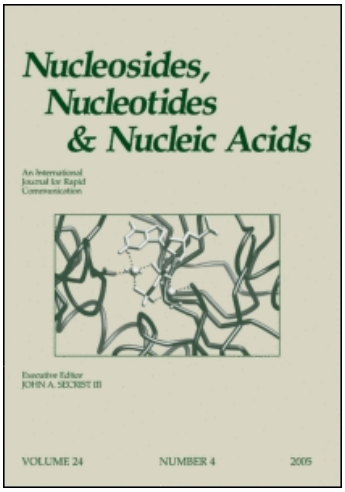

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INTERACTION OF (2' -5') AND (3' -5') LINKED 2-AMINOADENYLYL-2-AMINOADENOSINES WITH POLYURIDYLIC ACID

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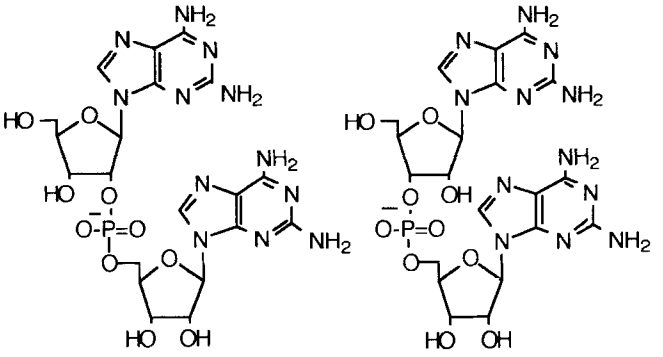
ABSTRACT: 2'-5' and 3'-5' linked 2-aminoadenylyl-2-aminoadenosines [(2'-5')n^{2}Apn^{2}A (**1**) and (3'-5')n^{2}Apn^{2}A (**2**)] were synthesized by condensation of 5'-*O*-monomethoxytrityl-*N*^{2}, *N*^{6}-dibenzoyl-2-aminoadenosine and *N*^{2},*N*^{6},2',3'-*O*-tetrabenzoyl-2-aminoadenosine 5'-phosphate using dicyclohexylcarbodiimide (DCC). The conformational properties of these dimers **1** and **2** were examined by UV, NMR and CD spectroscopy. The results reveal that the 2'-5'-isomer **1** takes a stacked conformation, which contains a larger base-base overlap and is more stable against thermal perturbation with respect to the 3'-5'-isomer **2**. Interactions of **1** and **2** with polyuridylic acid (Poly (U)) were also examined by *T*m , mixing curves , UV and CD spectra. Both the dinucleoside isomers **1** and **2** formed a complex of 1 : 2 stoichiometry with poly(U), which was much more stable than that of the corresponding ApA isomer

Since the findings[1, 2] that the DNA of cyanophage S-2L contains exclusively 2,6-diaminopurine (2-aminoadenine) in place of adenine, the effects of this modified base on the physicochemical and biochemical properties of nucleic acids have been of interest. 2-Aminoadenine can form three hydrogen bonds with thymine or uracil enhancing stability of the base pair.

In the deoxyribonucleotide series, polynucleotides and oligonucleotides containing 2-aminoadenine in place of adenine were synthesized and used for DNA structural studies, [3-8] elucidation of DNA-protein interactions[4] and for a variety of applications including improved hybridization probes.[9-11] In the ribonucleotide series, properties of poly-2-aminoadenylic acid [poly(n^{2}A)][12, 13] and random copolymers of adenylic acid and poly(n^{2}A)[14] have been investigated. They form both double and triple helices with complementary polyuridylic acid [poly(U)] depending on the conditions. In these studies,



(2'-5')n²Apn²A **(3'-5')n²Apn²A**

(1) **(2)**

FIGURE 1

however, the polynucleotides contain only 3'-5' phosphodiester linkage. No investigation of the effects of 2'-5' phosphodiester linkage on the complex formation is reported.

Because dinucleoside monophosphates are the simplest models of nucleic acids, it is worthwhile to investigate the physicochemical properties of a system containing dimers which have well defined chemical structure when compared with polynucleotides prepared enzymatically. We attempt to study interaction of 2'-5' linked 2-amino-adenylyl-2-aminoadenosine [(2'-5')n²Apn²A (**1**)] and 3'-5' linked 2-aminoadenylyl-2-aminoadenosine [(3'-5')n²Apn²A (**2**)] with poly(U) and compare the results with the interactions of corresponding isomers of ApA with poly(U). In this study, we focused on the system of triple stranded complexes in the presence of magnesium ions because only the triple stranded complexes were present under these conditions and the complexes showed stability convenient for physicochemical analysis.

In this paper, we report synthesis and properties of the phosphodiester linkage isomers, **1** and **2**, and their interactions with poly(U) as examined by UV, NMR and CD spectroscopy. The 2'-5' isomer (**1**) takes more stacked conformation than the 3'-5' isomer (**2**) as observed in the case of ApA isomers. Both isomers form a n²Apn²A•2poly(U) complex in the presence of 10 mM $MgCl_2$ with Tm's at around 27°C which are much higher than those for the ApA isomers. In the thermal melting process, both complexes show a single transition whereas poly(n²A)•2poly(U) is reported to show a biphasic melting curve.

