OPTICAL PROPERTIES OF CHEMICALLY SYNTHETISED POLYNUCLEOTIDES CONTAINING D- OR L-RIBONUCLEOSIDES

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1. Introduction

The stereochemistry and topology of nucleic acid structure is determined by the asymetric configuration of the nucleosides due to the D-sugar and by the 3′-5′ phosphodiester linkage between them [1, 2]. It was therefore of interest to construct nucleic acid structures which contained L-ribose as the sugar and/or 2′-5′ phosphodiester linkages. In this communication, the optical properties of chemically synthetised poly (A) and poly (U)** containing either D- or L-ribonucleosides and a random distribution of 2′-5′ and 3′-5′ linkages will be reported.

2. Material and methods

The synthesis of nucleosides containing L-ribose has been described [3]. The chemical condensation of randomly linked (2′-5′ and 3′-5′) homo-L-polynucleotides as well as the corresponding D-compounds by polymerisation of the corresponding 2′,3′-cyclic phosphates by a modification of the procedure of Michelson [4] will be described elsewhere [5]. The polynucleotides thus prepared have been exhaustively dialysed against tap water and gel filtrated on a G-200 Sephadex column in 0.05 M triethylammonium hydrogen carbonate, pH 7.5. After lyophilisation, $S_{20,w} \sim 1.5$ was obtained which indicates an average chain length of about 20 to 25 nucleotides.

Enzymatically synthetized poly (A) and poly (U) were purchased from P.L. Laboratories (Milwaukee, USA). Optical methods were described in [6].

3. Results

3.1. *Optical properties of chemically synthetised polynucleotides*

From the results on the optical activity of nucleosides Ulbricht et al. [7] have deduced certain empirical rules on the conformation of these compounds. It is thus not surprising that the Cotton effects of nucleosides containing L-ribose are the mirror images of those of the D-series [8].

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** *Abbreviations:*
In order to distinguish between the different types of polynucleotides used in the present work the usual abbreviation for the enzymatically synthetised 3′–5′ linked polyadenylic acid (poly(A)) has been used, while the chemically synthetised polyadenylates which contain a random distribution of 2′–5′ and 3′–5′ linkages have been called D-poly (A), if the sugar moiety was D-ribose and L-poly (A), if the sugar was L-ribose. Corresponding abbreviations have been used for the polyuridylic acids. CD: Circular dichroism; λ_p, λ_c, λ_t: wavelengths of peak, crossover and trough of the CD spectrum.

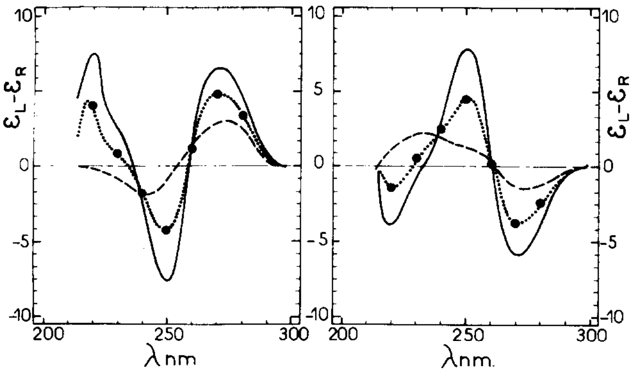

Fig. 1. Circular dichroism spectra of (a) D-poly(A) (——), D-poly(U) (- - -), an equimolar mixture of the two polymers (. . . . .); the circles indicate the summation of the two polymers. (b) The same experiment with L-poly(A) and L-poly(U) at 25°.

If such compounds are incorporated into a polynucleotide chain an inverted Cotton effect is expected. Fig. 1 shows the CD spectra of the random homopolymers (D-poly(A), D-poly(U), L-poly(A), L-poly(U)) and the corresponding equimolar mixtures. While D-poly(A) (fig. 1a) shows a CD spectrum which approaches that of enzymatically synthetised oligoadenylates [9], it should be noted that the optical activity is very much reduced (see table). Very similar dichroic bands but with inverted signs are observed for L-poly(A) (fig. 1b, table). If the amplitudes of the dichroic bands and the wavelengths of their extrema of the chemically synthetised polyadenylates are compared with those of poly(A) and adenyloligomers, it can be seen that D-poly(A) and (L-poly(A) with inverted signs) resemble partly ApA and partly 2′-5′-ApA, but not ApApA, and even less the higher oligomers.

