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Raman spectral studies of nucleic acids XVII. Conformational structures of polyinosinic acid

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ABSTRACT

Laser-Raman spectra of poly(rI) show the formation of an ordered complex in aqueous solutions of high ionic strength. This structure exhibits the A-helix geometry, contains stacked bases and is apparently stabilized by specific hydrogen bonding involving hypoxanthine C6=O groups. Thermal dissociation of the poly(rI) complex (T_m=45°C) yields single-stranded and disordered poly(rI) chains. A disordered structure also occurs for poly(rI) in aqueous solutions of low ionic strength. In oriented films, poly(rI) forms an ordered structure probably the same as that which occurs in solutions of high ionic strength. Raman intensities measured at 815 and 1100 cm^{-1} in spectra of poly(rI) and poly(rU)•poly(rA)•poly(rU) indicate that the correlation previously established for single- and double-stranded ribopolymer structures is valid also for these multi-stranded structures. X-ray diffraction and model-building studies confirm the A-helix structure.

INTRODUCTION

Polyinosinic acid [poly(rI)] associates to form a multi-stranded complex, the structure of which has been investigated by various techniques. The first X-ray diffraction data[1], obtained from fibers of poly(rI), led to the proposal that the ordered molecular structure was probably a triple-stranded and right-handed helix stabilized by hydrogen bonding between C6=O and N1-H groups of the hypoxanthine bases in adjacent strands (Fig. 1a). Later independent X-ray diffraction and molecular model-building studies by Arnott *et al.*[2] and by Zimmerman *et al.*[3] led to the conclusion that the complex contains four right-handed helical chains. However the models differed in two important respects. In the Arnott *et al.*[2] model the sugar rings are puckered C2'-endo (i.e. the conformation is of the B-type) and individual bases in the hydrogen-bonded quartet are not perpendicular to the common helix axis. In the Zimmerman *et al.*[3]

model the sugar rings are C3'-endo (i.e. A-type) and the quartet of bases share a common plane perpendicular to the helix axis. In both models G can replace I and additional N2-H---N7 hydrogen bonds formed. Polyriboguanylic acid is therefore isostructural with poly(rI).[5]

Aqueous solutions of poly(rI) have been studied by optical rotatory dispersion (ORD)[6] and circular dichroism (CD) techniques.[6,7] In the earlier spectroscopic study[6] the data were interpreted in terms of a structure with left-handed helical chains. Cech and Tinoco[7] however were able to show that the characteristic negative circular dichroism of poly(rI) is obtained with right-handed helices provided that the individual bases are, like the Arnott *et al.*[2] model, not perpendicular to the helix axis. Nevertheless the ORD and CD results do indicate that the multi-stranded complex of poly(rI) is stable in solutions of high ionic strength (0.1 M NaCl). A single-stranded and "poorly stacked" structure was proposed for poly(rI) in aqueous solutions containing less than 0.1 M NaCl.[6]

Infrared[8] and Raman spectroscopy[9,10] have been employed previously to identify the keto tautomeric structure of hypoxanthine and to demonstrate the stacking of hypoxanthine bases in poly(rI) and related model compounds.

Of the techniques employed to study aqueous poly(rI), Raman spectroscopy would appear to offer the greatest promise for distinguishing A and B-helix geometries as well as to detect base stacking and hydrogen bonding interactions.[11] Raman data of both aqueous and solid samples may also be compared to determine whether conformational properties of poly(rI) are the same or different in the two states. These and similar questions are addressed in this communication. We also report the Raman spectrum of a triple-stranded and right-handed polyribonucleotide complex, *viz.* poly(rU)•poly(rA)•poly(rU), to show that multi-strandedness of itself does not invalidate the conformational correlations established previously for double-stranded ribopolymer complexes.[12,13]

MATERIALS AND METHODS

Details of the preparations of films of poly(rI) and fibers

of poly(rU)·poly(rA)·poly(rU) are discussed elsewhere.[2,14] In all samples, the polynucleotide molecules were oriented more or less unidirectionally so that the helical axes were roughly parallel to one another along a known direction of the film or along the fiber axis.

