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Polynucleotides. XXXIV.[1] Ultraviolet Absorption and Circular Dichroism of ApUpG Analogs containing Modified Adenosine Residues

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Ultraviolet absorption and circular dichroism properties of ApUpG and its analogs in which the adenosine is modified are reported. The adenosine analogs include formycin (F), 8,5′-S-cycloadenosine (^{s}A), 8,2′-S-cycloadenosine (A^{s}), 8,5′-O-cycloadenosine (^{o}A), 8,2′-O-cycloadenosine (A^{o}), 8-bromoadenosine (Br-A) and 8-oxyadenosine (HO-A). S- and O-Cyclonucleosides and F take torsion angles of anti type, while Br-A and HO-A assumed to take *syn* conformation in these analog trinucleotides. All analogs have very weak stacking interaction and show a tendency to form an aggregate at low temperature.

Introduction

Adenylyl-(3′,5′)-uridylyl-(3′,5′)-guanosine (ApUpG or AUG[3]) is the trinucleotide which codes for initiation or internal methionine in protein biosynthesis.[4] To elucidate the conformational requirement for AUG as a codon for methionine, ApUpG analogs containing modified adenosine residues of different torsion angles about the glycosidic linkage have been synthesized[5] and the activity for stimulation of Met–tRNA binding to ribosomes has been examined in our laboratory.[6] In these ApUpG analogs, the adenosine residue was replaced by formycin (F), 8,5′-S-cycloadenosine (^{s}A), 8,2′-S-cycloadenosine (A^{s}), 8,5′-O-cycloadenosine (^{o}A), 8,2′-O-cycloadenosine (A^{o}), 8-bromoadenosine (Br–A) and 8-oxyadenosine (HO–A). These adenosine analogs are assumed to have a variety of torsion angles about glycosidic bond and for some of them χ values[7] (or ϕ_{CN}[8]) have been determined by X-ray analysis.[9–12] Torsion angles of ^{s}A and ^{o}A are fixed in the *anti-syn* boundary region.[11] Br–A and HO–A are assumed to have a *syn* conformation by X-ray[12] and circular dichroism (CD) studies.[13] In F, the torsion angle is supposed to be easily changeable. In X-ray analysis, hydrobromide of F shows a *syn* conformation,[14] though neutral monohydrate of F has a torsion

1) Part XXXIII: Morio Ikehara, J. Yano, E. Fukuda, and S. Uesugi, *J. Amer. Chem. Soc.*, in press. (1974).
2) Location: *133–1 Yamadakami, Suita, Osaka, 565, Japan.*
3) Abbreviations for ApUpG analogs are illustrated in the Chart.
4) J. Lucas-Leonard and F. Lipmann, *Ann. Rev. Biochem.*, **40**, 409 (1971).
5) *a*) M. Ikehara, T. Nagura, and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **22**, 123 (1974); *b*) M. Ikehara, T. Nagura, and E. Ohtsuka, *ibid.*, **22**, 2574 (1974); *c*) M. Ikehara, T. Nagura, and E. Ohtsuka., *Nucleic Acid Res*, **2**, 1345 (1975)
6) E. Ohtsuka, T. Nagura, K. Shimokawa, S. Nishikawa, and M. Ikehara, *Biochim. Biophys. Acta*, **383**, 236 (1975).
7) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).
8) J. Donohue and K.N. Trueblood. *Biopolymers*, **2**, 363 (1960).
9) Formycin monohydrate, $\chi=109.8°$, P. Prusiner, T. Brennan, M. Sundaralingam, *Biopolymers*, **12**, 1196 (1973).
10) ^{s}A (2′,3′-isopropylidene derivative) $\chi=42°$, K. Tomita, T. Tanaka, M. Yoneda, T. Fujiwara and M. Ikehara, *Acta. Crystallog.*, **A28**, S45 (1972).
11) A^{s} 3′,5′-cyclic phosphate, $\chi=122°$, T. Tomita, T. Tanaka, M. Yoneda, T. Fujiwara, and M. Ikehara, *Acta, Crystallog*, **A28**, S45 (1972).
12) Br-A, $\phi_{CN}=199°$, S.S. Tavale, and H.M. Sobell, *J. Mol. Biol.*, **48**, 109 (1970).
13) M. Ikehara, S. Uesugi, and K. Yoshida, *Biochemistry*, **11**, 830 (1972).
14) G. Koyama, K. Maeda and H. Umezawa, *Tetrahed. Letters*, **1966**, 597.

