Volume 3 no.10 October 1976 Nucleic Acids Research

Models of triple-stranded polynucleotides with optimised stereochemistry

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Received 28 May 1976

ABSTRACT

Detailed models are presented for the triple-stranded polynucleotide helices of poly(U)·poly(A)·poly(U) (two forms), poly(U)·poly d(A)·poly(U), poly d(C)·poly d(I)·poly d(C), poly d(T)·poly d(A)·poly d(T) and poly(I)·poly(A)·poly(I). The models were generated using a computerized, linked-atom procedure which preserves standard bond lengths, bond angles and sugar ring conformations, constrains the helices to have the pitches and symmetries observed in X-ray diffraction experiments, and optimises the non-bonded interatomic contacts including hydrogen bonds. The possible biological significance of such complexes is discussed.

INTRODUCTION

Triple-stranded complexes containing three coaxial helical chains connected by hydrogen-bonded bases can be formed from a variety of simple polynucleotides. In cases like poly d(T)·poly d(A)·poly d(T) two of the chains are hydrogen-bonded in the Watson-Crick manner: therefore local segments of a native DNA could participate in such complexes and it is of some interest to determine the effect of involving a third strand with such a duplex. Since, in the case mentioned, the second poly d(T) chain cannot be involved in Watson-Crick pairing, and in other cases like poly(I)·poly(A)·poly(I) none of base-pairings can be of the standard kind, it is also of interest to determine the conformational consequences of having unusual base-base hydrogen bonds.

Although these complexes are, in many cases, susceptible to the same general methods of structure analysis as the more familiar double-stranded forms, several factors combine to make the extension of the technique problematic. Earlier determinations of nucleic acid structures[3,4] have used a linked-atom

description of the structure, that is, one in which bond lengths and angles are fixed and only the bond torsion angles are treated as explicit variables.[5] This reduction of the number of parameters in the description allows the optimisation of the fit between the predicted X-ray diffraction of the model and the quite limited number of observed data. In triple-stranded complexes, however, there no longer exists the simplification allowed by the symmetry between the strands of a double helix: the three strands each have different base hydrogen bonds and one cannot assume even that the sugar-phosphate backbones of the two strands joined by Watson-Crick base pairing have the same conformation. Coupled with this three-fold increase in the number of parameters to be determined is a scarcity of quantifiable X-ray diffraction data, caused by limited ordering in the samples studied.

To overcome these problems we must increasingly use stereochemical information obtained from simpler systems. One well-established and powerful constraint of this sort is the avoidance, where possible, of over-short interatomic distances between non-bonded atoms. This paper describes the use of a computer program to use this sort of information to predict probable molecular structures with standard bond lengths and angles for six triple-stranded polynucleotides. All but one of these have provided sufficient X-ray data for earlier analyses,[3,4,8,9] thus affording a basis for assessment of the present method. Since nucleic acid systems of current biological interest are more complicated than the duplex structures that have received most attention heretofore it may be that the new strategy we will describe could form the basis for accurate modelling of those complex systems.

METHOD AND PROCEDURE

The linked-atom least-squares procedure (LALS) has been described in detail earlier.[3,4,5,6,7] Briefly, it involves analytic minimisation of

$$\Phi = \sum_j k_j \delta_j^2 + \sum_h \lambda_h G_h \qquad (1)$$

The first summation includes several classes of terms which are, together, to be minimised. The k_j are weights and the δ_j are differences between model values and standard (i.e. known

or observed) values of various structural features. In the case of interatomic contacts (a contact being a separation *less* than the standard value), the standard values and weights are derived in advance by fitting a non-bonded van der Waals energy function.[6] Where hydrogen bonds are known or suspected to exist, the standard value for calculating δ_j is reduced to the known length of the hydrogen bond, whilst the original standard value is still used for determining whether the contact exists.[6]

These terms, therefore, tend to drive apart atoms which are too close and to make hydrogen bonds close to standard lengths.

Other terms in the first summation include recognition of the known relationship that the angle (γ) between the helix axis and the normal to the plane of the bases in a polynucleotide bears to the projected height (h) of a residue on the helix axis.[10]

The parameters varied in order to minimise Φ are, in the present case, primarily single bond torsion angles. There are also some geometrical parameters of no immediate physical significance needed to orient and position the structure and to allow sufficient flexibility to the hydrogen bonds between the strands.

The second summation in equation 1 is of linear Lagrange constraints ($G_h = 0$) on the parameters necessary to maintain the observed helical symmetry. The λ_i are undetermined multipliers.

In each minimisation, consequently, 32 parameters were varied, upon which 27 constraints were applied of which 9 were linearly dependent on others, leaving 14 net degrees of freedom. The number of data used to determine these in this type of minimisation is not an obviously obtainable number, since one has to arbitrarily decide whether the lack of short contacts constitutes data, and if so, at what interatomic separation such data cease to be significant. The number of *non-zero* contributions to equation 1 was typically around 100 to 300, but it must be stressed that conventional considerations concerning the data-to-parameter ratio are not necessarily valid in this case, and a more appropriate value for 'number of

data', bearing in mind the desirability of a balanced distribution of δ_j, might be several times greater.

Six complexes, listed in table 1, were analysed in the present study. Five of these have been investigated previously using other data and methods[3,4,8,9] and have been shown to adopt similar hydrogen-bonding schemes, in which the third strand occupies what would be the major groove of an *A*-type polynucleotide duplex, and participates in Hoogsteen-type interactions with one of the two Watson-Crick linked strands (Figure 1). This, and the standard C3-*endo* sugar ring conformation[11] observed in all polynucleotides of the *A* genus, were assumed for the present study. Each of the six can be oriented sufficiently to show X-ray diffraction which yields the helix symmetry and the helix pitch, and hence the turn angle (t) and projected residue height (h). (Table 1).