Samples were deuterated by storage for several days in a sealed hygrostatic cell (75% relative humidity) which contained a saturated solution of $NaClO_3$ in D_2O.[15]

After spectral determinations of films or fibers were completed the samples were dissolved to about 40 μg/μl in either H_2O or D_2O, as required. Na_2SO_4 was added to solutions for use of its 980 cm^{-1} line as a Raman intensity and frequency standard. Salt concentration was adjusted in the range 0 to 1.5 M by direct addition of NaCl to the solutions. Spectra were recorded in the temperature range 0 to 80°C by use of a thermostatically controlled cell, described previously.[16] Solution pH (or pD) was always within the range 6.8 ± 0.4.

The Raman instrumentation used in this work consists of a Coherent Model CR2 argon-ion laser and Spex Ramalog spectrometer. Further details are given elsewhere.[10,12,16] Spectra of solids were excited with approximately 200 milliwatts of 514.5 nm radiation and of solutions with 400 mW of 488.0 nm radiation.

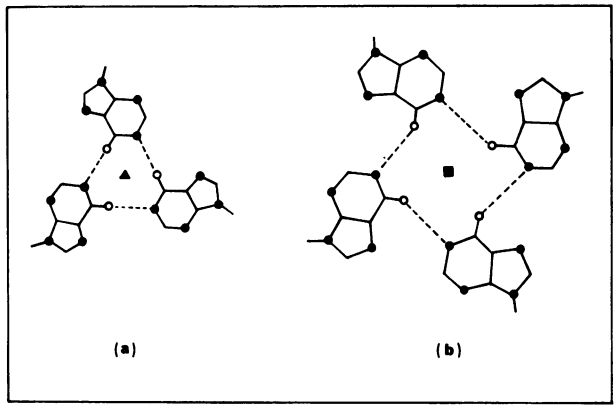

Figure 1: Hydrogen-bonding schemes proposed in (a) the triple-stranded model of poly(rI), ref. 1, and (b) the four-stranded models of poly(rI), refs. 2 and 3. Possible differences in furanose ring puckering are not shown. (N and O atoms are indicated by filled and open circles; see also refs. 1-5).

RESULTS AND DISCUSSION

1. Poly(rI).

Raman spectra of non-deuterated and deuterated films of poly(rI) are shown in Fig. 2. Raman lines in the region 750-850 cm^{-1}, which are due to vibrations of the phosphodiester backbone, are informative of the polynucleotide chain conformation.[11] Thus the lines of comparable intensity near 815 and 795 cm^{-1} in each spectrum of Fig. 2 show that the film of poly(rI) consists of a mixture of roughly equal amounts of ordered (A-helix) and