For the preparation of such modified oligonucleotides the use of the N^2-isobutyryl-N^6-benzoyl derivative of 2,6-diaminopurine was reported.[3,4] This compound

was found to be deprotected very slowly with ammonia. Replacing the benzoyl group in N^6 position by *N,N*-dibutylformamidine[15] or *N*-methyl-2-pyrrolidine amidine[10] groups increases the lability to ammonia but long treatment (2-3 days) is still needed for the complete deprotection. Recently a more labile phenoxyacetyl (Pac) group has been reported.[16,17] The deprotection was performed with concentrated ammonia at 60°C for 2 hr.[17]

In the previous papers,[18,19] we reported that synthesis of dinucleoside monophosphates containing 2-aminoadenine and 8,2'-S-cyclo-2-aminoadenosine were achieved by deprotection of N^2,N^6-diacetyl- or N^2,N^6-dibenzoyl-2,6-diaminopurine with 40% aqueous methylamine at room temperature for 2-3 hr. In this work, N^2 and N^6 position of 2,6-diaminopurine were also benzoylated and deprotected by treatment with 40% aqueous methylamine at room temperature for 4 hr after condensation.

Synthesis of (2'-5')n^{2}Apn^{2}A (1) and (3'-5')n^{2}Apn^{2}A (2)

The dinucleoside monophosphates **1** and **2** were synthesized by the phosphodiester method with suitable protected nucleoside and nucleotide units. 2-Aminoadenosine (n^{2}A)[20-26] was treated with benzoyl chloride in pyridine to give N^2,N^6,2',3',5'-*O*-pentabenzoyl-2-aminoadenosine. Selective debenzoylation of the sugar moiety by treatment with 1 M sodium hydroxide at 0°C gave N^2, N^6-dibenzoyl-2-aminoadenosine (**3**). Treatment of **3** with monomethoxytrityl chloride in DMSO and pyridine yielded 5'-*O*-monomethoxytrityl-N^2, N^6-dibenzoyl-2-aminoadenosine (**4**). 2-Aminoadenosine 5'-phosphate (pn^{2}A)[14] was treated with benzoyl chloride in pyridine at 0°C and converted to a pyridiniun salt to give the fully protected pyridinium N^2, N^6, 2', 3'-*O*-tetrabenzoyl-2-aminoadenosine 5'-phosphate (**5**). The condensation of **4** and **5** was achieved by using dicyclohexylcarbodiimide (DCC) as the condensing reagent at 30°C for 72 hr. Deprotection of the benzoyl and momomethoxytrityl groups was carried out with 15 M methanolic ammonia at 30°C for 12 hr and 80 % aqueous acetic acid at 25°C for 2 hr. Debenzoylation at the N^2 position was achieved by use of 40% aqueous methylamine at 25°C for 4 hr. Column chromatography on Dowex 1x2 (formate form) and further on DE-23 cellulose (bicarbonate form) gave (2' -5')n^{2}Apn^{2}A (**1**) and (3' -5')n^{2}Apn^{2}A (**2**) in yields of 46 % and 16 %, respectively. The structures of these dimers **1** and **2** were confirmed by properties on paper electrophoresis (PEP), UV, CD and NMR spectra as described later. These dinucleoside monophosphates were completely hydrolyzed with crude snake venom phosphodiesterase to give approximately equal amounts of n^{2}A and pn^{2}A, respectively, on PEP with authentic samples.

Proton Magnetic Resonance Spectra of the Dimers

The proton NMR spectral data of the dimers are presented in Table 1, together with the data for pn^2A and n^2A. The signal assignments were made by decoupling, nuclear Overhauser effect (NOE) and DQF-COSY experiments. The proton signals for each sugar residue were assigned by tracing the crosspeaks of the COSY spectra. Two sets of sugar resonances of a dimer can be assigned to the specific sugar residues since 5'-methylene protons of a free 5'-terminal residue usually resonate at the highest field. Assignment of the base proton signals was made by observing NOE's between H8 and sugar protons (H1' and H2'). When the chemical shift data of the $-pn^2A$ protons of each dimer and pn^2A are compared, H8 and H1' of $-pn^2A$ residue of the 2'-5' isomer show considerably larger upfield shifts than those of the 3'-5' isomer (0.40 and 0.21 ppm vs. 0.24 and 0.06 ppm). These upfield shifts are assumed to be caused by the shielding effect of the base of n^2Ap- residue and are usually observed when a dimer takes a right-handed stacked conformation. This result suggests that $(2'\text{-}5')n^2Apn^2A$ has more extensive base-base overlapping than the 3'-5' isomer. A similar result is also obtained in the case of ApA isomers.[27]

Ultraviolet Absorption Spectra of the Dimers

The ultraviolet (UV) absorption spectral properties of the dimers in 0.01 M phosphate buffer (pH 7.0) at room temperature are presented in Table 2. The molar extinction coefficients of the dimers were determined from the hyperchromicity observed upon complete digestion by alkaline hydrolysis.

These dimers as well as n^2A show two peaks around at 256 and 280 nm regions. The isomer **1** shows higher hypochromicity at both wavelengths suggesting that the 2'-5' isomer has a more stacked conformation than the 3'-5' isomer. A similar trend is also observed for ApA isomers.[28]

Circular Dichroism Spectra of the Dimers

Circular Dichroism (CD) spectra of the dimers at various temperatures are shown in Figure 2. The spectral data at 0 °C are presented in Table 3. Both dimers show very similar spectral patterns except for the 240-260 nm region: two pairs of positive and negative bands centered at around 285 and 220 nm. The 2'-5' isomer shows definitely larger bands in the short wavelength region. A similar trend is also seen in the case of ApA isomers.[28] These results suggest that two n^2A bases in these dimers stack on each other.

