Irregularities in the Circular Dichroism of Oligoribonucleotides

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Marked anomalies, previously unresolved, exist in the CD spectra of oligoadenylates and oligouridylates at the pentamer or tetramer stage, indicating that these molecules differ in conformation from the preceding and the following member of the series. The CD of oligoadenylates retains a positive Cotton effect even at 80 °C. Caution must be exerted when predicting oligonucleotide structures from CD spectra.

Oligonucleotides serve as model compounds for nucleic acids and they assume independent regulatory functions in critical processes such as ribonucleotide reduction or protein synthesis [1 – 3]. As it becomes increasingly clear that ordered three-dimensional structures of both oligo- and polynucleotides must not be monotonous along the entire nucleotide chain but can include discontinuities (*e.g.*, pieces of Z-DNA, hinge regions in tRNA and in mixed helical/unstacked oligonucleotides [4 – 9]) more systematic knowledge of the distribution of such discontinuities, and of the methods of their detection is desirable. We here show that the circular dichroism (CD) spectra of two homooligoribonucleotide series also indicate the existence of anomalous conformations at certain short chain lenghts.

Materials and Methods

Oligoadenylates and oligouridylates were prepared by limited alkaline hydrolysis of polyadenylic or polyuridylic acid, respectively [10, 11] followed by removal of the terminal phosphate residues with alkaline phosphatase. The individual oligomers (dimer to octamer) were rigorously purified by column chromatography on DEAE cellulose (5 mM to 1 M triethylammonium bicarbonate gradient, pH = 7) and were characterized by paper electrophoresis and by the 2′(3′)nucleotide/nucleoside ratio determined after alkaline or ribonuclease hydrolysis.

Oligonucleotide solutions were made, at 0 °C, in 10 mM Tris-HCl buffer, pH = 8.5. Spectral measurements were carried out immediately after temperature equilibration. Concentrations (approximately 0.5×10^{-4} M, $A_{260} < 1$) were determined in a Cary 15 spectrophotometer after hydrolysis of an aliquot sample with snake venom phosphodiesterase to account for the hypochromic effect of oligomers. Molar absorption coefficients of the monomers: adenylic acid, $\varepsilon_{mon} = 16000\ M^{-1}\ cm^{-1}$ at 259 nm; uridylic acid, $\varepsilon_{mon} = 10000\ M^{-1}\ cm^{-1}$ at 262 nm. Tris buffer and triethylammonium ions did not detectably influence UV or CD spectra.

CD spectra were recorded with a Cary 61 spectropolarimeter in 1 cm quartz cells, using the 0.05 °C or 0.10 °C scale and 5 nm/min scanning speed. The temperature of the cuvettes (±0.1 °C) was controlled with a YSI tele thermometer. The base line was recorded after every other spectrum and the nucleotide spectra were corrected for variations. Under these conditions the reproducibility of CD spectra was within 3 – 5% at wavelengths above 220 nm. The spectra are expressed in terms of molar ellipticity $[\theta]$ (in degree · cm² · dmol⁻¹), defined by the equation

$$[\theta] = \frac{100 \cdot \vartheta}{l \cdot c} = \frac{100 \cdot \vartheta \cdot \varepsilon_n}{l \cdot A}$$

where ϑ is the measured ellipticity in degrees, l is the light path in cm, and c is the concentration in mol per liter which is obtained from the absorbance A (including hypochromicity, see above) and the molar absorption coefficient ε_n of an oligonucleotide $= \varepsilon_{monomer} \cdot n$ (n = chain length).

Results and Discussion

Molar ellipticities of the long-wavelength Cotton effects of oligonucleotides ApA to $(Ap)_6A$ and UpU to $(Up)_7U$, measured in the 5 ° – 80 °C temperature range are summarized in Table I. The general appearance of these CD spectra, characterized by positive bands centered at 270 nm and negative troughs at 250 or 242 nm, respectively, is well-known from previous studies [12 – 17]. A representative example is given in Fig. 1, contrasting the weak circular dichroism of monomeric adenosine phosphate with the strong exciton-type spectrum of ApApA. Note

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Table I. Molar ellipticity $[\theta]$ (in degree · cm^{-2} · $dmol^{-1}$) of the long-wavelength Cotton effects of oligoadenylate ($Ap_{n-1}A$) and oligouridylate ($Up_{n-1}U$) solutions at pH 8.5 and selected temperatures. Data at intermediate temperatures were also collected (cf. Fig. 2). $[\theta]_{mon}$ is the molar ellipticity per monomer residue (=$[\theta]/n$).