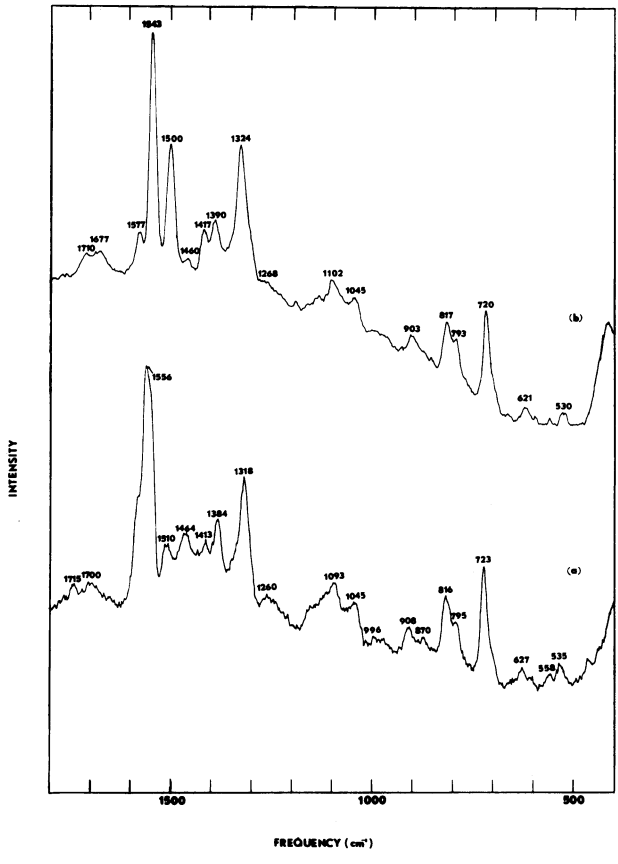

Figure 2: Raman spectra of (a) non-deuterated and (b) deuterated films of poly(rI). In both (a) and (b), the incident beam was at 45° to the plane of the film and the scattered radiation was collected at 90° to the incident beam direction. Raman frequencies of the prominent lines are listed in cm^{-1} units. Conditions: slit width $\Delta\sigma$ = 10 cm^{-1}, scan rate r = 25 cm^{-1}/min, rise time τ = 3 sec, excitation wavelength λ = 514.5 nm.

disordered chain conformations.[13] There is no line near 835 cm^{-1} to indicate a B-helical conformation in the film of poly(rI).[9] These results indicate that, in the film under study, about 50% of the poly(rI) molecules exist in the ordered structure, and further that the ordered structure which is present is of the A-helix (C3'-endo) type rather than of the B-helix (C2'-endo) type.

In the double-bond region (1600-1750 cm^{-1}) of Fig. 2b, two Raman lines are detected, with frequencies at 1677 and 1710 cm^{-1}. These are assignable to C6=0 groups of hypoxanthine.[8] The appearance of two distinct carbonyl group frequencies is consistent with the presence of hypoxanthine residues which exist in either ordered or disordered conformational states, as suggested above. The 1677 cm^{-1} Raman frequency is close to the C6=0 group frequency found in infrared spectra (1674 cm^{-1}) of disordered poly(rI).[8] Other Raman lines in the spectra of Fig. 2 are due to purine ring vibrations expected of the ribonucleotide of hypoxanthine and their assignments are straightforward.[10]

Raman spectra of D_2O solutions of poly(rI), containing 0.035 M Na_2SO_4 (i.e. "low" salt concentration), are shown in Fig. 3a at 10 and 70°C. At this low ionic strength, the spectra of poly(rI) exhibit (i) the backbone frequency characteristic of a disordered chain (793 cm^{-1}), (ii) a single C6=0 group frequency (1674 cm^{-1}) and (iii) no temperature dependence. We may conclude that poly(rI) at these conditions is a disordered chain, containing neither appreciable base stacking nor base pairing interactions. It seems clear that the hypoxanthine bases of aqueous disordered poly(rI), for which C6=0 groups may be hydrogen bonded to solvent D_2O molecules but not to N1-D groups (Fig. 1), exhibit the same carbonyl group frequency (1674 cm^{-1}) in both infrared and Raman spectra. This is consistent with results obtained on poly(rU) and other disordered polyribonucleotides.[11,12,17]

At high salt concentration (Fig. 3b), aqueous poly(rI) exhibits a backbone frequency of 815 cm^{-1}, a carbonyl group frequency of 1710 cm^{-1} and temperature-dependent Raman intensities (hypochromism), most notably at 1495, 1326 and 718 cm^{-1}. These results indicate that in solutions of high ionic strength

