Oligonucleotide conformations. 4. Temperature and solvent effects on different uridylyl-uridines^o

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SUMMARY

The temperature dependence of the circular dichroism (CD) spectra in hydroalcoholic solutions of five uridylyluridine derivatives was studied. The use of the hydroalcoholic solutions permitted the study of CD spectra as low as -140°C which put in evidence a large negative Cotton effect in the case of U2'P5'Urd which has been interpreted as being due to a left handed helix stack. If either one of the bases of UpU is blocked into the *syn* conformation no stacking occurs. Methylation of the bases in N³ renders the bases more apolar and stabilizes the stacking interaction.

INTRODUCTION

Most conformational studies of dinucleoside phosphates in solution have employed nuclear magnetic resonance^{1,2} or optical methods^{3,4}, especially ultraviolet absorption spectroscopy and circular dichroism (CD)^{5,6}.

The stacking of heterocyclic bases constitutive of nucleic acids each over its neighbour is a temperature sensitive phenomenon. An important observation was made some time ago: the shorter the length of the oligonucleotide chain, the lower the mid-transition temperature^{7,8}. Thus, the study of short-chain oligonucleotides has required the use of solvents which will not freeze even at low temperatures. This has formerly been realised by the use of solvents of high ionic strength (KF or LiCl)^{7,9}. But recently it had been shown¹⁰ that Li⁺ ions perturbed the properties of oligonucleotides, probably by alteration of the hydration shell of the molecule. For this reason we have chosen to use hydromethanolic solvents which have at very low temperatures dielectric constants quite similar to that of water at 20°C^{11,12}. In order to study the base-base interactions of uridine dinucleoside phosphates and some of their analogues.

^o Part 3 is ref. (17).

MATERIAL AND METHODS

Uridylyl-uridine ($U_{3,p5,Urd}$) was from Sigma (St. Louis, MO, USA). 3-Methyl-uridylyl-3-methyl-uridine ($m^3U_{3,p5,m^3Urd}$), uridylyl-6-methyl-uridine ($U_{3,p5,m^6Urd}$) and 2',5'-uridylyl-uridine were prepared according to a general method¹³. 2,2'-O-anhydro-uridylyl-uridine ($ahU_{3,p5,Urd}$) was a gift of Dr.J.C. Catlin¹⁷.

The purity of these compounds was verified before use by thin layer chromatography. Eventual purification was performed by column chromatography, either on Sephadex G 10 in pure water, or on Sephadex A 25¹⁴ with a linear gradient of ammonium carbonate (pH 6.5, 0.01 M to 0.5 M). Methanol Merck "Uvasol" was used throughout for the hydroalcoholic solutions. An empirical formula has been established for the correction of volume expansion of the solvent as a function of temperature¹².

The optical signal was the amplitude of the two first high wavelength CD bands of opposite sign and was recorded on a Roussel-Jouan dichrograph II equipped with a low-temperature cell holder. The temperature range accessible with this device is from -180 to + 40°C. For temperatures above +40°C a Haake thermostat was used. Temperatures were recorded by a thermocouple immersed into the solution connected with a chart recorder and a digital nanovoltmeter. The accuracy of the temperature measurements was within 0.1°C. CD spectra were recorded every 7° approximately.

The spectral data at each alcohol concentration were treated according to two models: 1) the classical two-state model and 2) the oscillating dimer model^{15,16}. In the first case, the change of the optical signal is a symmetric function of $1/T$.

$$-\Delta G = RT \cdot \log_e K = RT \cdot \log_e \frac{A_{\infty} - A_T}{A_T - A_0} = T \cdot \Delta S - \Delta H$$

where K is the temperature dependent equilibrium constant, A_T the optical signal at temperature T , A_{∞} the high temperature limit, A_0 the low temperature limit of the optical signal, ΔS and ΔH the entropy and enthalpy of the transition. Alternatively, the dinucleoside phosphate was assimilated to an oscillating dimer¹⁵.

Spectral data as a function of temperature were processed by a digital computer, using the procedure of Powell et al.⁵ with some modifications as described elsewhere¹⁷ in order to calculate the parameters of the thermal transition.

RESULTS

In this work the optical and thermodynamic parameters of $U_{3,p5,Urd}$, the

Fig.1. Representation of the direct CD spectra (top) and difference CD spectra (bottom) of $U_{3,p5},Urd$ at different temperatures in phosphate buffer pH 6.88 (a), 50% MeOH (b) and 70% MeOH (c).

	a	b	c	
□	Tmin	Tmin	Tmin	
*	5°	-51°	-107°	(°C)
◇	50°	-22°	- 53°	
+	90°	22°	42°	
×	Tmax	Tmax	Tmax	

"natural" mother compound will be compared with the modified analogues.