The intensities of the CD bands decrease with increasing temperature due to destacking. It appears that the stacking conformation of the 3'-5' isomer is more sensitive to thermal perturbation than that of the 2'-5' isomer since the CD change is more profound for the 3'-5' isomer. In other words, the 2'-5' isomer has a more stable stacked

TABLE 1 ^{1}H-NMR Data for the Dimers and Related Compounds

Compound		Chemical shift ppm (Hz)[a] H8	H1'	H2'	H3'	H4'	H5'	H5"
(2'-5')n^{2}Apn^{2}A 1	n^{2}Ap-	7.86	6.02 (4.5)	5.11	4.64	4.22	3.84	3.72
	-pn^{2}A	7.82	5.73 (3.1)	4.34	4.14	4.36	4.06	3.96
(3'-5')n^{2}Apn^{2}A 2	n^{2}Ap-	7.90	5.74 (3.6)	4.63	4.33[b]	4.31[b]	3.84	3.75
	-pn^{2}A	7.98	5.88 (4.4)	4.62	4.50	4.33[b]	4.16	4.15
n^{2}A		8.00	5.87 (6.0)	4.73	4.42	4.27	3.93	3.84
pn^{2}A		8.22	5.94 (5.8)	4.77	4.52	4.36	4.05	4.04

[a] $J_{1'2'}$ is shown in parentheses.

[b] We were not able to definitely assign H3' and H4' resonances of (3'-5')n^{2}Apn^{2}A because of serious overlapping of the signals.

[c] Samples except n^{2}A were measured as the Na salt in D_2O.

conformation. This is consistent with the NMR and UV data that suggest stronger and more extensive stacking interactions for the 2'-5' isomer. A similar phenomenon is also observed for ApA isomers.[27,28]

Interaction with Poly uridylic Acid

The UV absorption properties in mixtures of continuous variation were used to determine the stoichiometry of the interaction between the dimers and polyuridylic acid (poly(U)). The mixing experiments were done in 0.01 M sodium phosphate buffer (pH 7.0), 0.1 M NaCl, 0.01 M $MgCl_2$ at 0°C by measuring UV spectra. The mixing curves monitored at three wavelengths are shown in Figure 3. The mixing curves for both

TABLE 2 Ultraviolet Spectral Properties for the Dimers and Related Compounds

Compound	$\lambda_{max}^{pH7.0}$	$\varepsilon_{max} \times 10^{-3}$ [c]	$\lambda_{min}^{pH7.0}$	$\varepsilon_{min} \times 10^{-3}$ [c]	Hypochromicity[d] (%)
(2'-5')n^{2}Apn^{2}A[a]	256.5	8.30	237.0	4.80	14.0
1	279.5	8.20	267.0	6.80	18.0
(3'-5')n^{2}Apn^{2}A[a]	256.5	8.70	267.5	6.50	9.4
2	279.0	8.50	328.0	5.70	15.4
(2'-5')n^{2}Apn^{2}A• 2poly(U) [b]	258.0	6.20	238.0	3.10	
(3'-5')n^{2}Apn^{2}A• 2poly (U) [b]	258.0	5.10	238.0	2.60	
n^{2}A [a]	256.0	9.60	236.0	5.40	
	280.0	10.40	266.0	8.10	

[a] Spectra were measured in 0.01 M phosphate buffer (pH 7.0) at 25°C.
[b] Spectra were measured in 0.01 M phosphate buffer (pH 7.0) 0.1 M NaCl and 0.01 M $MgCl_2$ at 0°C.
[c] Calculated from the hyperchromicity observed upon alkaline hydrolysis.[27] The ε values are expressd in terms of per base residue.
[d] The hypochromicity values are calculated[27,28] comparing the ε_{max}'s of the dimer and the monomer n^{2}A.[25]

isomers show a discontinuity at 63 - 67 mole % poly(U). These results suggest the formation of a n^{2}Apn^{2}A•2poly(U) complex for both isomers under these conditions. Similar results are also obtained for ApA isomers.[28]

Properties of n^{2}Apn^{2}A•2poly(U) Complexes

Thermal stability of the dimer•2poly(U) complexes was examined by measuring UV-temperature profiles (Figure 4). Both complexes show sharp transition profiles with *T*m's at 27.5°C (2'-5' dimer complex) and 26.5°C (3'-5' dimer complex). These *T*m values are 13 - 16°C higher than those reported for the corresponding complexes of ApA isomers (11.3°C for the 2'-5' dimer complex and 13.6°C for the 3'-5' dimer complex in 0.01 M Tris buffer (pH 7.5), 0.01 M $MgCl_2$).[28] This much higher thermal stability of n^{2}Apn^{2}A•2poly(U) complexes should be due to the third hydrogen bond formed between