Table I: properties of the systems studied.

Complex	Abbreviation	Helix Symmetry	Pitch (nm)	Turn angle t (°)	Residue height h (nm)	Base skew γ (°)
Poly(U)·poly(A)·poly(U)	UAU-11	11_1	3.34	32.7	0.304	12.0
Poly(U)·poly(A)·poly(U)	UAU-12	12_1	3.65	30.0	0.304	12.0
Poly(U)·poly d(A)·poly(U)	UdAU	11_1	3.34	32.7	0.304	12.0
Poly d(C)·poly d(I)·poly d(C)	dCdIdC	11_1	3.48	32.7	0.316	10.0
Poly d(T)·poly d(A)·poly d(T)	dTdAdT	12_1	3.91	30.0	0.326	8.5
Poly(I)·poly(A)·poly(I)	IAI	12_1	3.95	32.7	0.329	8.0

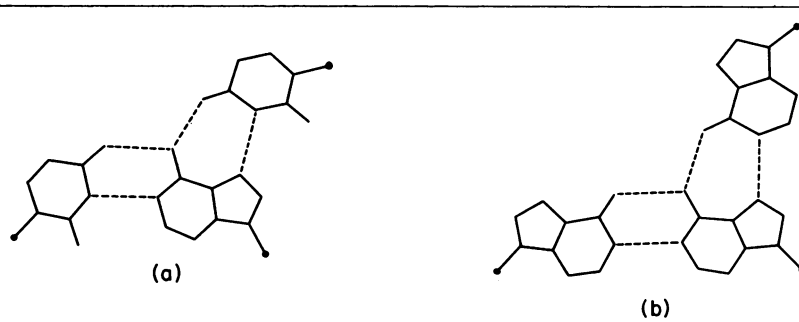

Figure 1: Triplex hydrogen-bonding schemes: (a) pyrimidine:purine:pyrimidine, (b) poly(I)·poly(A)·poly(I).

For each complex, six torsion angles in each strand were varied (Figure 2). Five of these define the sugar-phosphate backbone conformation, and the sixth is about the sugar-base bond.

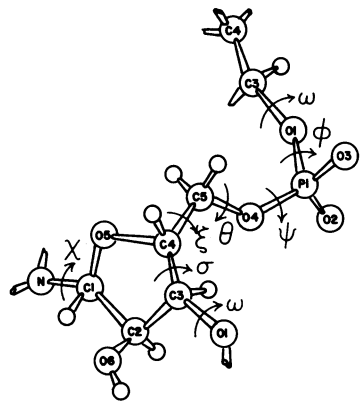

Figure 2: Atom and conformation angle nomenclature for a nucleotide residue. It should be noted that the above nomenclature we use differs from that in recent work, to correspond with that in the earlier studies considered in this paper. The conformation angles shown are defined as

ω = θ[C4-C3-O1-P1]
ϕ = θ[C3-O1-P1-O4]
ψ = θ[O1-P1-O4-C5]
θ = θ[P1-O4-C5-C4]
ξ = θ[O4-C5-C4-C3]
χ = θ[C2-C1-N-(C2pyr,C4pur)]

RESULTS AND DISCUSSION

In Table II are shown the values obtained for the varied torsion angles, and the differences between these and those obtained in the earlier studies. Although there are in some cases moderately large differences, it must be realised that the earlier structures were derived from quite limited numbers of data, supplemented (in most cases) by only statistical information concerning probable chain conformation angles.

Table III lists the hydrogen-bond lengths of the present models: none of these is exceptional.

Table IV shows the remaining short contacts in the present models. These are the interatomic distances between pairs of atoms not bonded (or hydrogen-bonded) to each other or to a common third atom, more than 0.02nm less than the sum of the

Table II: varied conformation angles (degrees) and differences from earlier studies.

Complex	ω	φ	ψ	θ	ξ	χ	Δω	Δφ	Δψ	Δθ	Δξ	Δχ
poly(U)·	-152	-74	-55	173	47	83	13	-15	4	-16	3	-3
poly(A)·	-156	-69	-76	176	64	89	-2	9	16	-4	-16	13
poly(U)	-149	-76	-63	174	53	83	-25	29	-23	33	2	17
poly(U)·	-156	-75	-28	171	24	87	16	-12	6	0	-3	4
poly(A)·	-163	-67	-66	-179	53	91	5	-16	22	4	-23	12
poly(U)	-149	-83	-40	167	37	85	-5	-6	-10	12	3	10
poly(U)·	-152	-74	-57	172	49	83	15	-18	2	-16	7	-1
poly(dA)·	-160	-67	-71	178	58	92	-6	9	22	-3	-23	17
poly(U)	-149	-76	-62	174	53	84	-31	24	-4	32	-13	-21
poly(dC)·	-155	-70	-61	176	51	83						
poly(dI)·	-151	-72	-82	173	72	84						
poly(dC)	-153	-72	-65	178	54	84						
poly(dT)·	-156	-74	-46	174	39	86	12	-13	17	-12	-8	-3
poly(dA)·	-161	-68	-66	180	55	91	3	-2	-16	1	15	-1
poly(dT)	-160	-77	-53	172	47	85	-5	-1	-5	0	4	-1
poly(I)·	-152	-77	-57	171	52	84	8	-11	16	-12	-11	4
poly(A)·	-154	-75	-62	173	55	85	6	-9	11	-10	-8	5
poly(I)	-153	-75	-70	177	60	80	10	-14	-13	-11	15	-3

The left hand part of this table lists the final values of the conformation angles in the present study. The right hand part lists the differences between these values and the earlier studies cited in the text. It should be noted that the earlier studies used a variety of different methods and data and that comparisons must be made with this in mind.