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angle in a *syn–anti* boundary region[9] similar to A^s. Since poly (F) forms a complex with poly (U),[15] F seems to able to take also an *anti* conformation. This may be verified also by the fact that poly (laurusin phosphate), which is deaminated form of poly (F), forms complexes either with poly (A) or poly (A^s).[16] Adenosine is known to take an *anti* conformation and χ is determined to be 9.9° in crystal.[17]

NpUpG
N;

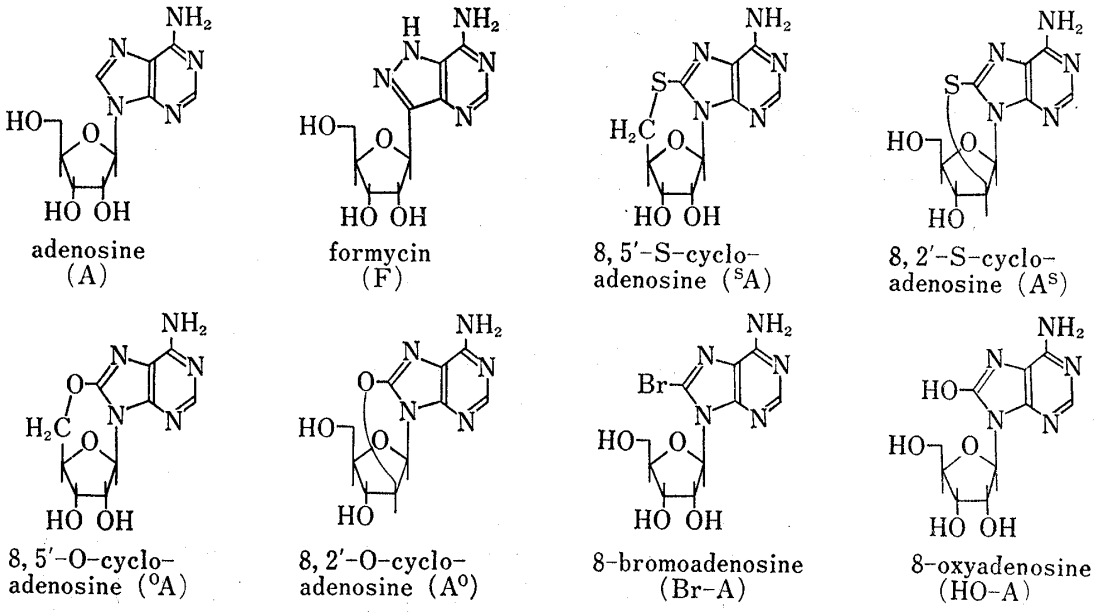

Chart 1

In the binding experiments,[6] FUG gave even more stimulation than AUG and °AUG showed 20—30% stimulation compared with AUG at 10 mM Mg^{2+} concentration, while other AUG analogs, in which modified adenosine residues were assumed to be *syn-anti* boundary region or *syn* conformation, gave very small or no stimulation. In conclusion, it was suggested that an *anti* AUG codon might be necessary for complex formation between trinucleotide, Met-tRNA and ribosomes in *E. coli* system and the formycin residue in FUG might take an *anti* conformation.

In this paper, we wish to report on the spectroscopic properties, hypochromicity, ultraviolet (UV) absorption and circular dichroism of these AUG analogs and discuss the conformation of these molecules.

Experimental

Materials——ApUpG and its analogs were synthesized chemically by condensation of the Ap component and the U-G component.[5] The triethylammonium salt of the trimers was used for optical measurements. Snake venom phosphodiesterase was purchased from Worthigton Biochemical Corp.