$U_{3,p5},Urd$. The CD spectra of this compound in phosphate buffer, 50% and 70% methanol are presented in Fig. 1 at different temperatures: three intermediate experimental spectra and the two computed spectra at very high and very low temperature, respectively. Isosbestic points are neatly put in evidence which suggest a distribution between two optically distinct states, completely structured and completely unstructured. Difference spectra (obtained by subtraction of the high temperature spectrum from the others) underline the

Fig. 2. U_3-p_5-Urd : fraction of unstacking as a function of absolute temperature at different concentrations of MeOH in solution.

effect of stacking on the dinucleoside phosphate spectrum. This effect corresponds to an excitonic band arising from the coupling between the B_{2u} transitions of the two successive bases²¹. In Fig. 2 the fraction of unstacked bases as a function of absolute temperature is presented in accordance with the two-state model. If drawn as a function of $1/T$, one obtains a S-shaped curve, symmetrical in $1/T_m$, a finding which is consistent with the two-state model. The van t'Hoff plots for all methanol concentrations are linear, which again is in agreement with the two-state model (Fig. 3). The data for the oscillating dimer model which are not linear are plotted in Fig. 4 and will be discussed together with the data obtained for the other dinucleoside phosphates.

$m^3U_3-p_5-m^3Urd$. This dinucleoside phosphate is less polar than the preceding compound because of the methylation on N^3 . The experimental and limit (computed) spectra are shown in Fig. 5. The isosbestic points are as neat as had been observed for the mother compound which again suggests the applicability of the two-state model, i.e. a distribution between two limit states, each

Fig. 3. Van t'Hoff plots for U_3p_5Urd at different concentrations of MeOH in solution

Fig. 4. Representation of the temperature data according to the oscillating dimer model. The slope of the curves is κ , the force constant of the oscillator.

Fig. 5. Direct CD₃ spectra (top) and difference CD spectra (bottom) of $mU_3'p_5'mUrd$ at different temperature in 20% methanol (a) and 80% methanol (b).

(a)	T_{min}	-13.2°C	+20.0°C	+42.2°C	T_{max}
(b)	T_{min}	-133.0°C	-45.0°C	+40.0°C	T_{max}
symbol	$\square$	$\times$	$\diamond$	$+$	$\times$

intermediate spectrum being a linear combination of the two extreme spectra. Direct CD spectra show a high wavelength positive band, the ellipticity of which is greater than for the corresponding temperatures of $U_3'p_5'Urd$. Plotting the fraction of unstacked bases as a function of $1/T$ again shows the characteristic symmetric S-shaped curve (not shown) as seen for the mother compound; the van t'Hoff plots at all methanol concentrations are linear (Fig. 6a). The oscillating dimer plots are not linear and the oscillating dimer force constant¹⁵ appears to be a function of temperature.

$U_2'p_5'Urd$. It differs from the mother compound by the nature of the phosphodiester linkage. In contrast to $U_3'p_5'Urd$, the first long wavelength band is

Fig. 6. Van t'Hoff plots at different concentrations of MeOH in solution for (a) m^3U_3,p_5,m^3Urd and (b) U_2,p_5Urd .

negative and centered at about 275 nm (Fig. 7) in the spectra measured at low temperatures, while the CD spectra above $+40^\circ C$ have their high wavelength band of positive ellipticity. In addition, this spectrum looks very much like the corresponding spectrum at high temperature of U_3,p_5Urd . It must be noted that for methanol concentrations lower than 50 % v/v the inflexion point of the transition (T_m , temperature of the mid transition) was below the accessible temperature range. Thus it was not possible to compute the optical and thermodynamical data below 50 % methanol. The computed melting curves and the van t'Hoff plots (Fig. 6b) within the range 50 to 80 % methanol again showed the applicability of the two-state model.

ahU_3,p_5Urd and U_3,p_5,m^6Urd . The first of these two dinucleoside phosphates with a *syn*-like structure in the 3'-bound nucleoside did not show any significant CD changes in the same temperature ranges as the three preceding compounds. The absence of a stacked structure of this compound in solution was also indicated by the absence of an excitonic band, characteristic of coupling between homologous transition moments of two adjacent bases. The same phenomenon was observed with U_3,p_5,m^6Urd where the 5'-bound nucleoside is constrained in a *syn* conformation due to the methylation on C⁶ (Fig. 8).