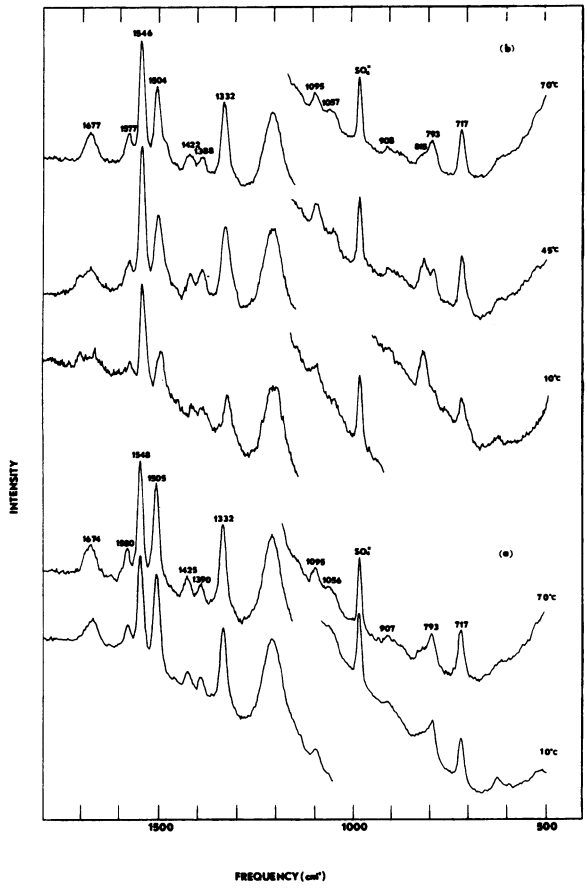

Figure 3: Raman spectra of D_2O solutions of (a) poly(rI), 40 μg/μl in 0.035 M Na_2SO_4 (pD7), at 10 and 70°C; and (b) poly(rI), 40 μg/μl in 1.5 M NaCl + 0.035 M Na_2SO_4 (pD7), at 10, 45 and 70°C. Conditions: $\Delta\sigma$ = 10 cm^{-1}, r = 25 cm^{-1}/min, τ = 3 sec, λ = 488.0 nm. [Note that D_2O solutions were not maintained at 70°C for more than 30 min and therefore substantial deuterium exchange of 8-CH groups has not taken place (ref. 20).]

poly(rI) forms at low temperature an ordered structure which contains the A-helix backbone, specific hydrogen bonding of C6=0 groups and base stacking interactions. Upon denaturation of the ordered structure of poly(rI) (i.e. with the transition from 10 to 70°C shown in spectra of Fig. 3b), the following changes take place in the Raman spectrum: the intensity of the line at 717 cm^{-1} increases by 50%, that at 1326 cm^{-1} increases

by 80% while its peak shifts to 1332 cm^{-1}, that at 1495 cm^{-1} increases by 80% while its peak shifts to 1504 cm^{-1} and that at 1541 cm^{-1} does not change appreciably while its peak shifts to 1544 cm^{-1}; the carbonyl group frequency shifts from 1710 to 1677 cm^{-1}; and the backbone frequency shifts from 815 to 793 cm^{-1}. In other words the spectrum of the ordered (high ionic strength) form of poly(rI) is converted upon heating to 70°C to a spectrum nearly identical to that of the disordered (low ionic strength) form of poly(rI) (*cf.* Figs. 3a and 3b).

The Raman intensity ratios, I_{1544}/I_{1500} and I_{1544}/I_{1329}, both decrease sharply with thermal denaturation of the multi-stranded poly(rI) complex. Plots of these ratios vs. temperature (Fig. 4) show clearly a sharp (cooperative) transition, with median melting temperature (T_m) of 45 ± 1°C. Thus, for the change in state

$$[\text{poly(rI)}]_n \rightarrow n\ \text{poly(rI)},$$

we find that $T_m = 45 \pm 1$°C. The Raman results are in agreement with earlier CD and UV melting profiles,[6] but do not allow determination of the value of n. (Arnott *et al.*[2] and Zimmerman *et al.*[3], independently proposed n=4 from X-ray data).

2. Poly(rU)·poly(rA)·poly(rU).

Raman spectra of non-deuterated and deuterated fibers of the triple-stranded complex poly(rU)·poly(rA)·poly(rU) are shown in Fig. 5. The spectra are very similar to those of poly(rA)·poly(rU),[12] with the exception that here the Raman lines originating from the poly(rU) component are about twofold more intense. The most interesting aspect of Fig. 5 is the fact that the ratio $I_{814}/I_{1100} = 1.65$ in both spectra, indicating a fully ordered A-helical structure in both non-deuterated and deuterated fibers.[13] Therefore the Raman spectra confirm the results of X-ray diffraction studies which show that the third polynucleotide strand [poly(rU)] is bound to double-stranded poly(rA)·poly(rU) with conservation of the A-helix geometry in each strand.[18]

These results lend further credence to the proposal that the 815 cm^{-1} line identifies the A-helix backbone geometry in a polyribonucleotide, regardless of helix-strandedness,[19] and that the Raman intensity ratio I_{814}/I_{1100} is directly proportional to the number of ordered nucleotide subgroups.[13]