TABLE 3 Circular Dichroism Spectral Data for the Dimers and Related Compounds

Compound	Wavelength λ max (nm)	[θ] x 10^{-4}	λ min (nm)	[θ] x 10^{-4}
(2'-5')n^2Apn^{2}A	225.7	5.85	212.0	-4.88
1	294.8	1.00	275.8	-1.75
(3'-5')n^2Apn^{2}A	226.3	4.34	213.0	-3.94
2	294.4	1.13	249.4	-0.43
			275.2	-1.44
(2'-5')n^2Apn$_2$A• 2Poly(U)	265.8	3.06	246.1	-1.30
			292.3	-0.09
(3'-5')n^2Apn^{2}A• 2Poly(U)	265.7	3.19	246.4	-0.94
			293.5	-0.57
Poly (U)	220.0	1.09	246.6	-1.50
	260.2	5.61		

Measured solutions contained 8 x 10^5M total nucleotide in 0.01 phosphate buffer (pH 7.0), 0.1 M NaCl and 0.01 M $MgCl_2$ at 0°C.
The [θ] values are presented in terms of per base residue value.

a 2-amino group of n^2A residue and a 2-carbonyl group of U residue. It was a rather unexpected result that the (2'-5') n^2Apn^{2}A complex showed a slightly higher *T*m than the (3'-5')n^2Apn^{2}A complex. Stabilization by the third hydrogen bond and stronger stacking interaction may overwhelm the disadvantage due to the unfavorable backbone configuration for incorporation into a triple-stranded RNA structure.

CD spectra of the dimer•2poly(U) complexes at 0°C and 40°C are shown in Figure 5, together with the addition spectra which were obtained by summation of the component spectra. For both complexes, the observed spectra at 0°C, where formation of the triple-stranded complexes are complete, are markedly different from the corresponding addition spectra. The CD change upon complex formation occurs mostly in a negative direction and is especially prominent in the short wavelength region. In the case of the 3'-5' dimer complex, a distinct negative band is observed in the longest

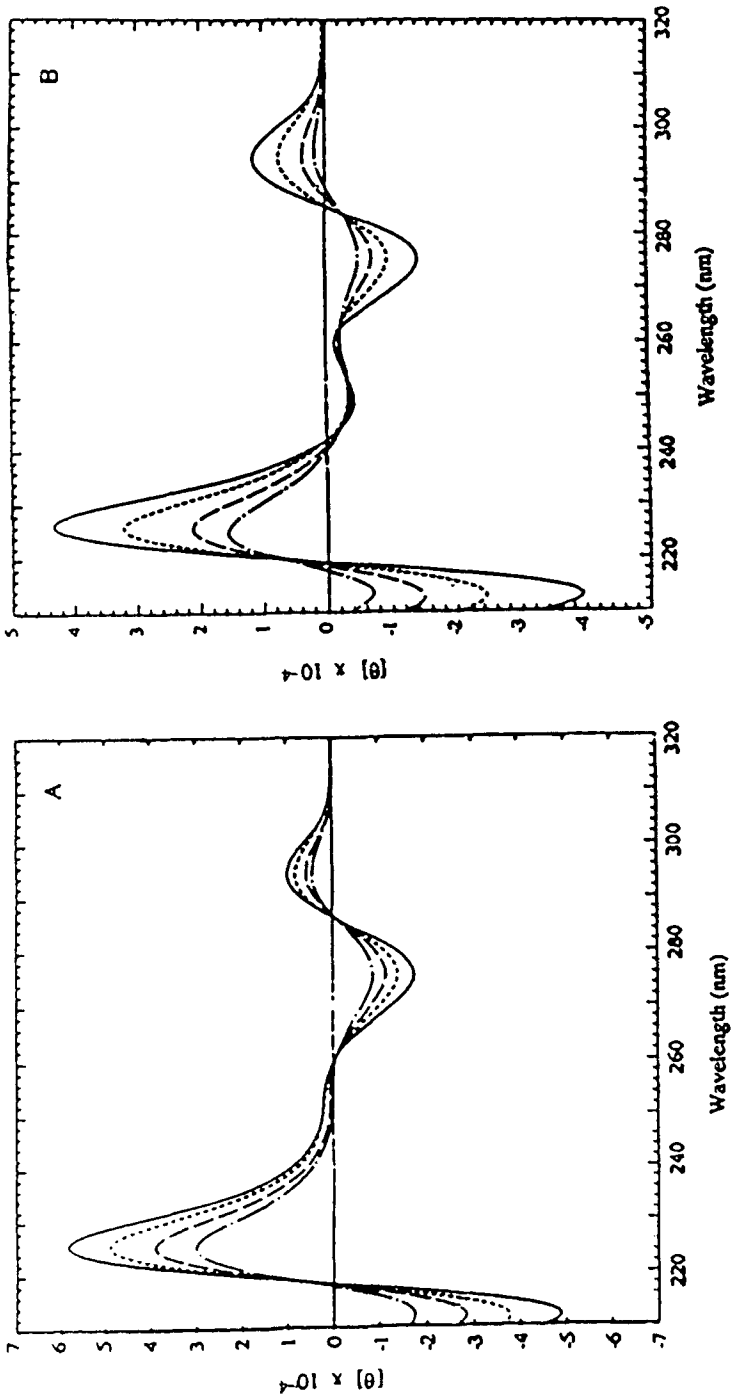


FIGURE 2 : CD spectra of (2'-5')n^{3}Apn^{3}A (A) and (3'-5')n^{3}Apn^{3}A (B). Measued at 0°C (——), 20°C (·····), 40°C (– – –) and 60°C (– · – · –) in 0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl and 0.01 M $MgCl_2$. The total nucleotide concentration was 8×10^{-6} M.