UV Absorption and Hypochromicity Measurements——UV absorption spectra were taken on a Hitachi EPS-3T spectrophotometer. Molar extinction coefficients (ε), hyper-and hypochromicity of the trimers were obtained from enzymatic digestion by snake venom phosphodiesterase. Reaction mixture 150 μl contained 3—3.5 OD_{260} units of each trimers, 0.13 M ammonium bicarbonate and 10 μl of venom phosphodiesterase (10 mg per ml). It was incubated at 37° for 3 hr. Examination by paper chromatography showed complete hydrolysis to component monomers under these conditions. The reaction mixture was diluted to a certain

15) D.C. Ward and E. Reich, *Proc. Nat. Acad. Sci. USA.*, **61**, 1494 (1968).
16) M. Ikehara and T. Tezuka. *Nucleic Acid. Res.*, **1**, 907 (1974).
17) T.F. Lai and R.E. Marsh, *Acta Crystallog.*, B **28**, 1982 (1972).

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volume with 0.1 M KF, 0.05 M phosphate buffer (pH 7.0) and the UV absorption spectra was measured. UV absorption spectrum of the control solution without enzymatic digestion was also taken under the same condition. Hyper- and hypochromicity were calculated comparing areas which were formed by two UV absorption curves before and after the digestion from 230 to 330 nm.

CD Measurements——CD spectra were taken with a JASCO ORD-UV5 spectropolarimeter equipped with a CD attachment. The instrument was calibrated with d-10-camphorsulfonic acid. Measurements were carried out in a 10 mm path-length cell which was thermostated with a JASCO low temperature device. The data are presented as molar ellipticity $[\theta]$ per residue. The concentration of the samples is 1.4—1.6 absorption units per ml at λ_{max}.

Results and Discussion

Ultraviolet Absorption Properties

UV absorption spectra of ApUpG and its analogs at three different pH's are shown in Fig. 4—11 and spectral data are summarized in Table I. Molar extinction coefficients (ε), hyper- and hypochromicity were obtained from the results of enzymatic digestion by snake venom phosphodiesterase. Typical absorption spectra before and after digestion are shown in Fig. 1—3.

Table I. Ultraviolet Absorption Data for AUG Analogs[a]

Compound	pH 2	λ_{max}[b] (ε_{max})[c] pH 7	pH 12
AUG	258.5 (11700)	258 (11400)	260 (10200)
FUG	258.5 (8000)	257.5 (8000)	261 (6800)
^{s}AUG	275 (10300)	270—275[d] (9300)	273—275[d] (9200)
A^{s}UG	266 (11000)	262—268[d] (11000)	269 (9300)
	272 (10900)		
°AUG	260.5 (11200)	258.5 (11100)	261.5 (10600)
A°UG	259 (10800)	257.5 (11300)	258.5 (10100)
Br-AUG	262 (11600)	260 (11100)	264 (10700)
HO-AUG	261.5 (9800)	259 (10000)	269 (9000)

a) Spectra were taken at room temperature. ε was obtained from the results of enzymatic hydrolysis.
b) expressed in nm
c) expressed in a per residue value
d) Peak was very broad.

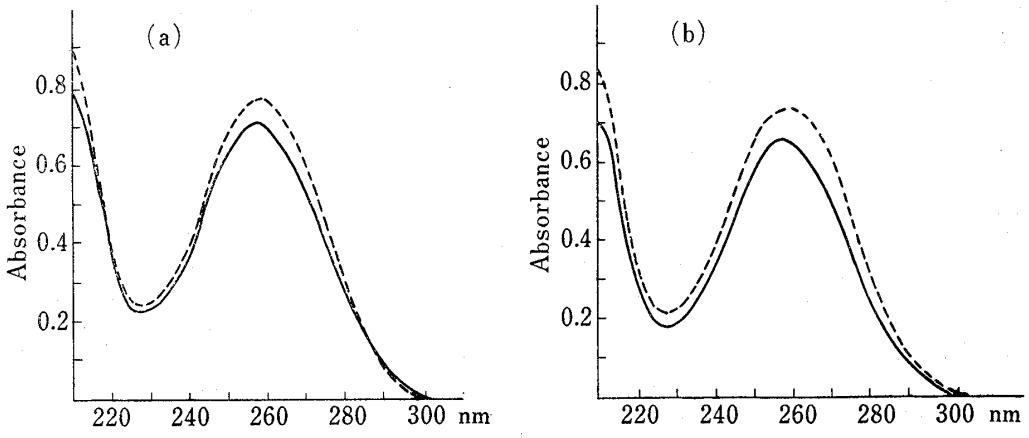

Fig. 1. UV Absorption Spectra before and after Digestion of AUG and° AUG by Snake Venom Phosphodiesterase