Table III: hydrogen-bond lengths (nm). For notation see Table IV.

	UAU-11	UAU-12	UdAU	dCdIdC	dTdAdT	IAI
N1 A - N3 W'	0.28	0.28	0.28	0.28	0.28	-
N1 A - N1 W	-	-	-	-	-	0.28
N6 A - O4 W	0.30	0.29	0.30	-	0.31	-
N6 A - O6 W	-	-	-	-	-	0.30
O6 A - N4 W	-	-	-	0.29	-	-
N7 A - N3 H	0.30	0.29	0.30	0.30	0.30	-
N7 A - N1 H	-	-	-	-	-	0.29
N6 A - O4 H	0.27	0.29	0.27	-	0.29	-
O6 A - N4 H	-	-	-	0.27	-	-
N6 A - O6 H	-	-	-	-	-	0.29

van der Waals radii of the two atoms. The bracketed figures show these differences. Also shown are the final values (Σ) of the first summation of equation 1: these are on an arbitrary scale but show the relative stereochemical acceptability of the different structures.

Clearly the 12-fold poly(U)·poly(A)·poly(U) model is much less satisfactory than the others. This structure has always proved awkward to modelbuild using standard assumptions, and it may well be that some distortion of the sugar rings from the standard C3-*endo* shape is present in this system.

Of the other models a common feature of all except the all-

Table IV: Short contacts (nm) in the models. A indicates the central strand, W the Watson-Crick paired strand and H the Hoogsteen strand. The letters c, s and b signify chain, sugar and base atoms. Asterisks indicate atoms in the next (towards +z) residue.

	UAU-11	UAU-12	UdAU	dCdIdC	dTdAdT	IAI
W-strand						
C3 c W - O4 c W		0.27(0.03)				
C5 c W - O1 c W		0.27(0.03)				
O4 c W - HC6 b W			0.23(0.02)	0.23(0.02)		
C2 s W - C5 s W*		0.28(0.04)				
C2 s W - HC5 s W*		0.19(0.08)			0.24(0.03)	
O5 s W - C6 b W	0.27(0.03)	0.27(0.03)	0.27(0.03)	0.27(0.03)	0.27(0.03)	
O6 s W - C5 c W*		0.25(0.05)				
O6 s W - HC5 c W*	0.22(0.03)	0.15(0.10)	0.23(0.02)			
HC2 s W - C6 b W*	0.25(0.02)	0.25(0.02)	0.24(0.03)		0.19(0.03)	
HC2 s W - HC6 b W*	0.19(0.03)	0.18(0.04)	0.19(0.03)	0.19(0.03)		
A-strand/H-strand						
O2 c A - O2 b H	0.23(0.05)	0.23(0.05)	0.23(0.05)	0.26(0.02)	0.23(0.05)	
O5 s H - O2 c A*		0.25(0.03)				
H-strand						
O4 c H - HC6 b H	0.22(0.03)		0.22(0.03)	0.22(0.03)	0.23(0.02)	
HC5 s H - O6 s H*		0.21(0.04)				
O5 s H - C6 b H	0.27(0.03)	0.27(0.03)	0.27(0.03)	0.27(0.03)	0.27(0.03)	
HC2 s H - C6 b H		0.24(0.03)				
HC2 s H - HC6 b H		0.19(0.03)				
A-strand						
HC5 c A - O6 s A*		0.21(0.04)				
Σ	153	242	150	133	161	111

purine poly(I)·poly(A)·poly(I) is the contact between the phosphate O2 on the A strand and the base O2 on the H strand. Although this too may be an artefact of the standard assumptions, it has been shown that this interaction contributes to the anomalous infra-red spectrum from the base oxygen atoms of triple-stranded complexes,[12] and it may therefore be at least partly a real feature of these molecules.

Generally, however, these models are stereochemically acceptable (with the exception of 12-fold poly(U)·poly(A)·poly(U)): much more so than those refined against X-ray data, whilst differing not greatly from the latter. We therefore conclude that this method is a valid and useful one for constructing such models.

Atomic coordinates of all six models are given in Table V, and a view perpendicular to the helix axis of a typical structure is shown in Figure 3.

We would further predict that models similar to those we have built for the pyrimidine:purine:pyrimidine systems would be possible and indeed probable for any such complexes. None of the DNA strands appears to have any serious interactions at the site of the ribose O6 atom, and it would seem therefore