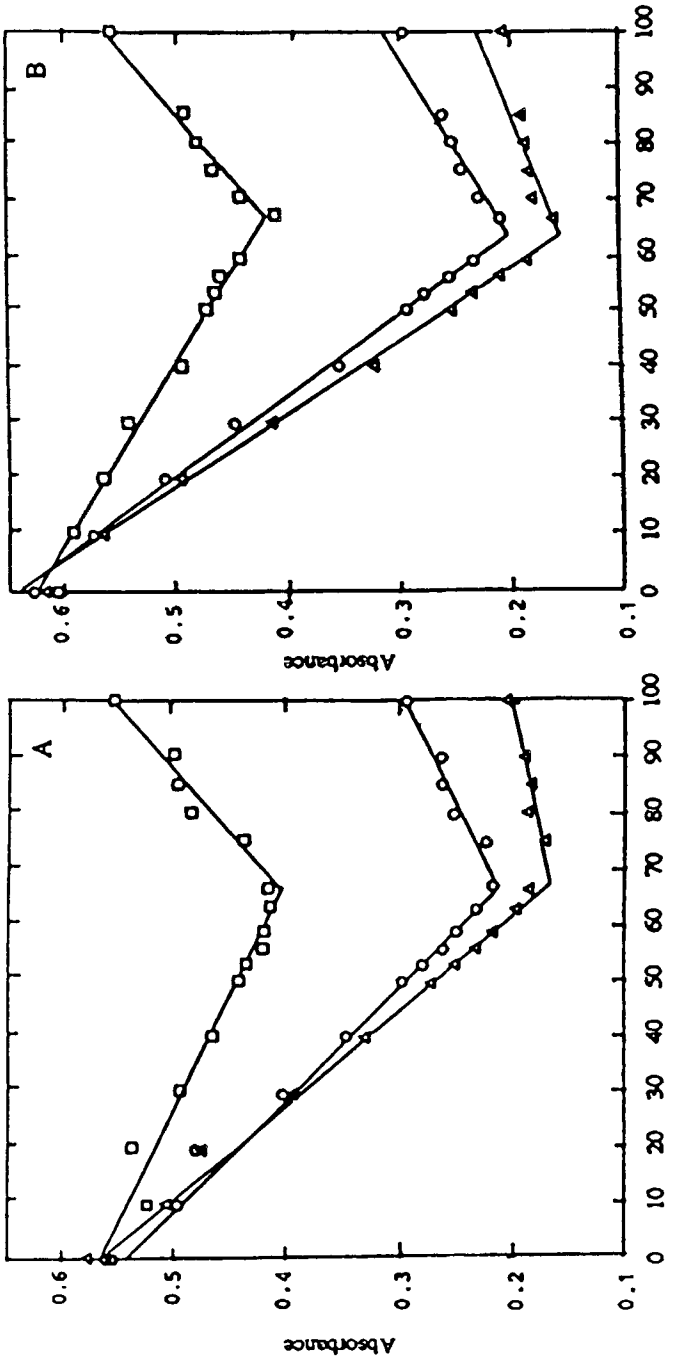


FIGURE 3 : Mixing curves for (2'-5')n^{2}Apn^{2}A and Poly(U) (A), for (3'-5')n^{2}Apn^{2}A and Poly(U) (B). Monitored at 260 nm (□——□), 275.5 nm (○——○) and 280 nm (△——△). The experiments were carried out at 0°C in 0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl and 0.01 M $MgCl_2$. The total nucleotide concentration was 8 x10^{-6} M.

