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A RNA·DNA hybrid that can adopt two conformations: An x-ray diffraction study of poly(rA)·poly(dT) in concentrated solution or in fibers

(transcription/replication)

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ABSTRACT **The RNA·DNA hybrid poly(rA)·poly(dT) can adopt two conformations, depending upon its degree of hydration. Fibers yield a conventional A′ RNA-like pattern at 79% relative humidity. In contrast, under highly solvated conditions this material yields an x-ray diffraction pattern with striking similarities to that of B DNA and clearly different from the RNA-like diffraction patterns previously obtained from fibers of other hybrids. A structural model is proposed for the solution form of the hybrid that has several similarities to models proposed for B DNA. The hybrid model accommodates the 2′-hydroxyl groups of the poly(rA) strand in intrachain hydrogen bonds to the adjacent ribose moieties.**

The structure of RNA·DNA hybrids is potentially of major functional importance in such processes as transcription and replication. Although hybrids have generally been assumed to be similar to RNA in conformation on the basis of earlier x-ray fiber diffraction studies (1, 2), we questioned whether hybrids *in solution* would adopt an RNA-like structure. The structures proposed for hybrids have features typical of double-stranded RNA in fibers (reviewed in ref. 3). For example, the structures have 11 or 12 base pairs per helical turn, with the base pairs highly tilted and spaced at relatively small intervals along the helix axis. These characteristics are very different from those of DNA in concentrated solutions (4) or in fibers held at high relative humidity (5). Under such conditions DNA assumes the B form,* which has 10 base pairs per helical turn; the base pairs are essentially untilted and are spaced at relatively large intervals along the helix axis.

The synthetic RNA·DNA hybrid poly(rA)·poly(dT) has been our major focus. In view of the tendency of the analogous DNA·DNA complex poly(dA)·poly(dT) to retain a B or "wet DNA" conformation under conditions in which DNA duplexes usually adopt an RNA-like conformation (6, 7), it seemed possible that the corresponding hybrid might also tend to form a B DNA-like conformation. In fact, in concentrated solution or in fibers at high relative humidity, poly(rA)·poly(dT) does form a structure whose general x-ray diffraction pattern and helical parameters appear to be quite similar to those of B DNA. This observation reopens the question of RNA·DNA hybrid conformation in general and especially under the highly solvated conditions that may be particularly relevant to biological processes.

MATERIALS AND METHODS

Poly(rA) and poly(dT) (from P-L Biochemicals and Miles, respectively) were each dissolved in 0.01 M Tris·HCl, pH 8.0/0.1 mM EDTA, and shaken at room temperature for several minutes with an equal volume of neutralized redistilled phenol. The remaining steps were at 0–5°C. Aqueous phases were dialyzed vs. several changes of 0.01 M sodium phosphate, pH 7.6/1 M NaCl/20 μM EDTA. For a typical preparation of poly(rA)·poly(dT), 8 μmol (on a nucleotide basis; extinction coefficients from ref. 8) each of poly(rA) and of poly(dT) were mixed in a total volume of 4 ml of 0.1 M NaCl. The solution was adjusted to pH 8.8 to 9.2 with 1 M NaOH and held overnight to ensure the absence of the acid form of poly(rA) (9). Two volumes of cold 95% (vol/vol) ethanol were added; the precipitate was collected by centrifugation, washed three times with 8 ml of 67% ethanol, redissolved in distilled water, and lyophilized.

Fibers were formed at room temperature from lyophilized hybrid dissolved in a small amount of distilled water between two glass supports. Diffraction patterns were collected as described (4), using specimen-to-film distances of 28–33 mm. Wetted samples were prepared as before. Spacings of meridional reflections were determined on short exposures of an appropriately tilted sample (4).

Molecular models were initially constructed from wire models (5 cm/Å). Small changes in atomic positions were made computationally to maintain proper covalent bond angles and distances. Covalent bond angles were maintained within 4° and covalent bond distances were maintained within 0.07 Å of those in models for B DNA (10) [for the model of the poly(dT) strand] and A RNA (11) [for the model of the poly(rA) strand]. Transforms were calculated as described (12).

RESULTS

X-ray Diffraction Patterns. Fibers of poly(rA)·poly(dT) exposed to an intermediate relative humidity (79%) gave a striking crystalline pattern (Fig. 1*A*) similar to that recorded for poly(rI)·poly(dC) (2) and for poly(rI)·poly(rC) (13). The pattern corresponds to a 12-fold helix (Table 1).