Poly(U)·Poly(dA)·Poly(U)
(11-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.1294	-.9204	.0701	.9295	-82.00	.1579	.9458	-.0327	.9589	80.52	-.8224	.4595	-.2509	.9421	150.81
P	.1392	-.8654	.2200	.8765	-80.86	.1818	.9011	-.1845	.9193	78.60	-.7538	.4445	-.3946	.8751	149.47
O2	.1545	-.9786	.3140	.9908	-81.03	.0635	.8268	-.2335	.8293	85.61	-.8477	.4894	-.4998	.9789	150.00
O3	.0259	-.7740	.2469	.7744	-88.08	.2223	1.0185	-.2650	1.0424	77.69	-.7002	.3075	-.4102	.7647	156.29
O4	.2754	-.7817	.2141	.8288	-70.59	.3057	.8012	-.1689	.8575	69.11	-.6328	.5485	-.3828	.8374	139.08
C1	.5218	-.5293	.0673	.7433	-45.41	.5251	.5180	-.0524	.7376	44.61	-.3318	.7201	-.2277	.7929	114.74
C2	.5937	-.5300	.2018	.7959	-41.76	.6052	.5236	-.1820	.8003	40.87	-.2982	.7794	-.3641	.8345	110.94
C3	.5273	-.6487	.2714	.8360	-50.89	.5540	.6535	-.2442	.8567	49.71	-.4244	.7466	-.4438	.8587	119.61
C4	.5079	-.7469	.1559	.9032	-55.78	.5358	.7427	-.1214	.9159	54.19	-.5341	.7653	-.3390	.9332	124.91
C5	.3965	-.8481	.1732	.9362	-64.95	.4351	.8550	-.1356	.9593	63.03	-.6623	.6881	-.3631	.9550	133.91
O5	.4764	-.6614	.0421	.8151	-54.23	.4899	.6511	-.0178	.8148	53.04	-.4731	.7179	-.2154	.8598	123.39
O6	.7302	-.5572	.1754	.9185	-37.35	--	--	--	--	--	-.2850	.9193	-.3462	.9625	107.23
N1	.4038	-.4385	.0630	.5961	-47.36	.3118	.0548	.0077	.3165	9.97	-.2818	.5810	-.2089	.6457	115.87
C2	.4281	-.3055	.0385	.5259	-35.51	.4356	.1003	.0260	.4470	12.96	-.1512	.5664	-.1688	.5862	104.95
N3	.3163	-.2244	.0352	.3879	-35.36	.4830	.2217	.0082	.5314	24.65	-.1084	.4361	-.1524	.4494	103.96
C4	.1856	-.2644	.0540	.3230	-54.94	.3860	.3052	-.0344	.4921	38.33	-.1836	.3221	-.1723	.3708	119.69
C5	.1702	-.4058	.0790	.4400	-67.25	.2551	.2745	-.0579	.3747	47.10	-.3194	.3476	-.2142	.4721	132.58
C6	.2771	-.4869	.0827	.5602	-60.35	.2155	.1417	-.0356	.2579	33.32	-.3636	.4733	-.2309	.5969	127.53
N7	--	--	--	--	--	.1859	.3869	-.1009	.4292	64.34	--	--	--	--	--
C8	--	--	--	--	--	.2756	.4805	-.1020	.5539	60.16	--	--	--	--	--
N9	--	--	--	--	--	.3995	.4385	-.0630	.5932	47.66	--	--	--	--	--
N6	--	--	--	--	--	.0903	.0976	-.0545	.1329	47.21	--	--	--	--	--
O2	.5401	-.2608	.0207	.5997	-25.78	--	--	--	--	--	-.0770	.6610	-.1487	.6655	96.65
O4	.0936	-.1824	.0487	.2051	-62.84	--	--	--	--	--	-.1343	.2105	-.1544	.2497	122.53

Poly(dT)·Poly(dA)·Poly(dT)
(12-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.1823	-.9346	.0544	.9522	-78.96	.2770	.9669	-.0185	1.0058	74.01	-.7287	.6505	-.1657	.9768	138.25
P	.1814	-.8757	.2031	.8943	-78.30	.2883	.9182	-.1705	.9624	72.57	-.6757	.6176	-.3130	.9154	137.57
O2	.1905	-.9865	.3008	1.0047	-79.07	.1618	.8530	-.2112	.8683	79.26	-.7656	.6787	-.4134	1.0232	138.44
O3	.0662	-.7843	.2197	.7870	-85.18	.3333	1.0307	-.2555	1.0833	72.08	-.6533	.4719	-.3265	.8060	144.16
O4	.3174	-.7914	.2047	.8527	-68.15	.4046	.8088	-.1601	.9043	63.43	-.5349	.6937	-.3137	.8760	127.64
C1	.5722	-.5278	.0740	.7784	-42.69	.6079	.5064	-.0572	.7912	39.80	-.1895	.7881	-.1757	.8106	103.52
C2	.6300	-.5354	.2149	.8268	-40.36	.6803	.5105	-.1913	.8506	36.88	-.1573	.8411	-.3150	.8557	100.59
C3	.5570	-.6571	.2714	.8614	-49.72	.6350	.6456	-.2465	.9056	45.47	-.2949	.8387	-.3813	.8891	109.37
C4	.5494	-.7494	.1498	.9292	-53.75	.6307	.7319	-.1204	.9662	49.25	-.3867	.8792	-.2659	.9605	113.74
C5	.4369	-.8509	.1507	.9565	-62.82	.5376	.8514	-.1254	1.0070	57.73	-.5506	.8328	-.2766	.9875	122.50
O5	.5297	-.6582	.0378	.8449	-51.18	.5845	.6405	-.0168	.8671	47.62	-.3258	.8170	-.1490	.8796	111.74
N1	.4551	-.4363	.0622	.6305	-43.79	.3666	.0562	-.0040	.3709	8.72	-.1701	.6410	-.1616	.6632	104.86
C2	.4816	-.3032	.0408	.5692	-32.19	.4944	.0921	.0071	.5029	10.56	-.0434	.5973	-.1313	.5989	94.16
N3	.3708	-.2216	.0305	.4319	-30.87	.5490	.2108	-.0081	.5881	21.01	-.0293	.4605	-.1191	.4614	93.64
C4	.2388	-.2611	.0394	.3539	-47.55	.4555	.3029	-.0393	.5470	33.63	-.1289	.3662	-.1343	.3882	109.39
C5	.2211	-.4026	.0619	.4594	-61.23	.3214	.2825	-.0543	.4279	41.32	-.2583	.4217	-.1661	.4945	121.49
C6	.3272	-.4843	.0724	.5845	-55.96	.2740	.1517	-.0356	.3132	28.97	-.2749	.5544	-.1785	.6188	116.37
N7	--	--	--	--	--	.2575	.4015	-.0864	.4770	57.33	--	--	--	--	--
C8	--	--	--	--	--	.3535	.4887	-.0897	.6031	54.12	--	--	--	--	--
N9	--	--	--	--	--	.4765	.4364	-.0622	.6461	42.48	--	--	--	--	--
N6	--	--	--	--	--	.1449	.1173	-.0473	.1865	38.98	--	--	--	--	--
O2	.5948	-.2589	.0315	.6487	-23.52	--	--	--	--	--	.0509	.6730	-.1159	.6749	85.67
O4	.1478	-.1787	.0285	.2319	-50.40	--	--	--	--	--	-.1041	.2461	-.1209	.2672	112.92
Me	.0806	-.4541	.0729	.4612	-79.93	--	--	--	--	--	-.3723	.3260	-.1843	.4949	138.80