Conditions for digestion are described in the text. Spectra were taken in 0.1M KF, 0.05M phosphate buffer (pH 7.0) at room temperature.
——: before digestion, ------: after digestion

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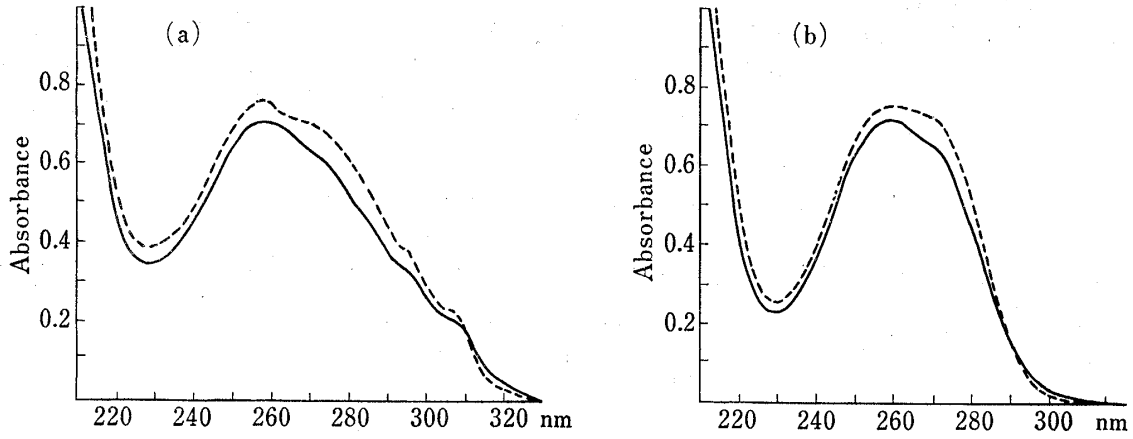

Fig. 2. UV Absorption Spectra before and after Digestion of FUG and HO-AUG by Snake Venom Phosphodiesterase

Conditions are the same as described in Fig. 1.
——: before digestion, ------: after digestion

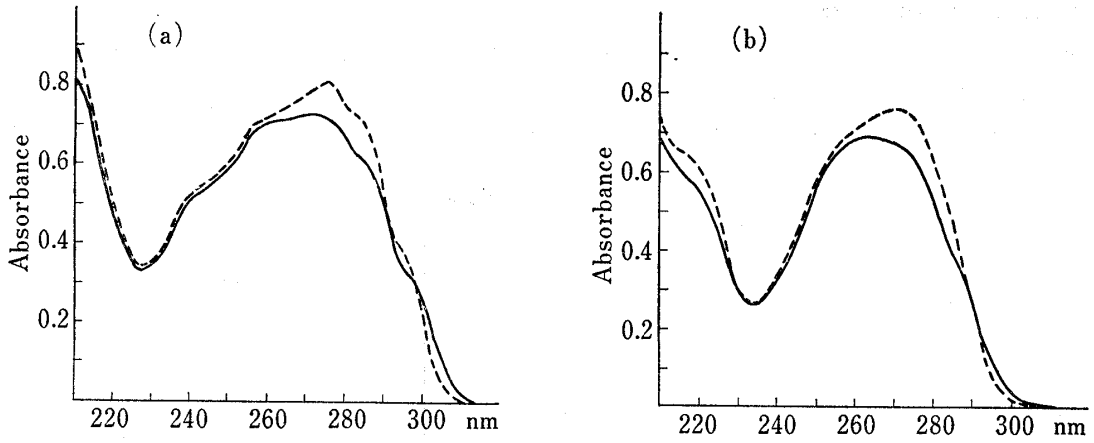

Fig. 3. UV Absorption Spectra before and after Digestion of ^{s}AUG and A^{s}UG by Snake Venom Phosphodiesterase