These results suggest that the polyadenylates obtained by chemical synthesis are random copolymers with 3′-5′ and 2′-5′ linkages with about a 50% participation of the latter. This does not exclude small stretches of three and even four nucleotides being continuously linked in 3′-5′ bonds. Also indicative of incomplete stacking and thus little single helix formation is the fact that the hypochromicity of the chemically synthetised polyadenylates is very much smaller than that of normal poly(A). While the latter shows an ϵ_{max} = 10,000 [10], D-poly(A) and L-poly (A) show ϵ_{max} = 13,500 which again ressembles the value for ApA of ϵ_{max} = 13,600[10].

The chemically synthetised polyuridylates do not show the exact mirror image relationship observed for the polyadenylates. The L-poly(U) shows a lower optical activity and the crossover wavelength is shifted to higher wavelengths. Although there is still a certain amount of base-base interactions, since the CD spectra are different from those of the monomers (fig. 1), the participation of 2′-5′ links may be different in the two cases.

If the chemically synthetised polyadenylates were heated, a rather flat non-cooperative heating curve was obtained. The CD spectrum decreased slowly (fig. 2) in a very similar way to oligo(A) [11]. A van t'Hoff plot from this heating curve (of rather limited value, since the upper and lower limits of the tempera-

Table
Circular dichroism of poly(A) and adenyloligomers at 25°.

Compound	λ_p	$\Delta\epsilon_p$	λ_c	λ_t	$\Delta\epsilon_t$	Reference
Poly(A)	2640	+15.0	2545	2475	−12.0	11
	2640	+21.9	2550	2480	−16.5	12
	2640	+19.0	2540	2480	−12.6	9
A_7	2650	+11.0	2560	2480	− 8.0	11
ApApA	2670	+ 5.8	2575	2500	− 6.5	11
ApA	2720	+ 7.5	2600	2500	− 7.2	12
	2710	+ 6.2	2595	2505	− 7.6	10
ApA(2′-5′)	2710	+ 5.3	2590	2500	− 3.4	13
L-Poly(A)	2710	− 6.0	2600	2500	+ 7.7	this work
D-Poly(A)	2720	+ 6.5	2590	2510	− 7.5	this work

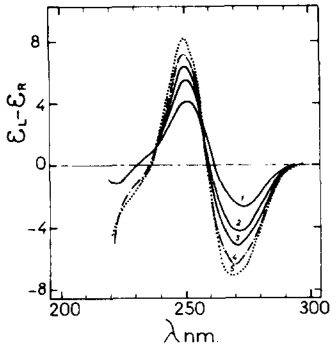

Fig. 2. Circular dichroism spectra of L-poly(A) at different temperatures in 0.15 M NaCl, pH 6.8. 5) −8°, 4) +8°, 3) +36°, 2) +52°, 1) +88°.

ture curve are difficult to establish) gives a value for $\Delta H^\circ = 7$ kcal/mole, while a parallel sample of poly(A) under identical conditions gave $\Delta H^\circ = 13$ kcal/mole, in agreement with the literature [14]. Again the low value for the free enthalpy change corresponds to the value obtained for dinucleotides [11, 13].

Acid titration of chemically synthetised poly(A) does not show a cooperative transition over a narrow pH range as is the case for enzymatically synthetised poly(A). While the latter forms a double helical protonated structure [15] with significantly changed optical properties [10, 11] and cooperative melting behaviour, none of these characteristic changes can be observed for the chemically synthetised polyadenylates. Instead a broad transition over more than one pH unit is observed (fig. 3) without shifts in the CD spectrum. The optical activity only becomes smaller. No melting is observed at pH 3.5 to 4.5 neither in CD nor in absorbance.

3.2. *Complex formation*

Attempts to form complexes between L-poly(A) or D-poly(A) with enzymatically synthetised poly(U) proved unsuccessful (fig. 4). No interaction could be observed between poly(U) and two synthetic polyadenylates, neither by absorbance nor by CD. Because of precipitation in 4 M LiCl no low temperature spectra could be recorded using enzymatically synthetised polynucleotides.