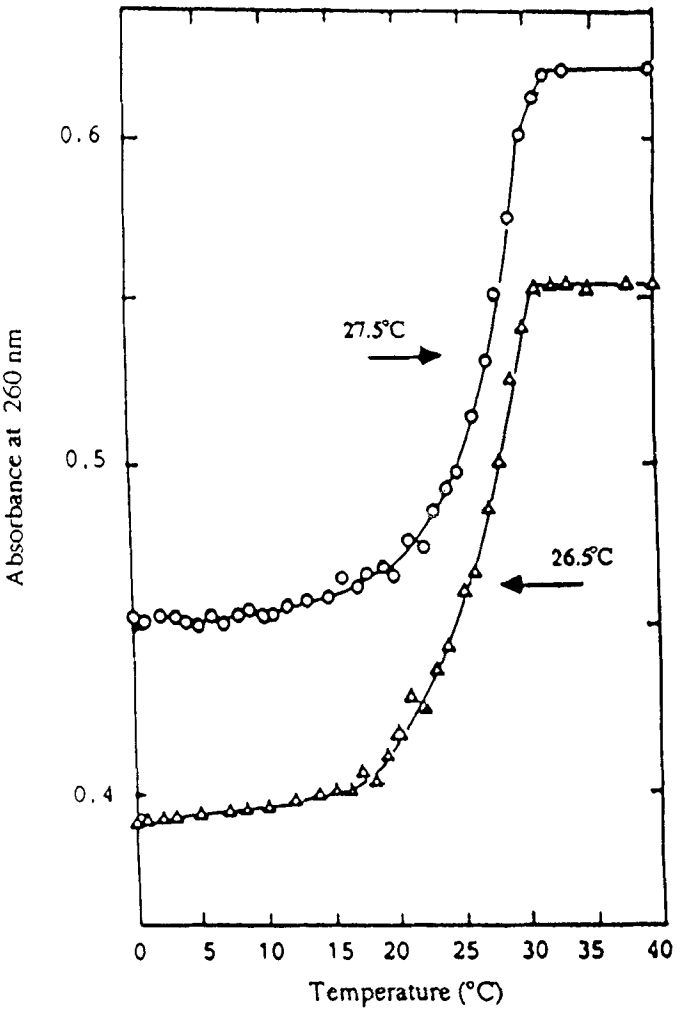


FIGURE 4 : UV Melting curves of (2'-5')n^{2}Apn^{2}A •2 Poly (U) (○—○) **and (3'-5')n^{2}Apn^{2}A •2Poly (U)** (△—△). Monitored with UV absorbance at 260 nm in 0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl and 0.01M $MgCl_2$. The total nucleotide concentration was 8×10^{-5} M.

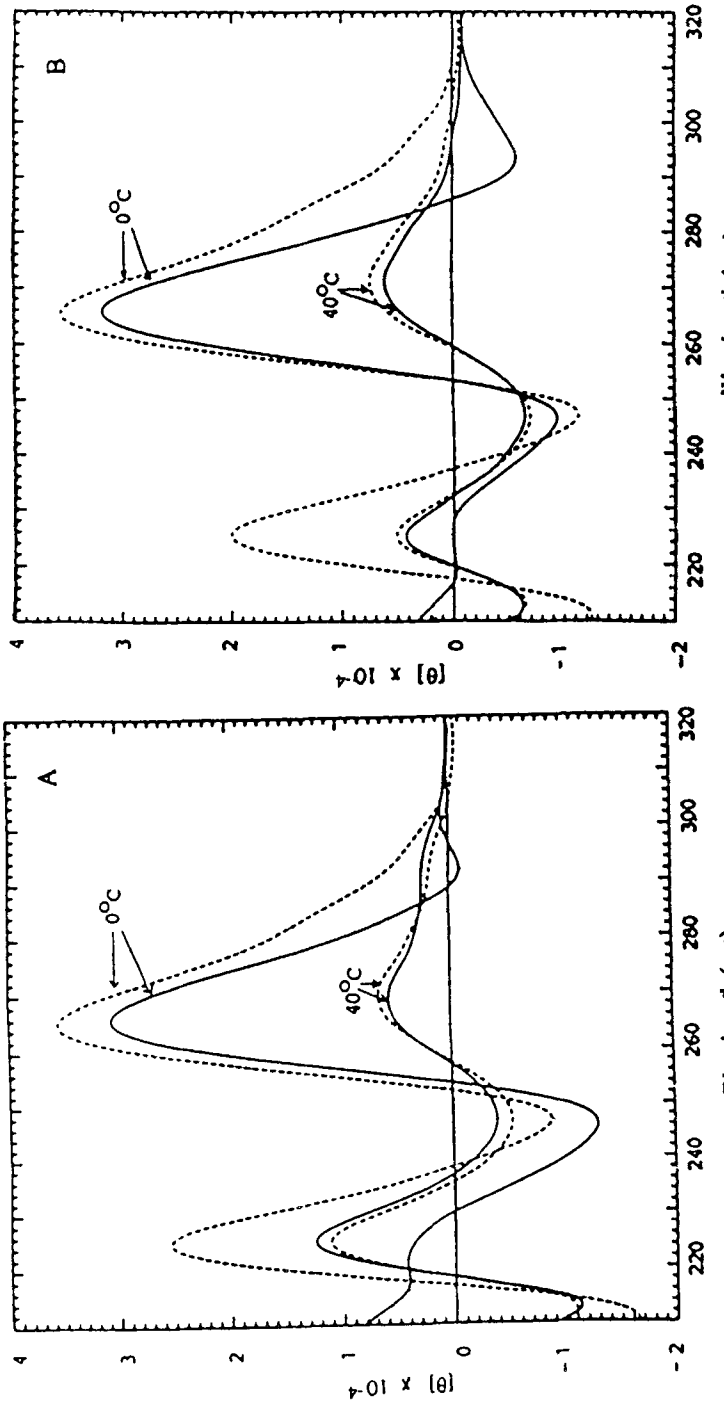


FIGURE 5 : CD spectra of (2'-5')n^{3}Apn^{3}A •2 Poly(U) (A) and of (3'-5')n^{3}Apn^{3}A •2Poly(U) (B). Measured at 0°C (——) and 40°C (——) in 0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl and 0.01M $MgCl_2$. The total nucleotide concentration was 8 x10^{-6} M. Addition spectra at 0°C (- - - -) and 40°C (- - - -) are also shown.

wavelength region. A similar negative band is also observed in the case of poly(n^2A)•2poly(U) complex.[12] The observed spectra at 40°C, where melting of the complexes are complete, are almost the same as the corresponding addition spectra suggesting no interactions between the dimers and poly(U).