Such fibers were allowed to take up large amounts of water, either by exposure for several days to an atmosphere at 98% relative humidity or by immersion in an excess of solvent. In both cases, the fibers were contained in a capillary so that swelling was limited to a range in which the molecular orientation in the unswollen fibers was largely retained. Although the diffraction patterns of the two types of sample were similar, the fiber swollen in a humid atmosphere (Fig. 1*B*) retained some crystallinity, whereas the sample prepared by immersion (Fig. 1*C*) had a con-

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* By "B-form DNA" or "B DNA" we mean the DNA structure present in those highly hydrated DNA samples that yield a diffraction pattern distinguished by helical parameters corresponding to 10 residues per turn and a general distribution of diffracted intensities as in ref. 4. Specific structural proposals such as sugar puckers, base tilts, etc. are considered as attributes of models for this structure.

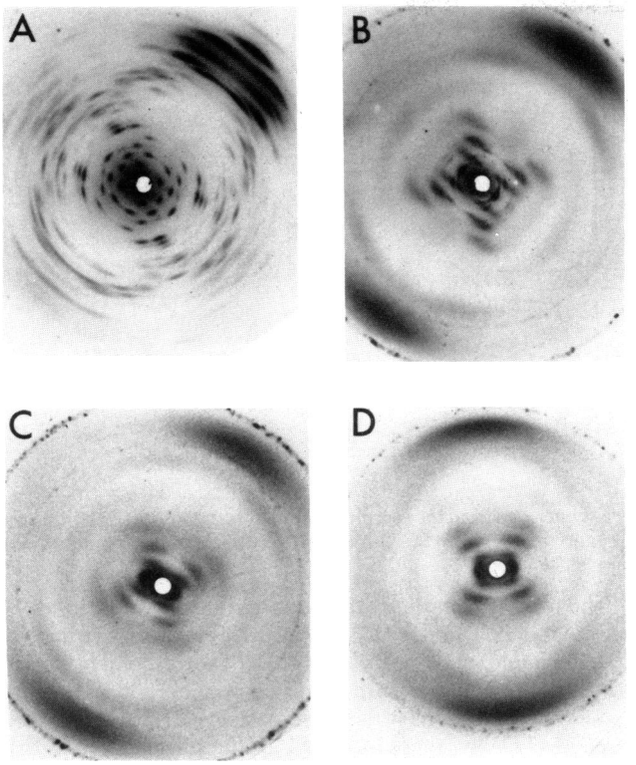

FIG. 1. X-ray diffraction photographs of poly(rA)·poly(dT) and DNA. *A*, *B*, and *C* are diffraction patterns from poly(rA)·poly(dT) fibers equilibrated at 79% or 98% relative humidity or after immersion in water, respectively. *D* is the pattern obtained from a DNA fiber after immersion in water. Samples *B*, *C*, and *D* were contained within capillaries so that the lateral expansion of the fibers was limited. The sample in *A* was tipped 15° from being perpendicular to the direct x-ray beam to show the meridional reflection on the 12th layer. The long axis of the other samples was essentially normal to the direct beam, and is either vertical (*D*) or tipped slightly from the vertical relative to this page (*A*, *B*, and *C*). The ring of diffracted intensity on these pictures is from a calcite standard.

tinuous transform. In both cases the pattern was totally changed from that of the lower humidity form mentioned initially to a pattern with similarities to that of B DNA. For comparison, the diffraction pattern of a DNA fiber immersed under similar conditions to those used to prepare the hybrid sample of Fig. 1*C* is shown in Fig. 1*D*. The helical parameters derived from wetted fibers of poly(rA)·poly(dT) are similar but not identical to those of wetted DNA fibers (Table 1).

Table 1. Helical parameters of poly(rA)·poly(dT) and DNA as fibers or in concentrated solution*

Fiber	Pitch, Å	Axial rise per residue, Å	Residues per turn	Example
Poly(rA)·poly(dT)				
79% rel. hum.	36.0	3.03	11.9	Fig. 1*A*
98% rel. hum.	34.5	3.20, 3.45†	10.8, 10.0†	Fig. 1*B*
Wetted	33.7	3.46	9.7	Fig. 1*C*
DNA				
98% rel. hum.	33.1	3.33	10.0	—
Wetted	33.0	3.34	9.9	Fig. 1*D*

rel. hum., relative humidity.
* The estimated errors for the hybrid samples are generally less than or equal to those of the comparable DNA samples described in ref. 4.
† The two values correspond to assuming a meridional localization for the intensity on layers 11 and 10, respectively, as discussed in the text.