Nucleotide (chain length n)		Adenylates					Uridylates				
		$[\theta]_{mon} \times 10^{-3}$	$[\theta] \times 10^{-3}$				$[\theta]_{mon} \times 10^{-3}$	$[\theta] \times 10^{-3}$			
		5 °C	5 °C	25 °C	50 °C	80 °C	5 °C	5 °C	25 °C	50 °C	80 °C
AMP, UMP	$n=1$		−4	−4	−4	−4		9	9	9	9
dimer	2	21	43	27	13	6	19	38	32	26	20
trimer	3	24	73	44	19	6	18.5	56	46	38	29
tetramer	4	29	118	72	32	8	**13**; 17[a]	**52**	**45**	**36**	34
pentamer	5	**18**; 30[a]	**90**	**58**	**27**	**7**	17	83	70	57	53
hexamer	6	37	226	148	71	20	17.5	104	87	71	61
heptamer	7	36	255	170	80	23	17.5	122	102	85	74
octamer	8						18	153	125	105	88

[a] calculated for $n=3$ (cf. text).

that the CD spectrum of AMP is temperature-independent while melting of the ordered trimer ApApA at elevated temperature greatly reduces, but even at 80 °C does not fully cancel the positive Cotton effect. While in general the ellipticities of oligonucleotides increase by a similar amount for each nucleotide added to a chain, the adenosine pentamer, $(Ap)_4A$, and uridine tetramer, $(Up)_3U$, are abvious exceptions in that their molar ellipticity is *lower* than that of the preceding shorter oligonucleotide. This property has been reproduced with different oligonucleotide preparations and is not found for other chain lengths.

Also included in Table I is the per residue ellipticity, $[\theta]_{mon}=[\theta]/n$, calculated for the lowest temperature where base interactions should be strongest. One observes an increase for adenylates from the dimer to hexamer, whereas oligouridylates exhibit a rather constant $[\theta]_{mon}$ value which is about twice the ellipticity of uridine 3′- or 5′-phosphate. The high values of per residue ellipticity *vs.* mononucleotide ellipticity indicate the exciton interaction of stacked chromophores [18]. $(Ap)_4A$ and $(Up)_3U$ remain exceptional in this set of data. However, if one considers a trimer as the basic conformational unit of these two oligomers the $[\theta]_{mon}$ values would fit the pattern of the entire series in a reasonable way.

The temperature dependence of the molar ellipticities shows normal noncooperative melting curves (Fig. 2). Data for ApA closely agree with the previously reported picture [19]. The CD spectra of oligouridylates approach the additive value of mononucleotides ($[\theta] \approx n \cdot [\theta]_{UMP}$) at temperatures above 50 °C. For oligoadenylates one observes a much steeper decrease of the Cotton effects with increasing temperature but still a significant positive $[\theta]$ at 80 °C. These oligomer spectra retain the red-shifted envelope at 270 nm (Fig. 1) and do *not* approach the mononucleotide's ellipticity which is negative. The anomalies of pentaadenylate and tetrauridylate (almost) disappear at high temperature.

Consequences of the above observations are twofold: The dependence of an oligonucleotide CD on

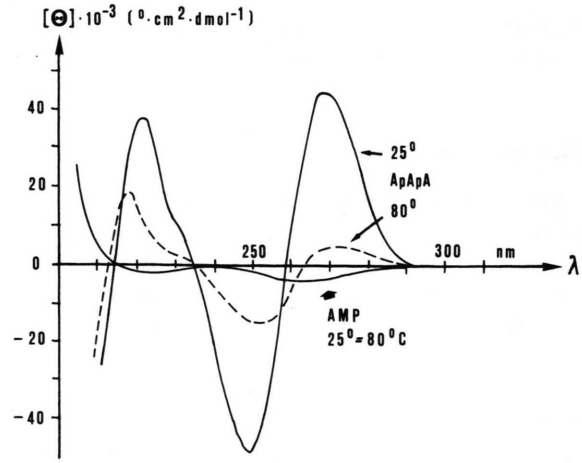

Fig. 1. Circular dichroism spectra of adenosine 5′-phosphate (AMP) and of adenylyl (3′-5′)adenylyl(3′-5′)adenosine (ApApA) in water (pH=8.5) at 25 ° and 80 °C, respectively.