In the case of poly(n^2A)•2poly(U) complex, the complex shows a two-step melting profile in the presence of relatively low concentration of NaCl (~0.25 M) as monitored by absorbance at 260 nm.[12] The first (lower) *T*m corresponds to a transition for the triple- to double-stranded complex (3 → 2 transition) and the second one corresponds to a transition for the double-stranded complex to single-stranded components (2 → 1 transition). Difference between the two *T*m's decreases with increasing salt concentration. The high salt concentration may preferentially stabilize the three-stranded complex neutralizing the crowded negative charges of the sugar-phosphate chains. Poly(A)•2poly(U) complex also shows a biphasic melting at low salt concentration although the difference in the *T*m's is much smaller. It is also reported that a single transition is observed for poly(A)•2polu(U) at 260 nm in the presence of 0.001 M $MgCl_2$ and 0.1 M NaCl.[29] In the case of n^2Apn^{2}A•2poly(U), only a single transition was observed under the present conditions (0.01 M $MgCl_2$, 0.1 M NaCl) as monitored by absorbance at 260 nm. No biphasic profile was observed when monitored at different wavelengths. A single transition is also observed in the case of ApA•2poly(U) complex under similar conditions.[28] We think that the transition corresponds to a 3 → 1 transition. If there is a hidden 3 → 2 transition, the *T*m should be lower than that of 2 → 1 transition. However, CD spectrum of each n^2Apn^{2}A•2poly(U) complex at 20 °C where the melting is about to start, was almost the same as that at 0°C, where the 2 : 1 stoichiometry had been confirmed. These results suggest that n^2Apn^{2}A•2poly(U) complexes melt with a cooperative 3 → 1 transition. More stable interactions between n^2Apn^{2}A and the first poly(U) may cooperatively stabilize association of the second poly(U).

EXPERIMENTAL

General Methods: UV absorption spectra were measured with a Hitachi 340 spectrometer. Mixing curves and UV melting data (*T*m) were obtained on a Hitachi U-3000 spectrometer. ^{1}H-NMR spectra were taken, otherwise mentioned, with a Bruker AMX-400 at 25° C. Samples were used as the Na salt in D_2O. NOE difference spectra were taken directly subtracting the FIDs for each scan. CD spectra were measured with a JASCO J-720A spectropolarimeter. Analytical HPLC was performed on a Hitachi HPLC 655A system apparatus with a Senshu ODS-2251-N chromatographic

column. Thin layer chromatography (TLC) was performed on plates of silica gel (Merk 60 F_{254}). For column chromatography, silica gel (Merk 60H) was used. Paper electrophoresis (PEP) was performed using 0.05 M triethylammonium bicarbonate (TEAB) buffer (pH 7.5) at 900 V/40 cm for 1 hr on Toyo filter paper No.51A. Poly (U) was purchased from Yamasa Corp. (Lot No. 401083). The molar extinction coefficients of (2'-5')n^{2}Apn^{2}A (**1**) and (3'-5')n^{2}Apn^{2}A (**2**) were determined[27,28] from the results of alkaline hydrolysis experiments.

Synthesis of N^2,N^6-Dibenzoyl-2-aminoadenosine (3)

To a suspension of 2-aminoadenosine[20-26] (0.95 g, 3.4 mmol) in pyridine (100 mL) was added benzoyl chloride (4.7 mL, 40 mmole) in an ice bath. After 1 hr, the mixture was poured into dichloromethane (56 mL) and water (40 mL) containing sodium bicarbonate (3 g). The aqueous layer was extracted with dichloromethane (25 mL x 3) and the organic layer was washed with water 2 times and concentrated to a gum. The gummy residue was dissolved in a mixture of ethanol (10 mL) and pyridine (7.6 mL) and the solution was treated with a mixture of 2 M sodium hydroxide (13 mL) and ethanol (13 mL) at room temperature for 15 min. The reaction mixture was neutralized with 2 M hydrochloric acid (13 mL) at 0°C and concentrated. The residue was dissolved in dichloromethane and purified by silica gel column chromatography (4 x 9 cm) using a dichloromethane-ethanol (19 : 1) elution system. The eluate was evaporated to give a foamy material (0.5 g, 31 %). mp. 220-222°C. TLC: $CHCl_3$- EtOH (19 : 1) Rf 0. 14. UV: λ max 50% EtOH 256 and 300(sh) nm, λ max H^+ 256, 310 (sh) nm, λ max OH^- 271 (sh), 312 (sh) nm. Anal. Calcd. for $C_{24}H_{22}O_6N_6$: C, 58.77; H, 4.52; N, 17.14. Found: C, 58,18; H, 4,30; N,16.87.

5'-*O*-Monomethoxytrityl-N^2,N^6-dibenzoyl-2-aminoadenosine (4)

N^2,N^6-Dibenzoyl-2-aminoadenosine (**3**) (0.49 g, 1 mmole) was dissolved in DMSO (2 mL) at 40°C. To the solution pyridine (1.5 mL) and monomethoxytrityl chloride (0.39 g 1.26 mmole) were added and stirred at 25°C for 48 hr in a dark place. The reaction mixture was treated with methanol for 10 min at 25 °C and evaporated *in vacuo*. The residue was dissolved in dichloromethane (40 mL), washed with 0.01 M triethylammoniun bicarbonate (TEAB) buffer (pH7.5, 40 mL) and concentrated. The residue was purified by silica gel column chromatography (3 x 12.5 cm) using a chloroform-ethanol (19 : 1) elution system and recrystallized from methanol (0.21 g, 27.3%). mp. 176 - 178 °C, TLC: $CHCl_3$-EtOH 19:1 , Rf, 0.53. UV: λ max 50% EtOH 236, 255, 283, 299 nm, λ max H^+ 236, 255 nm, λ max OH^- 271, 312 nm. Anal. Calcd: for $C_{44}H_{38}O_7N_6$. CH_3OH: C, 67.99; H, 5.33; N, 10.57. Found: C, 68.08; H, 5.20; N, 10.37.