As emphasized by a referee, alternative helical parameters can be assigned for the wet samples of poly(rA)·poly(dT), depending on the indexing of the strong apparently meridional intensity at Bragg spacing of 3.45 Å. Although it appears to be localized on the meridian, this intensity in Fig. 1 *B*, *C*, or *D* is diffuse enough to have a possible nonmeridional origin. Highly solvated samples of poly(rA)·poly(dT) or of DNA such as were used for Fig. 1 *C* or *D* have never shown diffraction beyond the 10th layer when samples were appropriately tilted to allow diffraction at Bragg spacings between 2.8 and 3.5 Å. We therefore have concluded that the most likely interpretation of those patterns is in terms of 10-fold helices. However, the possibility exists that the intensity at 3.45 Å actually is off-meridional in origin and the true meridional reflection is too weak to be detected, perhaps occurring on the 11th or 12th layers and so indicating 11- or 12-fold helices. A possible precedent for such an interpretation was cited by the referee: the three-stranded structure poly(dA)·poly(rU)·poly(rU) (14) is an 11-fold helix with much weaker intensity on the meridional 11th layer than on the off-meridional 10th layer. However, this pattern also has a very strong 9th layer, which is not shown by any of the present samples. Indeed, it is very often the case that when the meridional reflection is weakened due to the tilting of bases, strong off-meridional diffraction results on at least two and sometimes on three of the layers immediately below the meridional layer. For these reasons, we believe that a 10-fold helix best characterizes the diffraction from the immersed samples of either DNA or poly(rA)·poly(dT).

When samples such as in Fig. 1*B* are appropriately tilted, there is weak intensity with a Bragg spacing of about 3.2 Å localized near or on the meridian. If that intensity is taken as truly meridional, such samples should be considered as containing an 11-fold helix. This remains a possible interpretation for this sample.

Model Building and Calculation of Predicted Diffraction Patterns. The diffraction patterns suggest that solvated poly(rA)·poly(dT) can assume a structure similar to that of B DNA. To strengthen this contention, it is critical to show that the hybrid can adopt the proposed conformation in a stereochemically acceptable fashion and that the proposed model gives both a distribution of diffracted intensities and derived helical parameters similar to those observed.

A meridional reflection with a spacing of 3.46 Å would indicate that the bases are not strongly tilted with respect to the helix axis. Accordingly, we started with the base pair coordinates

Table 2. Comparison of the dihedral angles (degrees) in the poly(rA)·poly(dT) model with those in other structures

Angle*	Poly(rA)·poly(dT)† Poly(rA) strand	Poly(rA)·poly(dT)† Poly(dT) strand	A RNA‡	B DNA§
χ (O4′-C1′-N9-C4 or O4′-C1′-N1-C2)	185	208	200	262
ω' (O5′-P-O3′-C3′)	283	324	286	264
ω (C5′-O5′-P-O3′)	272	180	258	314
ϕ (C4′-C5′-O5′-P)	174	96	180	214
ϕ' (P-O3′-C3′-C4′)	214	182	209	155
ψ (C3′-C4′-C5′-O5′)	75	166	48	36
ψ' (O3′-C3′-C4′-C5′)	97	152	83	156

* Conventions are those recommended in ref. 15.
† Taken from the coordinates in Table 3.
‡ Ref. 11.
§ Ref. 10.