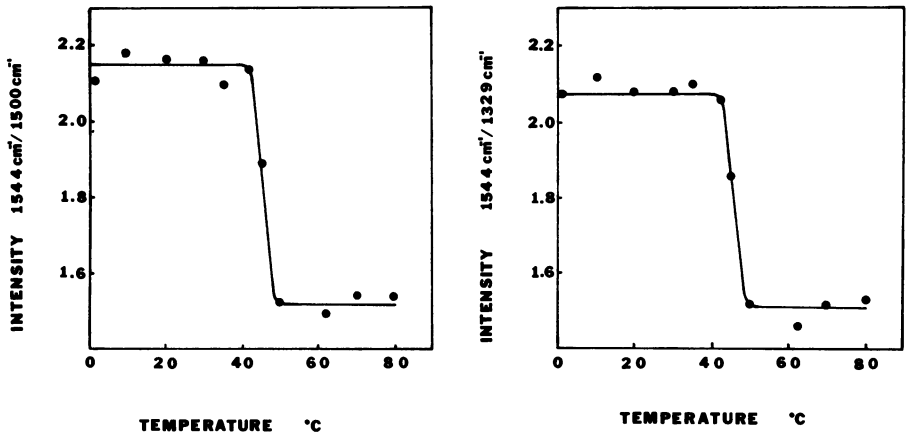

Figure 4: Left: Plot of the ratio of Raman line intensities at 1544 and 1500 cm^{-1} vs. temperature. Right: Plot of the ratio of Raman line intensities at 1544 and 1329 cm^{-1} vs. temperature.

RECONCILIATION WITH X-RAY AND MODELBUILDING STUDIES

We have recently reconsidered the structure of poly(rI) using improved computer modelling techniques and have concluded that the earlier rejection of A-type conformations[2] was not justified.

Using a method[21] in which we generate the models with standard bond lengths and angles which exhibit the least possible non-bonded interatomic contacts, we have built feasible four-stranded models with either sugar ring geometry.

The B-type model resembles closely that of Arnott *et al.*[2] with no conformation angles more than 11° different. The A-type model resembles that of Zimmerman *et al.*[3] rather less closely but is broadly similar. The main differences are that the bond lengths and angles are standard, the sugar rings have standard geometry, and the bases are not coplanar but (like the B-type model) have a saucer-like configuration.

The two models both show rather poor agreement with the Bragg X-ray data, the crystallographic residual $R'' = \Sigma(F_{obs} - F_{calc})^2/\Sigma F_{obs}^2$ being 0.46 for the B-type model and 0.36 for the A-type, thus somewhat favoring the latter. The six measurable equatorial Bragg data are undoubtedly contaminated with continuous diffraction to an extent which is very difficult to measure or calculate and refinement of the models against them may not

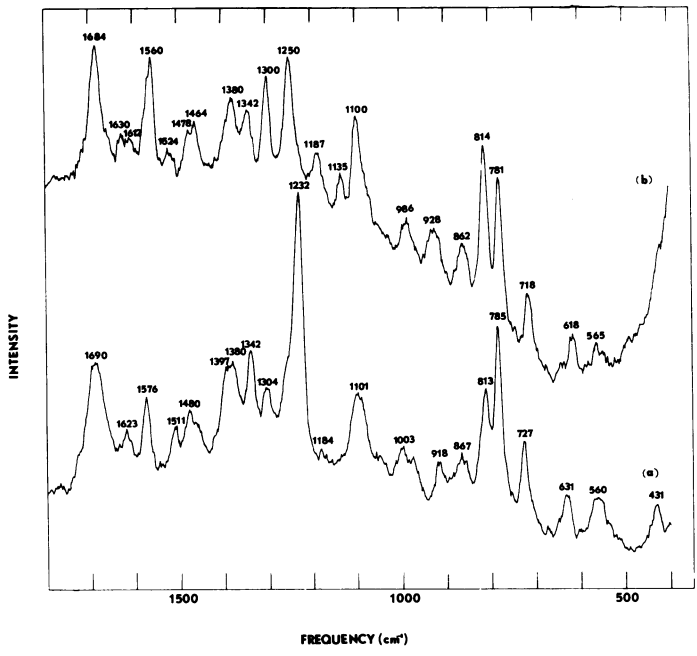

Figure 5: Raman spectra of (a) non-deuterated and (b) deuterated fibers of poly(rU)·poly(rA)·poly(rU)