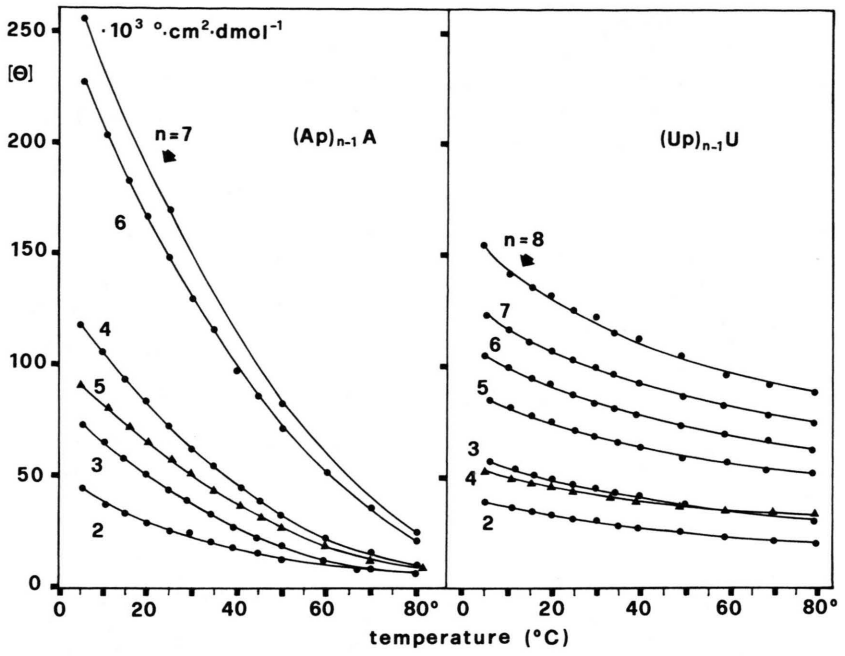

Fig. 2. Temperature dependence of the 270 nm Cotton effects of oligoadenylates (left) and oligouridylates (right). We forgo to construct van't Hoff plots from these data because a classical two-state stacking ⇄ unstacking equilibrium is inapplicable.

structure and temperature should be reassessed, and the specific properties of $(Ap)_4A$ and $(Up)_3U$ have to be considered. For oligoribonucleotides it is very unlikely that CD irregularities are caused by major conformation changes within the monomers (*i.e.*, glycosyl torsion angle or sugar conformation) in view of the rigidity of ribonucleotides even at elevated temperature [20, 21]. It may then be concluded that vertical base stacking does not necessarily increase uniformly with increasing chain length but that even in homologous series some conformers other than the stacked helical array are energetically feasible. This situation is known in crystalline ApA and ApApA [6, 7] and in solutions of tetramers like m_2^6ApUpm_2^6ApU or d(TpApApT) which were recently analyzed in excellent NMR studies [8, 9]. In the early CD studies with incomplete sets of compounds and at much lower instrumental precision the irregularities at certain chain lengths have apparently been overlooked but an unusual chain length dependence of $[\theta]$ with a minimum at about the pentamer unit was, in fact, observed for oligoinosinates [16]. If individual, not regularly stacked conformations occur more often in this class of compounds it follows that the original assumption (composition of a nucleotide chain CD from contributions of constituent dimers) and the use of a CD spectrum alone for describing oligonucleotide conformations are of limited value.

For that very reason we cannot assign precise structures to $(Ap)_4A$ and $(Up)_3U$ which have not as yet been investigated by additional methods. A reasonable approximation would be stacked trimer units with two bases or one base unstacked for in that case the $[\theta]_{mon}$ value is normalized (Table I), and analogous situations are known in ApApA or $(dAp)_ndA$ where one terminal residue must not be stacked [6, 17]. Such conformations are compatible with the compound's simple melting curves (Fig. 2) which do not differ qualitatively from those of the other oligomers.

The residual positive ellipticity of oligoadenylates at high temperature where essentially no ordered structure is expected (Fig. 1) is remarkable. It parallels another long-known and as yet unexplained anomaly, namely the temperature-resistant hypochromicity of poly(A) [10]. A steady state with a small fraction of stacked dimers, constantly formed and dissolved, among the random population of conformers would be one explanation. It must also be pointed out that the circular dichroism of adenosines is particularly sensitive to structure variations at the

C5′ chain, producing negative as well as positive Cotton effects without gross conformational change [22]. In any case the high-temperature conformation of these oligonucleotides will include small deviations from ideal coil behaviour.

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