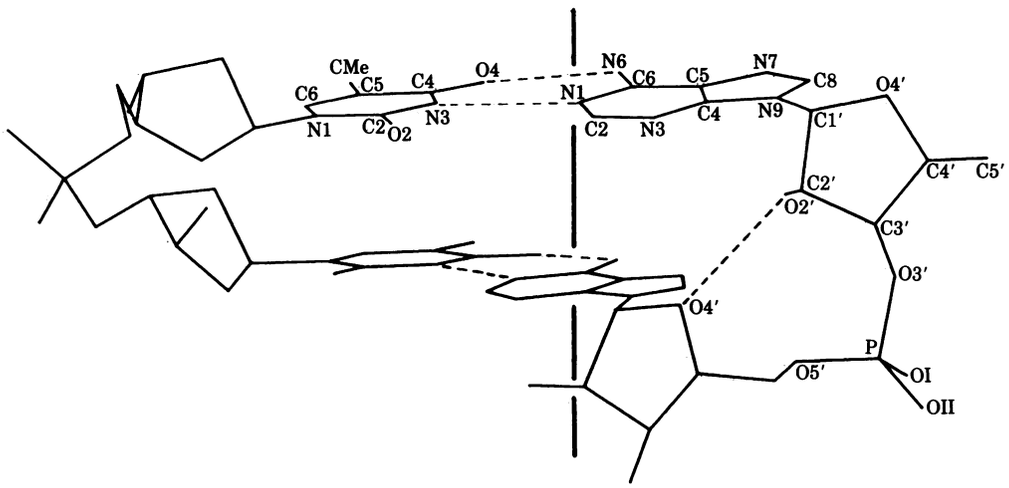

FIG. 2. A model for poly(rA)·poly(dT) in solution. A two-base-pair segment was generated from the coordinates in Table 2. The vertical line is the helix axis. Broken lines represent hydrogen bonds, including the intrastrand hydrogen bond between the 2'-hydroxyl group and the adjacent furanose ring oxygen of the poly(rA) strand, as well as the interstrand Watson–Crick hydrogen bonds.

from a current model for B DNA (10) set at the helical parameters observed for the wet hybrid samples, but with the base pair shifted 1.2 Å further away from the helix axis than in B DNA to relieve cose atomic contacts and to improve the transform.

We consider first the conformation of the backbone in the poly(rA) strand. Because of its 2'-hydroxyl group, this backbone is more constrained stereochemically than is that of the poly(dT) strand. In fact, the C3'-*exo* or C2'-*ndo* sugar conformations previously proposed for B DNA models cannot be accommodated in a model for the poly(rA) strand with the assumed helical parameters. Such conformations cause unacceptably close contacts between the 2'-hydroxyl group and the phosphate group. However, the C3'-*endo* sugar conformation, which is characteristic of polyribonucleotide structures, can be neatly accommodated in a structure with the helical parameters of the wet hybrid. Interestingly, this structure cannot be built for the slightly more cramped conditions of the B form of natural DNA. This result suggests that minor changes in helical parameters of the hybrid from those of B DNA may be to accommodate an RNA backbone into a B DNA-like helix. The dihedral angles at the glycosidic linkage and along the backbone of the poly(rA) strand in the current model are similar to those in current A RNA (11) or A DNA (1) models (Table 2). There is a relatively small range of such angles that do not engender unacceptably close atomic contacts. A striking aspect of the model is that in this range of dihedral angles there is a natural tendency to form a hydrogen bond between the ribose 2φ'·='-hydroxyl group and the furanose ring oxygen of the adjoining sugar in the same chain (Fig. 2). (O—H···O distance and angle are 2.87 Å and 101°, respectively.)

There is no structural reason why the poly(dT) strand could not have the same backbone conformation as does the poly(rA) strand. However, the calculated diffraction pattern for a hybrid helix with two C3'-*endo* backbones (dotted line, Fig. 3) is not in agreement with that observed (heavy bars, Fig. 3). Alternatively, a poly(dT) strand with the C3'-*exo* sugar proposed for B DNA (10) combined with the poly(rA) conformation just described resulted in a model (Table 3) that had a predicted diffraction pattern (solid lines, Fig. 3) fully consistent with the observed pattern (heavy bars, Fig. 3).

DISCUSSION

Poly(rA)·poly(dT) provides a detailed example of a RNA·DNA duplex that has the potential to adopt more than one conformation. In this regard, it is similar to many DNA duplexes.

The diffraction patterns of highly solvated fibers of poly(rA)·poly(dT) and of DNA are clearly similar. The structures in such samples of the hybrid and of DNA correspond to 9.7 and 9.9 residues per helical turn if we assume a meridional localization for the reflections with Bragg spacing of 3.45 Å and 3.34 Å, respectively. We propose a model for the solvated hybrid that has important similarities to those proposed for B DNA. In particular, the hybrid model is a 10-fold helix with its base pairs oriented essentially at right angles to the helix axis. The bases have the tilt and twist values of a current model for B DNA but the base pairs are slightly shifted out from the helix axis. The two strands in the model of the hybrid have different backbone conformations, neither of which is identical to those proposed for B DNA. The poly(dT) strand has a B DNA type of sugar pucker (C3'-*xo*) but has altered backbone conformational angles. The poly(rA) strand has a RNA type of sugar pucker (C3'-*endo*) and backbone conformational angles similar to those in A RNA or A DNA. The computed diffraction of the model accounts well for the observed diffraction pattern.