DISCUSSION

Figure 9 summarizes the thermodynamic parameters of the thermal transition at every methanol concentration tested for the first three dinucleoside

Fig. 7. Direct CD spectra (top) and difference CD spectra bottom of $U_2'p_5'Urd$ in 70 % methanol (a) and 80 % methanol (b) at different temperatures

(a)	T_{min}	-107°C	-28.3°C	+42.2°C	T_{max}	
symbol	□	×	◇	+	×	
(b)	T_{min}	-127.3°C	-59.0°C	+20.0°C	+42.8°C	T_{max}
symbol	×	×	◇	+	□	▲

phosphates. The values of the computed enthalpy, entropy and T_m values are plotted as a function of methanol concentration. No values between 0 and 50% methanol are shown for $U_2'p_5'Urd$ for the reasons explained above. The absolute values of ΔH and ΔS increase when increasing the methanol concentration in the solvent, to reach an optimum around 20% methanol. Above this methanol concentration ΔH and ΔS decrease and level off around 80% methanol. T_m 's show similar variations, only below 20% the variations are very small and not very significant. The thermodynamic parameters of the methylated UpU are always larger than those of UpU itself which indicates an increased stability

Fig. 8. Direct CD spectra at three temperatures in 7:3 MeOH:H₂O for U₃p₅,m⁶Urd.

of the stacking of the former, which can be accounted for by the difference in polarity of the bases. The solvent exclusion, especially water, from the hydrophobic interbase volume is thus increased, strengthening the stability of stacking. Above 20% methanol the solvation of the bases by methanol becomes predominant and thus decreases the stability of stacking.

The greater stability of stacking of m³U₃p₅,m³Urd over U₃p₅,Urd can also be seen through the relative importance of the exciton band around 275nm (Figs. 1 and 5, respectively). Model building with CPK models shows that the low temperature structured form is probably a right handed helix. The homologous transition moments B_{2u} form a positive angle between each other.

In the range between 50 and 80 % methanol for U₂p₅,Urd ΔH, ΔS and T_m vary in the same sense as the two other dinucleoside phosphates, but the stability of stacking is decreased compared with them (Fig. 9a). The CD difference spectra (Fig. 7) show an exciton band at 275 nm, the absolute value of which is lower than for the 3'-5' mother compound. The striking fact, however, is that this band is negative, which can be accounted for by a left handed helix in which the angle between the two B_{2u} bands will be negative. This structure can easily be constructed with CPK models. This idea is in good agreement with suggestions in the literature^{1,2,8} on other 2',5' di-

Fig. 9. Representation of thermodynamic parameters of the thermal transitions in different concentrations of methanol for $U_{3,p5},Urd$ and $U_{2,p5},Urd$ (a, left), $m^3U_{3,p5},m^3Urd$ (b, below)

$U_{3,p5},Urd$ $U_{2,p5},Urd$
 ΔH° —●—●— —■—■—
 ΔS° —○—○— —□—□—
 T_m —△—△— —▲—▲—

$m^3U_{3,p5},m^3Urd$:

ΔH° —●—●—
 ΔS° —○—○—
 T_m —△—△—

ΔH° in $kcal.mole^{-1}$
 ΔS° in $cal.mole^{-1}.deg^{-1}$
 T_m in $^\circ C$

nucleoside phosphates, that $A_{2,p5},Ado$ and $A_{2,p5},Cyd$ may be a left handed helix. At high temperature (above $+5^{\circ}C$), however, the left-handed helix may be only a small percentage according to these data² which is in agreement with our results.

In contrast with the preceding compounds, $ahU_{3,p5},Urd$ and $U_{3,p5},m^6Urd$, both of which have one of the nucleosides in a *syn* conformation show no detectable stacking. Model building clearly shows that it is impossible to put the two bases into a parallel stack in the case of $ahU_{3,p5},Urd$, but do not exclude stacking for $U_{3,p5},m^6Urd$. Apparently, the inversion of the base on the 5'-bound residue brings about a conformation which does not allow coupling with the transition moment of the 3'-bound base.

CONCLUSIONS

- 1) The use of hydromethanolic solvents has proven useful for the study of dinucleoside phosphate interactions. At a concentration of about 20% methanol, optimal stabilisation was observed.
- 2) Increase of the hydrophobic nature of the bases increased the stability of the stacking interaction
- 3) In contrast with $U_{3,p5},Urd$ which appears to form a right handed helix, the 2',5' analogue is probably stacked in a left handed helix as had been suggested before for other 2',5'-dinucleoside phosphates^{1,2}. Here the low-temperature spectra permitted the direct observation of the negative Cotton effect.
- 4) The *syn* conformation of either one of the two nucleosides prevents the formation of a stacked structure. This is in contrast with purine containing dinucleoside phosphates^{19,20}.

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