be meaningful. However, when each of the models was jointly refined against X-ray and short contact data, R" for the B-type model could only be reduced at the expense of worsened contacts, whereas the A-type model showed improvement in both. For these models, R" was 0.43 for the B-type and 0.23 for the A-type. The cylindrically averaged Fourier transforms of the two models are quite similar and show fair agreement with the observed diffraction, which in this instance appears not to be a very sensitive probe of the detailed conformation.

Stereochemically the B-type model retains the improbably close to eclipsed value of $\phi = \theta$[O4-P-O1-C3] reported earlier, and its short contacts (in the sense of Smith and Arnott[21]) are some 60% worse than those of the A-type model. In the now preferred A-type model the O6---N1 hydrogen bond has a length of 0.298 nm and the normal to each base plane makes an angle of 11° with the helix axis. The only contact shorter than 0.02 nm less than the sum of the van der Waals radii is between O4 and O5, 0.255 nm.

We therefore find that an A-type model is superior both on stereochemical grounds and in its fit with the X-ray data.

In Table 1 we list the conformation angles of the final A-type model, and in Table 2 its atomic coordinates. Fig. 6 shows the atom labelling scheme and Fig. 7 two views of the model. This model should be regarded as typical rather than definitive: clearly the finer details are dependent on our assumptions relating to hydrogen-bond geometry and the like.

TABLE 1: Conformation angles in the A-type model for poly(I). Atom labelling is shown in Fig. 6.

Conformation Angle	(degrees)
ω = θ[C4-C3-O3-P]	-156
ϕ = θ[C3-O3-P-O5]	-69
ψ = θ[O3-P-O5-C5]	-103
θ = θ[P-O5-C5-C4]	176
ξ = θ[O5-C5-C4-C3]	92
χ = θ[C2-C1-N9I-C4I]	72

TABLE 2: Atomic coordinates (nm and degrees) of one residue of one strand of poly(I). The other strands may be generated by adding 90, 180 and 270 degrees to the ϕ coordinates given. Successive residues in each strand may be generated by adding 0.6820 nm to z and 31.3 degrees to ϕ. The helix symmetry is 2_3^2, the unit cell c axis is 7.843 nm. The orientation about the helix axis (z) is arbitrary. The accuracy of these coordinates is not as great as that implied by the precision to which they are quoted: for details see ref. 21.

Atom	x	y	z	r	ϕ
P	0.9036	0.4509	-0.0466	1.0341	25.85
OP1	0.8147	0.5155	-0.1120	0.9640	32.32
OP2	1.0552	0.4937	-0.1142	1.1649	25.08
O5	0.9163	0.2919	-0.0352	0.9617	17.67
C5	1.0317	0.2095	-0.0603	1.0527	11.48
C4	0.9982	0.0640	-0.0337	1.0003	3.67
O4	0.8874	0.0576	0.0609	0.8893	3.72
C3	0.9485	-0.0161	-0.1541	0.9486	-0.97
O3	1.0559	-0.0647	-0.2335	1.0579	-3.51
C2	0.8674	-0.1271	-0.0874	0.8776	-8.34
O2	0.9529	-0.2255	-0.0319	0.9793	-13.31
C1	0.8038	-0.0526	0.0295	0.8055	-3.74
N9I	0.6675	0.0000	0.0000	0.6675	0.00
C8I	0.6260	0.1308	-0.0195	0.6395	11.80
N7I	0.4998	0.1430	-0.0439	0.5179	16.02
C6I	0.3195	-0.0387	-0.0597	0.3218	-6.91
O6I	0.2161	0.0230	-0.0840	0.2173	6.06
C5I	0.4507	0.0118	-0.0404	0.4508	1.50
C4I	0.5536	-0.0762	-0.0137	0.5588	-7.84
N3I	0.5482	-0.2115	-0.0023	0.5876	-21.10
C2I	0.4251	-0.2587	-0.0204	0.4976	-31.33
N1I	0.3161	-0.1783	-0.0475	0.3629	-29.43