Poly(I)·Poly(A)·Poly(I)
(12-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.0535	-.9875	.0274	.9889	-86.90	.0721	.9976	-.0206	1.0002	85.87	-.9938	.2225	-.1615	1.0184	167.38
P	.0604	-.9378	.1794	.9398	-86.32	.0825	.9498	-.1729	.9533	85.03	-.9282	.2292	-.3073	.9561	166.13
O2	.0602	-1.0547	.2702	1.0564	-86.73	-.0267	.8544	-.2026	.8548	91.79	-1.0341	.2481	-.4089	1.0635	166.51
O3	-.0453	-.8371	.2036	.8383	-93.10	.0910	1.0679	-.2616	1.0718	85.13	-.8396	.1124	-.3274	.8471	172.38
O4	.2038	-.8671	.1831	.8908	-76.77	.2228	.8728	-.1723	.9008	75.68	-.8400	.3622	-.2962	.9148	156.67
C1	.4751	-.6330	.0558	.7915	-53.11	.4871	.6297	-.0530	.7962	52.28	-.5918	.6149	-.1706	.8534	133.90
C2	.5451	-.6462	.1907	.8454	-49.85	.5590	.6430	-.1868	.8520	49.00	-.5845	.6814	-.3077	.8977	130.62
C3	.4675	-.7613	.2545	.8934	-58.45	.4855	.7613	-.2496	.9030	57.47	-.7004	.6139	-.3808	.9313	138.77
C4	.4406	-.8521	.1344	.9592	-62.66	.4599	.8510	-.1284	.9673	61.61	-.8047	.6003	-.2698	1.0039	143.28
C5	.3203	-.9435	.1464	.9964	-71.25	.3425	.9461	-.1402	1.0062	70.10	-.9066	.4896	-.2881	1.0304	151.63
O5	.4183	-.7592	.0244	.8668	-61.14	.4337	.7570	-.0201	.8724	60.19	-.7256	.5723	-.1506	.9242	141.74
O6	.6789	-.6844	.1641	.9640	-45.23	.6936	.6768	-.1581	.9691	44.30	-.6109	.8192	-.2880	1.0219	126.71
N1	.3603	-.1444	.0061	.3881	-21.84	.3566	.1356	-.0034	.3815	20.82	-.1210	.4532	-.0889	.4691	104.95
C2	.4811	-.2065	.0010	.5235	-23.23	.4733	.1993	.0036	.5136	22.84	-.1698	.5799	-.0952	.6043	106.32
N3	.4996	-.3348	.0153	.6014	-33.83	.4989	.3275	-.0104	.5968	33.29	-.2947	.6108	-.1171	.6781	115.76
C4	.3838	-.4006	.0361	.5547	-46.23	.3857	.3966	-.0349	.5532	45.80	-.3717	.5013	-.1332	.6240	126.56
C5	.2601	-.3436	.0422	.4310	-52.88	.2591	.3466	-.0451	.4327	53.23	-.3283	.3722	-.1279	.4963	131.41
C6	.2380	-.2075	.0273	.3158	-41.08	.2434	.2081	-.0284	.3203	40.52	-.1961	.3371	-.1048	.3899	120.18
N7	.1637	-.4396	.0647	.4691	-69.57	.1685	.4486	-.0711	.4792	69.41	-.4330	.2850	-.1486	.5184	146.64
C8	.2333	-.5532	.0717	.6004	-67.13	.2421	.5553	-.0757	.6058	66.45	-.5380	.3655	-.1659	.6504	145.81
N9	.3658	-.5318	.0547	.6455	-55.47	.3749	.5318	-.0547	.6506	54.82	-.5031	.4959	-.1570	.7065	135.41
N6	--	--	--	--	--	.1251	.1455	-.0358	.1919	49.33	--	--	--	--	--
O6	.1335	-.1435	.0304	.1960	-47.08	--	--	--	--	--	-.1437	.2264	-.0975	.2682	122.41

Table V: Atomic coordinates for triple-stranded polynucleotides. For each complex the W strand is to the left, the A strand in the middle and the H strand to the right. Atoms are named as in Arnott[5]. Coordinates of atoms in successive residues may be generated by applying to the listed coordinates a translation of h along and a rotation of t about the helix axis, z.