Hence, the model is adequate—but is it unique? The most obvious alternative structure that must be considered is the three-stranded helix, poly(rA)·poly(dT)·poly(dT), which could potentially arise by disproportionation of the two-stranded helix. A number of mixtures of synthetic polynucleotides, including the poly(rA) + poly(dT) system studied here, have been shown by physical-chemical techniques to form three-stranded structures (e.g., refs. 8, 16–18). Six examples, which do not include the current system, have been analyzed by diffraction methods, so that their helical parameters are known; all are 11- or 12-fold helices (for references see ref. 19). In particular, the DNA analogue of the hybrid under study here, poly(dA)·poly(dT)·poly(dT), forms a 12-fold helix with an axial rise per residue of 3.26 Å (6). Extensive solution studies of the various RNA, DNA, and hybrid systems indicate that the tendency to go from a two-stranded to a three-stranded form is favored as the ionic strength of the medium increases (8, 16–18). The critical ionic strength varies with the polymers involved. For the current system, poly(rA) + poly(dT), Riley *et al.* (8) state that no three-stranded material was detected below 2.5 M NaCl. Accordingly, we have prepared fibers from samples of poly(rA)·poly(dT) that have been precipitated with ethanol and washed extensively with 67% ethanol in order to remove free ions. In addition, attempts were made to form the three-stranded structure poly(rA)·poly(dT)·poly(dT) in order to observe its diffraction patterns. For this purpose, mixtures of the

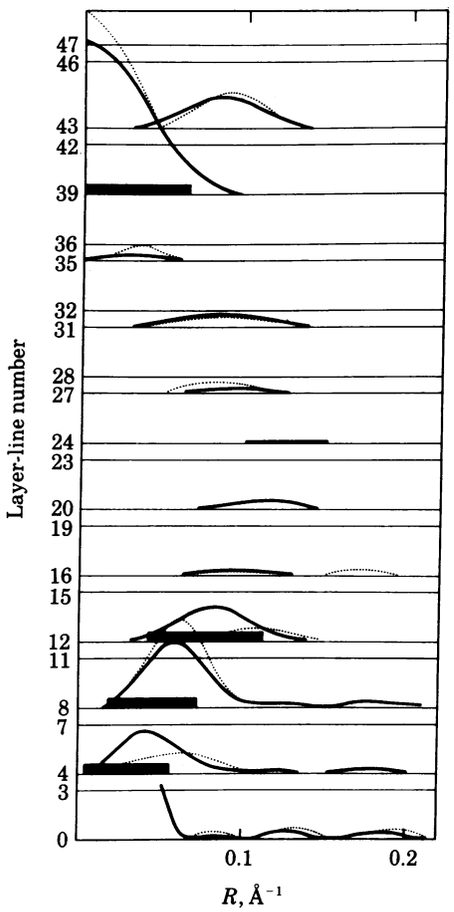

FIG. 3. Comparison of the observed intensities with those calculated for the poly(rA)·poly(dT) model. R, radial distance in reciprocal space. The 9.7 residue per turn helix proposed for wetted samples was approximated as a 39 residue per 4 turn helix for purposes of calculation. The solid bars indicate the positions where strong diffraction occurred. More diffuse intensity consistent with that calculated for layers 31 and 43 was also observed but not quantitated. The heavy lines are the calculated diffraction for the coordinates of Table 3 and represent a hybrid with two different backbone conformations. The dotted lines are the calculated diffraction assuming both strands have the backbone conformation proposed for the poly(rA) strand.