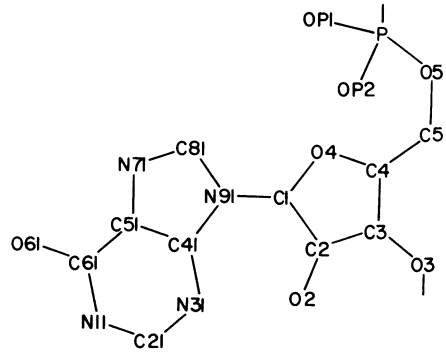

Figure 6: Atom labelling scheme for the inosine residue.

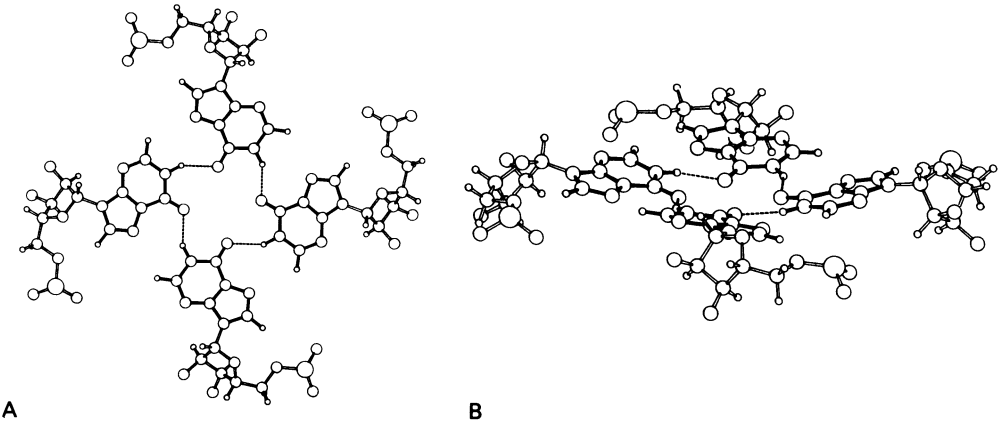

Figure 7: Views of a single base quadruplet from the final model (a) along the helix axis, (b) along a line 15° from the plane normal to the helix axis, showing the saucer-like appearance of the quartet of hydrogen bonded bases.

CONCLUSIONS

The present results indicate the following.

(1) In aqueous solutions of high ionic strength, poly(rI) forms an ordered complex in which the polynucleotide chains contain the A-helix backbone geometry. The complex also contains stacked bases which exclude solvent molecules from hydrogen bonding with C6=O groups of hypoxanthine. This complex exhibits a T_m of 45°C when in 1.5 M NaCl + 0.035 M Na_2SO_4 solution.

(2) In aqueous solutions of low ionic strength, poly(rI) may be classified as a disordered, single-stranded polynucleotide chain, containing no appreciable stacking of hypoxanthine bases and no specific base-base hydrogen bonding interactions. (This

disordered structure is also obtained upon thermal denaturation of the ordered poly(rI) complex, mentioned above.)

(3) In oriented films of poly(rI) the molecules are about equally distributed between ordered and disordered structural forms. The ordered structure present in the film, and presumably yielding the diffraction patterns reported elsewhere,[2,3] exhibits the A-helix (C3'-endo sugar ring pucker) and not the B-helix (C2'-endo) geometry. Therefore, the ordered structure of poly(rI), like that of other ordered polyribonucleotides, belongs to the A-helix family.

(4) The solvated hypoxanthine residue of disordered poly(rI) in D_2O solution exhibits its C6=O group frequency at 1675 ± 2 cm^{-1}, in both Raman and infrared spectra. Upon formation of the ordered multi-stranded structure of poly(rI), the C6=O group Raman frequency shifts upwards from 1675 to 1710 cm^{-1}, which suggests that inter-base hydrogen bonding (Fig. 1) is weaker than base-solvent hydrogen bonding.

ACKNOWLEDGMENTS

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