The accuracy of these coordinates is not necessarily that

Poly(U)·Poly(A)·Poly(U)
(11-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.0844	-.9186	.0808	.9225	-84.75	.2544	.9419	-.0427	.9757	74.89	-.7653	.5500	-.2576	.9425	144.30
P	.0962	-.8610	.2297	.8664	-83.63	.2728	.8937	-.1941	.9344	73.02	-.6972	.5269	-.4004	.8739	142.92
O2	.1035	-.9731	.3261	.9785	-83.93	.1482	.8292	-.2413	.8423	79.87	-.7847	.5809	-.5069	.9763	143.49
O3	-.0114	-.7622	.2535	.7623	-90.86	.3224	1.0062	-.2764	1.0566	72.23	-.6584	.3848	-.4146	.7626	149.69
O4	.2374	-.7862	.2236	.8212	-73.20	.3880	.7836	-.1784	.8744	63.66	-.5658	.6174	-.3879	.8375	132.50
C1	.5009	-.5503	.0747	.7441	-47.69	.5815	.4879	-.0568	.7591	40.00	-.2493	.7578	-.2324	.7977	108.21
C2	.5696	-.5541	.2108	.7947	-44.21	.6645	.4802	-.1846	.8198	35.85	-.2087	.8116	-.3692	.8380	104.42
C3	.4944	-.6677	.2799	.8308	-53.48	.6264	.6108	-.2539	.8749	44.28	-.3372	.7915	-.4494	.8604	113.07
C4	.4714	-.7656	.1648	.8991	-58.38	.6137	.7072	-.1358	.9364	49.05	-.4450	.8230	-.3457	.9355	118.40
C5	.3536	-.8596	.1805	.9295	-67.64	.5237	.8271	-.1578	.9789	57.66	-.5805	.7596	-.3699	.9560	127.39
O5	.4479	-.6795	.0496	.8139	-56.61	.5576	.6250	-.0293	.8376	48.26	-.3902	.7707	-.2211	.8639	116.85
O6	.7047	-.5900	.1877	.9191	-39.94	.8006	.4818	-.1454	.9344	31.04	-.1809	.9496	-.3525	.9666	100.79
N1	.3888	-.4524	.0669	.5965	-49.32	.3300	.0443	.0035	.3329	7.64	-.2145	.6143	-.2118	.6507	109.25
C2	.4219	-.3214	.0418	.5304	-37.30	.4574	.0791	.0212	.4641	9.81	-.0858	.5862	-.1727	.5925	98.33
N3	.3155	-.2336	.0353	.3926	-36.51	.5147	.1960	.0034	.5508	20.85	-.0573	.4523	-.1546	.4559	97.22
C4	.1822	-.2652	.0514	.3217	-55.51	.4250	.2876	-.0385	.5131	34.09	-.1446	.3469	-.1719	.3758	112.63
C5	.1574	-.4050	.0773	.4346	-68.76	.2918	.2682	-.0613	.3963	42.59	-.2773	.3864	-.2131	.4756	125.67
C6	.2590	-.4926	.0841	.5566	-62.26	.2412	.1391	-.0390	.2785	29.97	-.3078	.5159	-.2314	.6007	120.82
N7	--	--	--	--	--	.2321	.3861	-.1036	.4505	58.99	--	--	--	--	--
C8	--	--	--	--	--	.3295	.4718	-.1051	.5754	55.07	--	--	--	--	--
N9	--	--	--	--	--	.4496	.4193	-.0669	.6148	43.01	--	--	--	--	--
N6	--	--	--	--	--	.1126	.1059	-.0573	.1545	43.23	--	--	--	--	--
O2	.5369	-.2839	.0262	.6073	-27.87	--	--	--	--	--	-.0017	.6725	-.1548	.6725	90.14
O4	.0956	-.1777	.0433	.2018	-61.71	--	--	--	--	--	-.1075	.2309	-.1527	.2547	114.96