Similarly unsuccessful were our attempts to form complexes between D-poly(A) and D-poly(U) (fig. 1a) or between L-poly(A) and L-poly(U) (fig. 1b). Since the synthetic polynucleotides appeared to be completely soluble in 4 M LiCl the measurements were repeated at −20°. Again no interaction could be observed, the mixture spectra being the summation of the component spectra.

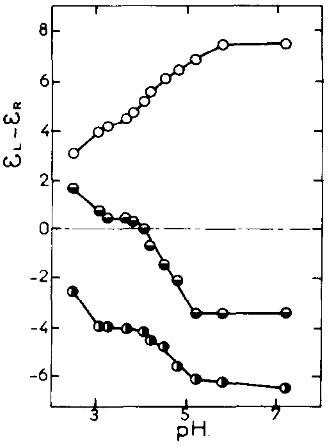

Fig. 3. Changes in circular dichroism of L-poly(A) with pH, in 0.15 M NaCl. ○ 250 nm, ● 225 nm, ◑ 270 nm.

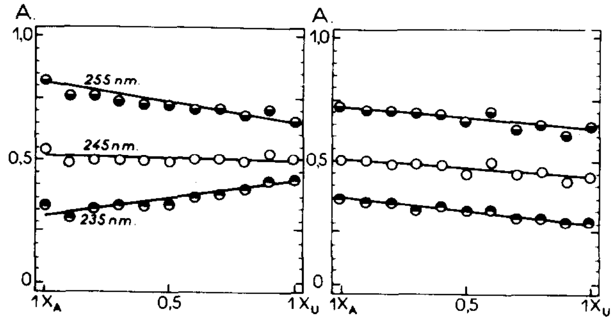

Fig. 4. Mixing curves between poly(U) and D-poly(A) (left) and L-poly(A) (right), in 0.15 M NaCl, pH 6.8 at indicated wavelengths, at 25°.

4. Discussion

The results presented on the optical properties of chemically synthetised polyadenylates demonstrate that not only the base-base interactions are necessary to form a stable single stranded helix, but that also interactions over more than the nearest neighbours are important. This is in agreement with the conclusions of Bush and Scheraga [12] who have criticised the dimer-stacking model and had shown that only "interior" nucleotides, i.e. not those at the end of a $3'$-$5'$ chain, contribute significantly to the optical activity and thus to the structure of a single stranded helix. From the work presented here it becomes clear that even short regions of $3'$-$5'$ linked nucleotides interrupted by $2'$-$5'$ linked residues are not sufficient to endow the polynucleotide chain with a structure ressembling the enzymatically synthetised polynucleotide. Thus the steric limitations of the $2'$-$5'$ nucleotide bond seem to limit the overlapping of the bases and other factors necessary for the constitution of a helix.

On the other hand the results on L-poly(A) in comparison with D-poly(A) indicate that the L-ribose is not an *a priori* obstacle for a polynucleotide structure. It should be possible in a L-poly(A) with all $3'$-$5'$ linkages to form a mirror image left-handed helix like enzymatically synthetised poly(A). Unfortunately, L-ADP is not a substrate for polynucleotide phosphorylase [5]. That L-poly(A) cannot interact with poly(U) containing D-ribose, comes as no surprise.

The fact that none of the four chemically synthetised polynucleotides is able to interact with its chemically synthetised partner nor with enzymatically prepared polymers again points to the absolute requirement for a continuous $3'$-$5'$ linked chain. Although one could image a helix-with-loops-model [16] between polynucleotide chains containing a random distribution of $2'$-$5'$ and $3'$-$5'$ linkages where only the latter will bind, this seems to be impossible in the present case, apparently due to the shortness of the $3'$-$5'$ linked regions. It would be interesting, if random polymers with a lower amount of $2'$-$5'$ bonds would interact. However, the different geometry of the $2'$-$5'$ bond compared with the $3'$-$5'$ linkage would certainly impose a certain strain on a possible helix formation. This may also explain the results of Michelson and Monny [17] that three-stranded complexes with $2'$-$5'$ linked oligo(A) or $3'$-$5'$ linked oligo (A), but not with mixed oligonucleotides were formed.

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