STRUCTURAL PARAMETERS OF SINGLE AND DOUBLE STRANDED HELICAL POLYRIBONUCLEOTIDES

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Summary: Precise structural parameters of polyribonucleotides single stranded helices are determined as well as those of double stranded helices of poly 2'-O-methyl A and of poly A at neutral and acid pH. Infrared linear dichroism investigations indicate the similarity of the conformation of the sugar-phosphate backbone of these single and double stranded helices. The angles of the phosphate group for single stranded helix at neutral pH is found to be oriented at 48° for the O2PO2 bisector and at about 65° for the O2---O3 line to the helix axis. Similar values were found for double stranded poly A helix at acid pH. These structural parameters obtained for the first time on single stranded polynucleotide helices are proposed to be valid for other similar helical chains such as poly A segments of nuclear or messenger RNA and single stranded CCA acceptor end of transfer RNA.

Accumulating data over the past years indicates that DNA and RNA may adopt both double and single stranded structures. In contrast to the double stranded helical DNAs and RNAs, whose structures are known with a high degree of precision from the investigation on fibers by x-ray diffraction (1,2), and on films by infrared dichroism (3,4), our knowledge of single stranded polyribonucleotides and nucleic acids is very restricted. In fact, the discovery and evidence of single stranded helical polynucleotides is based mainly on investigations in solution by sensitive optical methods, such as circular dichroism and optical rotatory dispersion (5), and small-angle x-ray scattering (5b). However, direct information on structural parameters of these helices, e.g. obtained by x-ray diffraction methods, is completely lacking.

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In this work we describe infrared dichroism investigaitons on single and double stranded polyribonucleotides poly A and poly 2'-O-methyl A (poly A_m), which yielded, for the first time, precise structural parameters, particularly of single stranded helices. From circular dichroism investigations, it is known that poly A_m at neutral pH forms single stranded helices analogous to that of poly A (6,7), but of greater conformational stability. For these reasons, the orientation of the film of poly A_m at neutral pH was successfully performed. At acid pH, poly A_m forms a very compact polycrystalline gel which could not be oriented. In contrast, poly A at acid pH forms a much more fluid gel and was very well oriented.

Poly A_m and poly A spectra, at neutral pH, are shown in Figs. 1 and 2, respectively. These two spectra are very similar in frequencies and relative intensities of absorption bands in the spectral region of 1500 - 1800 cm^{-1} arrising from inplane vibrations of the base residues. Particularly, there is no trace of a 1668 cm^{-1} band in the deuterated neutral poly A_m film (Fig. 1, curve 1), nor of a 1718 cm^{-1} band in undeuterated poly A_m film (Fig. 1, curve 2). This indicates the complete single strandedness of this polymer. In fact, these bands at 1668 cm^{-1} and 1718 cm^{-1} are known to be characteristic of protonated, base paired adenine residues, (8a, 8b) and more generally, of base pairing in all known polynucleotides (9).

The 1630 cm^{-1} absorption band of deuterated neutral poly A_m is the most dichroic in the spectrum, with dichroic ratio $R\ (\frac{\perp}{/\!/}) = 1.22 \pm 0.04$, and one may thus assume with reasonable certainty that the adenine residues are perpendicularly oriented to the helix axis. Thus the transition moment of the corresponding inplane vibration makes an angle greater than 70° with the helix axis, i.e. between 70° and 90°. (There is no example of a lower angle between the bases and the helix axis in polynucleotides.) From an estimated mean value of $\theta_{1630} = 80°$ (with about 10° uncertainty),