polymers in the stoichiometric ratios appropriate for the three-stranded complex were held at salt concentrations between 0.1 and 5 M NaCl. Fibers formed from such samples [or by the procedure described to isolate poly(dA)·poly(dT)·poly(dT) (6)] uniformly gave the crystalline diffraction pattern of the 12-fold helix shown in Fig. 1A. When such fibers were swollen by immersion in a variety of solvents (0–5 M NaCl; 1 M Tris·HCl, pH 8) invariably the diffraction pattern resembled the B DNA-like pattern in which the ionic strength had been maintained at an extremely low level. It might be argued that the very high polymer and counterion concentrations implicit in forming fibers create a milieu equivalent to that formed at high salt concentrations, so that three-stranded complexes might be favored. However, in related systems in which both two- and three-stranded complexes have been demonstrated [e.g., poly(dA) + poly(dT) (6) or poly(rA) + poly(rU) (20, 21)], simply employing low concentrations of added salt yielded fibers with the two-stranded structures. We conclude that the diffraction pattern from solvated poly(rA)·poly(dT) is not a result of disproportionation to a three-stranded structure.

Is the proposed hybrid structure unique to poly(rA)·poly(dT)? There is particular reason to suspect this hybrid might exhibit a

Table 3. Coordinates of a model for poly(rA)·poly(dT) under highly solvated conditions

Group	Atom	X, Å	Y, Å	Z, Å	r, Å	ϕ, °
		Poly(rA) strand				
Base	N1	1.86	0.55	0.03	1.94	16.5
	C2	3.07	1.10	0.04	3.26	19.7
	N3	3.41	2.37	0.16	4.15	34.8
	C4	2.31	3.14	0.28	3.90	53.7
	C5	1.01	2.73	0.28	2.91	69.7
	C6	0.78	1.35	0.15	1.56	60.0
	N6	−0.44	0.80	0.14	0.91	118.8
	N7	0.15	3.81	0.43	3.81	87.7
	C8	0.95	4.83	0.50	4.92	78.9
	N9	2.27	4.50	0.42	5.04	63.2
Sugar	C1′	3.47	5.44	0.47	6.45	57.5
	C2′	4.25	5.44	−0.89	6.90	52.0
	C3′	3.71	6.75	−1.57	7.70	61.2
	C4′	3.29	7.68	−0.39	8.36	66.8
	C5′	2.11	8.54	−0.54	8.80	76.1
	O2′	5.62	5.45	−0.68	7.83	44.1
	O3′	4.72	7.38	−2.33	8.76	57.4
	O4′	3.01	6.81	0.74	7.45	66.2
	O5′	5.34	5.61	−3.93	7.74	46.4
Phosphate	P	4.70	7.08	−3.92	8.50	56.4
	OI	3.35	6.98	−4.42	7.74	64.4
	OII	5.53	8.10	−4.63	9.81	55.7
		Poly(dT) strand				
Base	N1	2.27	−4.50	−0.42	5.04	−63.2
	C2	2.52	−3.16	−0.27	4.04	−51.4
	O2	3.65	−2.70	−0.18	4.54	−36.5
	N3	1.40	−2.35	−0.23	2.74	−59.2
	C4	0.09	−2.76	−0.32	2.76	−88.1
	O4	−0.84	−1.95	−0.27	2.12	−113.3
	C5	−0.07	−4.19	−0.47	4.19	−91.0
	CMe	−1.48	−4.69	−0.58	4.92	−107.5
	C6	1.00	−5.01	−0.52	5.11	−78.7
Sugar	C1′	3.45	−5.42	−0.46	6.42	−57.5
	C2′	3.92	−5.94	0.94	7.12	−56.6
	C3′	4.33	−7.38	0.62	8.56	−59.6
	C4′	3.35	−7.75	−0.53	8.44	−66.6
	C5′	2.04	−8.42	−0.09	8.66	−76.4
	O3′	5.68	−7.37	0.20	9.31	−52.4
	O4′	3.06	−6.55	−1.23	7.23	−65.0
	O5′	5.96	−6.00	2.21	8.46	−45.2
Phosphate	P	6.70	−7.06	1.31	9.73	−46.5
	OI	7.04	−8.28	2.08	10.9	−49.6
	OII	7.90	−6.35	0.75	10.1	−38.8

These coordinates are for one base pair. Corresponding atoms in the next residue are related to those listed above by a translation of Z = 3.46 Å and a rotation of ϕ = 37.0°.

