that there is some correlation between the calculated and experimental dimensions in Figure 2, however, lends additional support to the contention that the single-stranded neutral poly(rA) helix is similar in conformational detail to the individual chains of the solid-state double-stranded acid form.

SUMMARY

According to the above analysis, the single-stranded helix of poly(rA) in dilute solution must be very similar in conformational detail to the helical structures of the individual chains deduced from the X-ray fiber diffraction analysis of the double-stranded acid form of the molecule.²⁶ Thus, at low

temperatures the preferred solution conformation of the phosphodiester bonds of the single-stranded poly(rA) molecule as well as that in a number of complementary double helical structures (such as poly(rA):poly(rU)) which exhibit similar Raman spectra¹³ must be the $\underline{g}^+\underline{g}^+$ domain. Other combinations of the $\omega'\omega$ rotations may describe helices able to accommodate an array of parallel adenine bases. These conformations, however, cannot be regarded as feasible stacked structures since the chain backbone fixes the attached bases in a left-handed stacking pattern and/or positions the bases at distances greatly exceeding the normal limits of stacking. The $\underline{g}^+\underline{g}^+$ rotations, on the other hand, not only accommodate the observed right-handed stacking pattern of the adenine bases^{1-3,5,6} and the 3.5 Å helical step height¹⁹ but also reproduce approximately the experimentally observed unperturbed dimensions³⁰ of partially ordered poly(rA) chains.

It is important to recognize that the approximate description of the single-stranded poly(rA) molecule outlined above in no way requires that the set of internal rotations along the chain conform exactly to the values observed in the poly(rA) double helix. It is interesting to observe, nevertheless, that the ordered chain residues which best approximate the observed dependence of the unperturbed dimensions of the partially stacked poly(rA) molecule with decreasing temperature are units described by helical parameters very similar to those of the poly(rA) double helix. The double helix chains are characterized by a step height $z = 3.8$ Å, a radius (phosphorus atoms) $r = 5.95$ Å, and a repeating angle $\theta = 45^\circ$.²⁶ The mean helical parameters of the two theoretical chains (3 and 4 from Table IV) which best approximate the partially stacked dimensions are $z = 4.1$ Å, $r = 5.51$ Å, and $\theta = 40.9^\circ$. The calculated unperturbed dimensions of poly(rA) molecules containing more loosely wound helical units similar to the RNA X-ray structures (where $r \sim 8$ to 10 Å and $\theta \sim 30$ - 36°)¹⁵ are not comparable in magnitude to the observed experimental values.

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