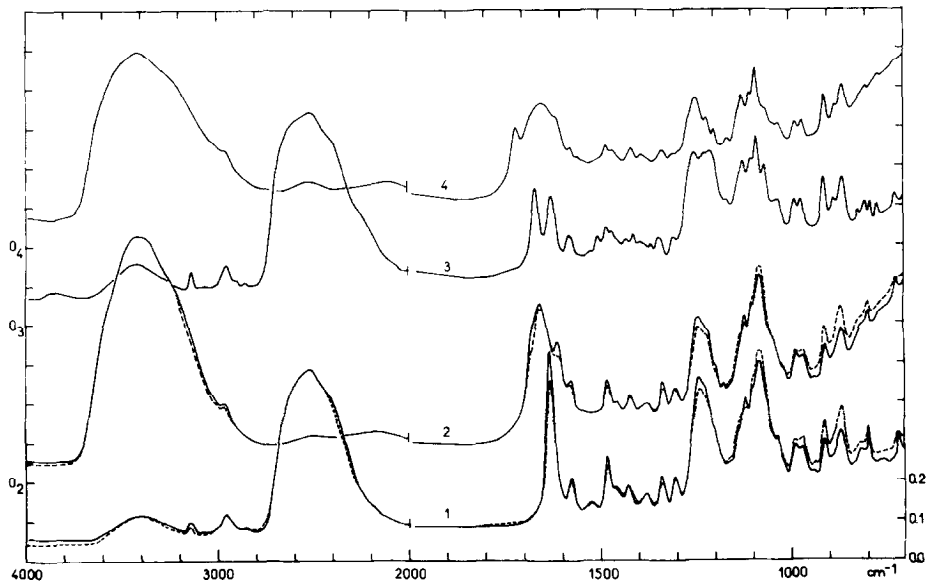

Fig. 1. I.R. spectra of poly A_m films in wet atmosphere. Oriented film of 2% Na poly A_m (1) and (2) aqueous solution at pH 6.5, neutral form, on AgCl plate, (1) deuterated, exposed to 100% r.h. D_2O, (2) undeuterated, exposed to 100% r.h. H_2O, (3) and (4) unoriented film obtained by drying a 2% Na poly A_m aqueous solution of pH about 5.0, which form a solid gel and cannot be spread, (3), deuterated, exposed to 100% r.h. D_2O, (4) undeuterated, exposed to 100% r.h. H_2O. Poly A_m was prepared as described by Rottman and Heinlein (15). The dichroic ratio $R\left(\frac{\perp}{/\!/}\right) = A\perp / A/\!/$ is related to the angle θ which the transition moment forms with the helix axis by: $R\left(\frac{\perp}{/\!/}\right) = \frac{\sin^2\theta + g}{2\cos^2\theta + g}$ where g is parameter which characterizes the semi cristalline state of the sample and which can be related to the f fraction of perfectly oriented chains by: $f = \frac{1}{1 + 3/2\ g}$

Details of measurements and corrections on the dichroic ratio have already been reported (24).

-----E vector of the light // to the orientation axis

____ E vector of the light $\perp$ to the orientation axis

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one can calculate the fraction of perfectly oriented chains: f = 14%±2%, and the corresponding orientation parameter g = 4.0±0.8. It is thus possible to calculate the phosphate group orientation. The dichroic ratio at about 1080 cm^{-1}, $R\left(\frac{\perp}{/\!/}\right)$ = 0.93±0.03, allows one to calculate the angle of OPO bisector with the helix axis: θ_{1080} = 48°±4°. This band corresponds to the symetric stretching of the PO_2^- group (9). At about

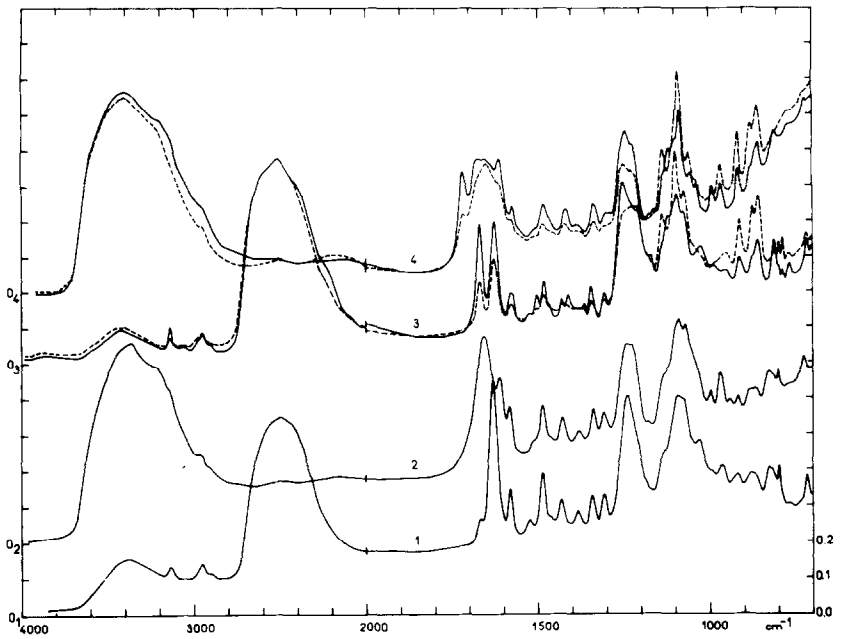

Fig. 2. I.R. spectra of poly A film in wet atmosphere at neutral and acid pH. (1) and (2) unoriented film obtained by drying at 2% Na poly A aqueous solution of pH about 6.5, (1) deuterated (2) undeuterated, (3) and (4) film oriented by unidirectional stoking of a 2% Na poly A aqueous solution of pH about 5.0 during drying, (3) deuterated, (4) undeuterated. Poly A was obtained from Miles Laboratories. Other conditions and conventions as on Fig. 1.