Pyridinium N^2,N^6,2',3'-*O*-tetrabenzoyl-2-aminoadenosine 5'-phosphate (5)

Pyridinium 2-aminoadenosine 5'-phosphate[14] (0.5 mmole) was coevaporated with pyridine (10 mL x 3) and suspended in pyridine (7.6 mL). Benzoyl chloride (2.6 mL) was added to the solution at 0°C and the mixture was kept at room temperature for 5 hr. The solution was added to a saturated solution of sodium bicarbonate (16 mL) in an ice bath and extracted with chloroform (20 mL x 3). The chloroform layer was washed with water and evaporated. The residue was rendered anhydrous by coevaporation with pyridine and treated with acetic anhydride (7.7 mL) in pyridine (7.7 mL) for 18 hr at 25° C. Acetic anhydride was evaporated *in vacuo* and methanol (10 mL) was added in an ice bath for 2 hr. The solution was evaporated *in vacuo* and 50% aqueous pyridine (21 mL) was added at 0°C. The solution was kept at 25°C for 18 hr and evaporated to make anhydrous pyridine solution. Coevaporation with pyridine was repeated three times and the pyridine solution (2.5 mL) was added to ether (75 mL) with vigorous stirring. The precipitate was centrifuged and washed with ether three times and then dried in a desiccator over P_2O_5. The N^2,N^6,2',3'-*O*-tetrabenzoyl-2-aminoadenosine 5'-phosphate (397 mg, 0.425 mmole) was dissolved in 50% aqueous pyridine (10 mL) and passed through a column (1 x 12 cm) of pyridinium Dowex 50 X2. The eluate and washing were combined and made anhydrous by coevaporation with pyridine and the anhydrous pyridine solution (4 mL) was added to ether (120 mL). The precipitate was washed with ether three times and dried *in vacuo* over P_2O_5. The yield was nearly quantitative. TLC: CH_2Cl_2- EtOH (19 : 1), Rf 0.08. UV : λ max 50%EtOH 237, 335 (sh) nm.

(2'-5') n^2Apn^2A (1) and (3'-5')n^2Apn^2A (2)

5'-*O*-Monomethoxytrytyl-N^2,N^6-dibenzoyl-2-aminoadenosine (**4**) (76.3 mg, 0.1 mmole) and pyridines N^2,N^6,2',3'-*O*-tetrabenzoyl-2-aminoadenosine 5'-phosphate (**5**) (191.3 mg, 0.1 mmole) were coevaporated with pyridine three times and the dry residue was dissolved in pyridine (0.6 mL). The mixture was treated with DCC (193,3 mg, 0.5 mmole) for 72 hr at 30°C. Water (9.7 mL) was added and the mixture was kept for 12 hr at 30°C. Cyclohexylurea was removed by filtration and the filtrate was extracted with *n*-pentane. The aqueous solution was concentrated and treated with 15 M methanolic ammonia (12 mL) for 16 hr at 30°C. The volatile materials were removed by evaporation and the residue was treated with 40% aqueous methylamine (15 mL) for 4 hr at 25°C. The solution was evaporated and coevaporated with methanol (5 mL x 3). The residue was treated with 80 % aqueous acetic acid (9 mL) for 2 hr at 25 °C. Acetic acid was evaporated and the residue was dissolved in water and extracted with ether. The aqueous solution (50 mL) was adjusted with 1 M ammonium hydroxide to pH 8 and applied to a column (1.5 x 38 cm) of Dowex 1x2 (formate form, 200 - 400 mesh). The

column was washed with water and eluted with a linear gradient of formic acid. The mixing chamber contained water (1.5 L) and the reservoir contained 0.3 M formic acid (1.5 L). The 2'-5' linked dimer **1** was eluted at 0.03 M formic acid (558 A_{260} units) and the 3'-5' linked isomer **2** was eluted at 0.05 M formic acid (198 A_{260} units). Each fraction was further applied to a column (1.5 x 45 cm) of DE-23 cellulose (biocarbonate form). After washing with water, the each column was eluted with a linear gradient of TEAB buffer (pH 7.5, 0 - 0.2 M, total 3 L). Chromatographically pure 2'-5' linked dimer **1** (384 A_{260} units, yield 46 %) eluted at 0.06 M TEAB buffer and 3'-5' linked isomer **2** (189 A_{260} units, yield 16 %) eluted at 0.06 M TEAB buffer were obtained as the triethylammonium salts. PEP: Rm_{AMP} 0..37 for **1** and 0.29 for **2**.

Hydrolysis of the dimers 1 and 2 with Snake Venom Phosphodiesterase

Each dimer sample (5 A_{max} units) was incubated with snake venom phosphodiesterase (1 mg/mL, 70 mL) in 0.05 M TEAB buffer (pH7.5, 150 mL) at 37°C for 12 hr. After quenching the reaction by heating at 100°C for 5 min, the digest was subjected to PEP at pH 7.5. The ratio of the nucleoside to nucleotide produced was determined by UV absorption measurement. Each dimer was hydrolyzed to give approximately equal amounts of the nucleoside and nucleotide.

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