Poly(U)·Poly(A)·Poly(U)
(12-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.1493	-.9219	.0955	.9340	-80.80	.3260	.9729	-.0532	1.0261	71.48	-.6637	.7118	-.2314	.9732	132.99
P	.1504	-.8593	.2427	.8723	-80.07	.3426	.9208	-.2036	.9824	69.59	-.6113	.6698	-.3767	.9069	132.39
O2	.1447	-.9677	.3432	.9785	-81.50	.2155	.8604	-.2495	.8870	75.94	-.7002	.7270	-.4803	1.0094	133.92
O3	.0453	-.7556	.2534	.7570	-86.57	.3967	1.0295	-.2881	1.1033	68.93	-.5919	.5232	-.3821	.7900	138.52
O4	.2946	-.7901	.2465	.8432	-69.55	.4532	.8066	-.1854	.9252	60.67	-.4692	.7430	-.3815	.8788	122.27
C1	.5620	-.5330	.0904	.7745	-43.48	.6412	.4979	-.0683	.8118	37.83	-.1235	.8110	-.2117	.8203	98.66
C2	.6144	-.5436	.2332	.8203	-41.50	.7158	.4963	-.2013	.8710	34.74	-.0829	.8574	-.3512	.8614	95.52
C3	.5305	-.6588	.2882	.8459	-51.15	.6769	.6319	-.2600	.9260	43.03	-.2179	.8622	-.4225	.8893	104.18
C4	.5201	-.7514	.1670	.9139	-55.31	.6741	.7210	-.1357	.9870	46.92	-.3107	.9124	-.3118	.9639	108.81
C5	.4002	-.8442	.1649	.9343	-64.63	.5861	.8440	-.1445	1.0275	55.22	-.4570	.8756	-.3265	.9877	117.56
O5	.5110	-.6602	.0537	.8349	-52.26	.6227	.6336	-.0310	.8884	45.50	-.2584	.8499	-.1910	.8883	106.91
O6	.7509	-.5805	.2244	.9491	-37.70	.8541	.4925	-.1709	.9860	29.97	-.0287	.9875	-.3371	.9880	91.67
N1	.4526	-.4331	.0740	.6265	-43.74	.3807	.0596	-.0072	.3854	8.90	-.1148	.6634	-.1927	.6733	99.82
C2	.4898	-.3030	.0504	.5759	-31.74	.5096	.0907	.0055	.5176	10.09	.0078	.6120	-.1582	.6120	89.27
N3	.3858	-.2133	.0357	.4409	-28.93	.5693	.2065	-.0120	.6056	19.94	.0120	.4749	-.1415	.4751	88.55
C4	.2511	-.2423	.0425	.3490	-43.98	.4803	.3014	-.0477	.5670	32.11	-.0936	.3874	-.1562	.3985	103.59
C5	.2220	-.3815	.0676	.4414	-59.81	.3458	.2860	-.0649	.4487	39.60	-.2182	.4508	-.1925	.5008	115.83
C6	.3212	-.4708	.0823	.5700	-55.70	.2927	.1578	-.0435	.3325	28.32	-.2251	.5838	-.2094	.6257	111.08
N7	--	--	--	--	--	.2874	.4064	-.1017	.4978	54.73	--	--	--	--	--
C8	--	--	--	--	--	.3870	.4895	-.1056	.6240	51.68	--	--	--	--	--
N9	--	--	--	--	--	.5072	.4331	-.0740	.6669	40.50	--	--	--	--	--
N6	--	--	--	--	--	.1626	.1283	-.0567	.2071	38.28	--	--	--	--	--
O2	.6062	-.2677	.0427	.6627	-23.82	--	--	--	--	--	.1069	.6813	-.1429	.6896	81.09
O4	.1670	-.1534	.0278	.2267	-42.57	--	--	--	--	--	-.0777	.2664	-.1386	.2775	106.25

Poly(dC)·Poly(dI)·Poly(dC)
(11-Fold Helix)

	X	Y	Z	R	φ	X	Y	Z	R	φ	X	Y	Z	R	φ
O1	.1335	-.9155	.0484	.9252	-81.70	.2217	.9605	-.0174	.9857	77.00	-.8055	.4692	-.2144	.9322	149.78
P	.1455	-.8592	.1977	.8714	-80.39	.2376	.9152	-.1702	.9455	75.45	-.7364	.4554	-.3581	.8659	148.27
O2	.1660	-.9715	.2920	.9856	-80.30	.1142	.8471	-.2151	.8548	82.32	-.8280	.5058	-.4628	.9702	148.58
O3	.0309	-.7703	.2265	.7709	-87.70	.2809	1.0309	-.2520	1.0685	74.76	-.6872	.3171	-.3765	.7568	155.23
O4	.2798	-.7725	.1879	.8216	-70.09	.3572	.8094	-.1594	.8847	66.18	-.6123	.5551	-.3428	.8265	137.81
C1	.5301	-.5169	.0578	.7404	-44.28	.5631	.5249	-.0407	.7698	42.99	-.3004	.7219	-.2057	.7819	112.59
C2	.5985	-.5202	.1942	.7930	-41.00	.6500	.5165	-.1660	.8302	38.47	-.2716	.7781	-.3445	.8242	109.24
C3	.5307	-.6405	.2596	.8318	-50.36	.6080	.6430	-.2408	.8850	46.61	-.4020	.7472	-.4176	.8485	118.28
C4	.5147	-.7365	.1416	.8985	-55.05	.5876	.7425	-.1267	.9469	51.64	-.5065	.7702	-.3083	.9218	123.33
C5	.4032	-.8382	.1538	.9302	-64.31	.4928	.8573	-.1556	.9889	60.11	-.6374	.6958	-.3254	.9437	132.49
O5	.4860	-.6487	.0289	.8105	-53.16	.5321	.6615	-.0191	.8490	51.19	-.4411	.7233	-.1869	.8472	121.38
O6	.7358	-.5465	.1709	.9166	-36.60	.7848	.5261	-.1228	.9448	33.84	-.2545	.9179	-.3296	.9526	105.49
N1	.4120	-.4265	.0522	.5931	-45.99	.3379	.0756	.0021	.3462	12.61	-.2530	.5818	-.1865	.6345	113.50
C2	.4366	-.2913	.0303	.5248	-33.71	.4676	.1101	.0216	.4804	13.25	-.1199	.5645	-.1507	.5771	101.99
N3	.3312	-.2058	.0244	.3899	-31.85	.5157	.2308	.0077	.5650	24.12	-.0729	.4383	-.1326	.4444	99.44
C4	.2060	-.2508	.0397	.3245	-50.59	.4207	.3191	-.0292	.5280	37.18	-.1538	.3326	-.1489	.3665	114.81
C5	.1784	-.3894	.0626	.4284	-65.38	.2890	.2901	-.0501	.4095	45.11	-.2915	.3484	-.1858	.4543	129.91
C6	.2853	-.4733	.0679	.5526	-58.92	.2421	.1555	-.0331	.2878	32.71	-.3361	.4755	-.2036	.5823	125.25
N7	--	--	--	--	--	.2200	.4034	-.0867	.4595	61.40	--	--	--	--	--
C8	--	--	--	--	--	.3132	.4989	-.0867	.5890	57.88	--	--	--	--	--
N9	--	--	--	--	--	.4346	.4499	-.0522	.6255	45.99	--	--	--	--	--
O6	--	--	--	--	--	.1288	.1111	-.0477	.1701	40.80	--	--	--	--	--
O2	.5535	-.2534	.0164	.6087	-24.60	--	--	--	--	--	-.0480	.6644	-.1364	.6662	94.14
N4	.1035	-.1673	.0341	.1967	-58.25	--	--	--	--	--	-.1079	.2096	-.1312	.2357	117.23