1240 cm^{-1} the band arises from antisymmetric stretching vibration (10) for which the dichroic ratio is $R\ (\frac{\perp}{/\!/}) = 1.09 \pm 0.05$ and yields the angle of orientation of the O-O line of the same group to the helix axis, $\theta_{1240} = 64° \pm 6°$. It is to be noticed that assuming that the bases are tilted from 70° to 90°, i.e. θ_{1630} equals 70° or 90°, the obtained extreme values for these OPO angles are not significantly different (Table I).

The double stranded poly A yielded the following values of angular orientation of phosphate groups $\theta_{1090} = 49° \pm 2°$, $\theta_{1240} = 66° \pm 3°$ which were obtained from our best oriented film (11) with $f = 60\% \pm 5\%$. This calculation was made by taking the base orientation $\theta_{1720} = 85° \pm 5°$ from x-ray diffraction studies (12).

Preliminary investigations of the Raman spectra of poly A_m indicated that they are very similar to those of poly A in the region of 700 cm^{-1}

TABLE 1. Structural parameters of single stranded (neutral pH) and double stranded (acid pH) poly A and poly A_m Orientation of the angle θ to the helix axis

	Phosphate group		Base
	02---03 line	02P03 bisector	
Band maximum	$\theta_{1240\ cm^{-1}}$	$\theta_{1090\ cm^{-1}}$	$\theta_{1630\ cm^{-1}}$
Poly A_m single stranded I.R.	64° (±6°)	48° (±4°)	80° (±10°)
Poly A double stranded I.R.	66° (±3°)	49° (±2°)	85° (±5°)
Poly A double stranded x-ray (after Rich et al., Ref. 12)	67.5°	49°	85°

to 900 cm^{-1}. The only major difference observed is in the region of 1050 cm^{-1}, where poly A_m shows a strong band. This band can be attributed to C-O stretch of ribose in 2' position, (13) which is now under the form of $CO-CH_3$. This Raman band is not observable in poly A. In the region of 807-815 cm^{-1}, one can observe a strong Raman band in both poly A and poly A_m. Thomas (14) and Small and Peticolas (13) have attributed the Raman 815 cm^{-1} band to symmetric stretching vibration of the O-P-O diester. The presence of the 815 cm^{-1} band in the Raman spectra of poly A and poly A_m represents the formation of a specific conformation of the sugar phosphate backbone in ordered double stranded polyribonucleotides, RNA (13), and in the A form of DNA (14b). Thus, despite of the absence of base pairing in these single stranded helices the sugar phosphate backbone adopts the same conformation as in double stranded ordered helices.

In conclusion, our infrared dichroism investigations on polyribonucleotides poly A_m and poly A enabled us to obtain structural parameters of single stranded helices. Despite the flexibility of these forms, which previously were considered to be random coils, and making use of greater

conformational stability of poly A_m, it was possible to obtain structural parameters concerning the phosphate-sugar backbone. The similarity of circular dichroic ultraviolet spectra of poly A and poly A_m at neutral pH allows us to consider their conformation to be similar (15). The orientation of the phosphate group in the single stranded helical poly A_m and also of poly A, is not distinguishable from its orientation in the double stranded helix of poly A, at acid pH (see Table I); it is also similar to that in the double stranded polyribonucleotides and RNA (16,11), and in the A form of DNA (4).

Our structural investigations may have many biological implications. Poly A sequences have been found in most messenger RNAs isolated from eukaryotic cells and animal viruses (17,18). These poly A sequences attached to messenger RNAs are relatively long, ranging in size from 50 to 250 nucleotides. Their attachment may have a role in conversion of nuclear RNA into messenger RNA (19,20). In solution, at the temperature of enzymatic experiments, i.e. at 25°C - 35°C, about half of the adenylic acid residues are in a stacked single stranded helical conformation (21).

Another example is provided by t-RNA. The presence of a single stranded segment was demonstrated in t-RNA at the 3'OH end containing the CCA acceptor sequence, by x-ray diffraction (22). The structural details of this portion of the t-RNA molecule were not elucidated. In solution the conformational stability of such sequences is similar to poly A (23,21). It is thus possible to propose that the CCA acceptor end of the t-RNA molecule, at a lower temperature, adopts a single stranded helical form similar to that described here for poly A_m, which at room temperature is only partially ordered.

Finally, this study presents an example of the usefulness of spectroscopic methods such as infrared linear dichroism (and also Raman scattering) which can provide structural parameters of flexible macromolecular chains very difficult to investigate by x-ray diffraction.

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