B DNA-like conformation, because the corresponding DNA·DNA duplex, poly(dA)·poly(dT), retains a B DNA-like conformation under humidity conditions that generally induce DNA to undergo a transition to the A form (6, 7).† However, there is no *a priori* reason why other DNA·RNA or RNA·RNA duplexes could not assume conformations related to that proposed for solvated poly(rA)·poly(dT). A survey of several other hybrids and RNA duplexes under highly solvated conditions has as yet yielded no clear examples of B DNA-like diffraction patterns from other polyribonucleotide-containing structures.

† Fibers of poly(dA)·poly(dT) also retain a B conformation when immersed in 80% (vol/vol) ethanol (unpublished observations), a treatment that rapidly converts natural DNA to the A conformation.

There is little evidence from the literature to indicate that hybrids have the potential to adopt other than RNA-like conformations. An important observation in this regard was made by Gray and Ratliff (22). They demonstrated that the circular dichroism spectra of two synthetic hybrids, poly(rA-rC)·poly(dG-dT) and poly(dA-dC)·poly(rG-rU), were different both from each other and from the circular dichroism spectra of the corresponding DNA·DNA and RNA·RNA duplexes. Upon addition of 60–80% ethanol, the circular dichroism spectra of both hybrids and of the DNA·DNA duplex approached the spectrum of the RNA·RNA duplex, which did not change. They concluded that hybrids are not restricted to RNA-like conformations in the absence of ethanol. A laser Raman study of poly(rA)·poly(dT) solutions was not inconsistent with the present model (23). A band associated with RNA conformations was noted, while another band (795 cm^{-1}) may represent a contribution from a non-RNA conformation. The region of the spectrum most clearly associated with the B conformation (835 cm^{-1}) was not sufficiently resolved to be evaluated.

On the other hand, there are a number of studies, including two diffraction analyses, which indicate that RNA·DNA hybrids can adopt RNA-like conformations. The diffraction pattern of poly(rI)·poly(dC) (at 75% relative humidity) is very similar to that of poly(rA)·poly(dT) (Fig. 1A) at a similar humidity value; this pattern was interpreted as a 12-fold helix of basically an RNA-like conformation (2). In a diffraction study of a DNA·RNA hybrid of mixed sequence, Milman *et al.* (1) obtained A form (RNA-like) patterns between 33% and 92% relative humidity. With both materials used in these diffraction studies, it is possible that an increase in the relative humidity or salt concentration of the fibers might have allowed such hybrids to adopt conformations other than the A or A′ conformations that were observed. The optical rotatory dispersion spectrum of the hybrid studied by Milman *et al.* was shown by Tunis and Hearst (24) to be relatively RNA-like, indicating that the RNA-like conformation of a mixed sequence hybrid might be retained in solution. Recently, Selsing *et al.* (25) have made model duplexes by annealing $(rC)_{11}$-$(dC)_{16}$ with poly(dG). These duplexes were designed to contain a short length of hybrid covalently joined to a short length of DNA-like duplex. The NMR spectrum of the complex is consistent with the presence of similar amounts of duplex in the A and B forms. This interpretation, of course, suggests that hybrids of such base sequence are present in an RNA conformation in solution. Unfortunately, comparable studies with other sequences are not available.

Overall, the evidence for the generality of a non-RNA type of conformation for RNA·DNA hybrids or RNA·RNA duplexes is incomplete at this point. It is consistent with the hypothesis that such a conformation is adopted only by hybrids possessing certain kinds of specific sequences, whereas other kinds of sequences may adopt a RNA-like conformation. There is an analogy in DNA duplexes, in which poly(dA)·poly(dT) does not adopt an A conformation under conditions in which natural DNA and certain other restricted sequence DNAs do adopt the A form.

Finally, we briefly consider some implications of our model for hybrid sequences. First, hybrid duplexes are joined to DNA duplexes at sites of initiation of DNA replication. A model has been proposed (26) for such a junction, assuming an A conformation for the hybrid and a B conformation for the DNA. This difference in conformations resulted in a striking structural discontinuity in the model at the juncture. In contrast, where hybrids adopt a B DNA-like conformation as proposed here, such a discontinuity would not occur. Second, the extra stability associated with RNA duplexes relative to DNA duplexes (9, 17) may be related to intrachain hydrogen bonds of the type proposed for the poly(rA) strand of the current model, in accord with earlier suggestions (27, 28). Last, it seems likely that the secondary structure of hybrid duplexes will turn out to have important functional consequences in transcription and replication. The ability of hybrids to adopt multiple conformations offers a potential means of control of such processes.

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