implied by the precision to which they are quoted. Because of the method used there is very strong correlation between the errors in coordinates of atoms closely joined by chemical bonds, and this correlation falls off as more variable structural features intervene between the atoms considered.

For this reason and others concerned with the statistical properties of the system, no error estimates are presented here. A fuller discussion is in preparation (ref. 19).

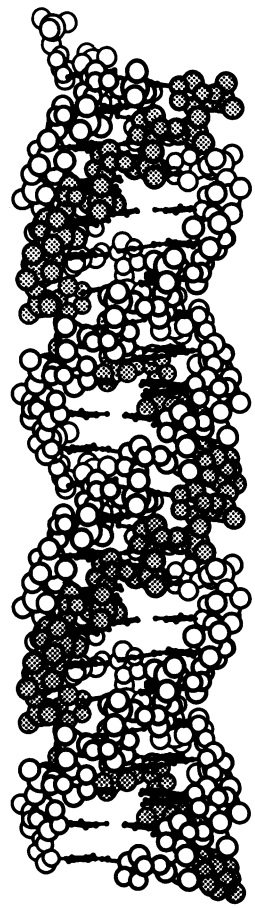

Figure 3: View perpendicular to the helix axis of a typical triplex structure, the poly(dT)·poly(dA)·poly(dT) of Selsing and Arnott (ref. 4). Notice how the third strand (shown shaded) fits in the major groove of a quite conventional *A*-DNA type structure.

that this conformational class would be available to DNAs, RNAs, and DNA-RNA hybrids: for example the triple-stranded complex of poly d(A-G)·poly d(C-T) and poly(C-U).[13]

No biological role for such triple-stranded complexes has yet been demonstrated, but we would be surprised if analogous structures were not exploited by living systems. It has indeed been suggested that DNA-RNA triplexes of this sort may serve to maintain chromosomes in folded conformations because folded *E. coli* chromosomes relax after treatment with ribonuclease speci-

fic for single-stranded RNA.[14,15] As shown in Figure 4, poly(Py) single-stranded RNA could form triplexes with two separated poly(Pu)·poly(Py) tracts in the chromosome; the bounded duplex would then loop-out. The triplex regions of this structure would be required to adopt *A*-type conformations; presumably the looped-out duplex would retain the *B*-DNA geometry, with kinks occurring at the junction between the *A* and *B* regions. Of course this scheme requires the presence of sizable oligo(Pu)·oligo(Py) tracts in the chromosome; it is noteworthy, therefore, that long sequences (∿750 base pairs on the average) of this type are found in the *Drosophila* genome.[16,17]

The discovery[18] that small RNA oligonucleotides are implicated in the initiation of DNA replication in the bacteriophage λ invites speculation that three-stranded structures are involved in this process. The geometry of three-stranded initiator regions, however, would preclude primer roles for these oligonucleotides since the 3'-hydroxyl group of the RNA, located in the groove of the DNA duplex (Figure 1), is not positioned correctly for chain elongation, since the oligo RNA would have the same 3'-5' polarity as the poly(purine) nucleotide stretch to which it would be attached. Rather we envisage the oligopyrimidine nucleotide tracts of such RNAs acting as inducers of an *A* conformation in a DNA that would normally have a *B* secondary structure. Triplex formation would require the local conformation to be *A*. The region of *A* conformation might well extend beyond the triplex region to a nearby site and enhance polymerase binding.

In conclusion, it seems to us that this method is a valid one for the postulation and evaluation of the structures of these interesting polynucleotides, a role played at present by

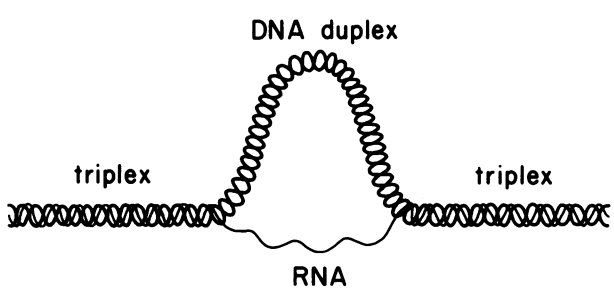

Figure 4: Looping out.

no more direct method. The paucity of X-ray data arises in part from the computational difficulty of extracting structural information from 'continuous' diffraction, but more fundamentally from the inherent disorder present in synthetic and, to an even greater extent, biological polynucleotide materials. It is of some importance then that such methods as the present one be developed.

ACKNOWLEDGEMENT

This work was supported by a grant (GM17371) from the National Institutes of Health of the USA.

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