Conditions are the same as described in Fig. 1.
——: before digestion, ------: after digestion

ApUpG shows hyperchromism in the short and middle wavelength region and hypochromism in the long wavelength region (above 285 nm) on digestion. The same phenomena are observed in all trimers studied except for ^{o}ApUpG and A^{o}pUpG. Among them Br–ApUpG (not shown here) gives a smooth curve with a shoulder in the peak. The other four trimers, especially FpUpG and ^{s}ApUpG, give more complex absorption curves. This complexity originates from the complex absorption spectra of the adenosine analogs.[18] ^{o}ApUpG shows hyperchromism over the entire range of wavelength on digestion. A similar result is obtained in the case of A^{o}pUpG (not shown here). Because some of the trimers studied possess complex absorption spectra, hyper-, and hypochromocity at a special wavelength is not useful for comparison of the data between them. So, calculation was made from the difference in areas which were formed by the two absorption curves before and after the enzymatic digestion from 230 to 330 nm. All trimers studied have minimum absorption wavelength around 230 nm and show no absorption in the region longer than 330 nm. The results are shown in Table II. The intagrated hypochromicity of ApUpG is 10%, while it shows hypochromicity

18) M. Ikehara, M. Kaneko, Y. Nakahara, S. Yamada, and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1381 (1971).

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of 8.3% at 260 nm which is close to the reported value (9.3%) by Inoue, *et al.*[19] Most of the trimers studied give hypochromicity of 6—10%. According to the classification by Cantor and Tinoco,[20] all of them should belong to the stacked conformation. If hypochromism originates only from base stacking interaction, we could say that °ApUpG is the most stacked one and A^{s}pUpG is the least stacked one.

Table II. Hyper- and Hypochromicity of AUG Analogs[a]

Compound	Hyperchromicity (%)	Hypochromicity (%)	Compound	Hyperchromicity (%)	Hypochromicity (%)
AUG	11	10	°AUG	18	15
FUG	11	10	A°UG	10	9
^{s}AUG	7	6	Br-AUG	9	8
A^{s}UG	9	8	HO-AUG	8	7

a) Trinucleotide was hydrolyzed with snake venom phosphodiesterase at 37° for 3 hr. UV absorption spectra were taken before and after hydrolysis in 0.1M KF, 0.05M phosphate buffer (pH 7.0). Hyper- and hypochromicity were calculated from the difference in areas formed by the two curves from 230 nm to 330 nm.

Circular Dichroic Properties

Circular dichroic spectra of the trimers at different conditions and the summation curves of the component monomers are shown in Fig. 4—11 together with the absorption spectra. The spectral data at 25° are given in Table III. The magnitude and shape of CD bands of

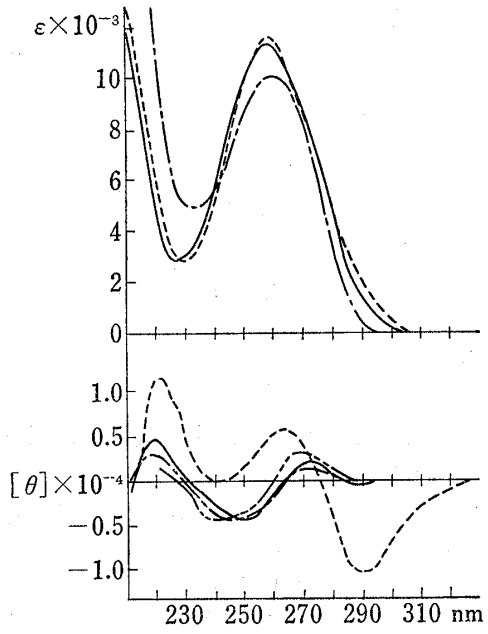

Fig. 4. UV Absorption and CD Spectra of AUG

UV absorption spectra were taken at room temperature.
------: at pH 2, ——: at pH 7, ———: at pH 12
CD spectra were taken in 0.1M KF, 0.01M phosphate buffer (pH 7.0).
——: at 25°, ———: at 60°, ————: mixture of monomer components at room temperature, -------: at 0° (the temperature of the solution was never raised to room temperature)

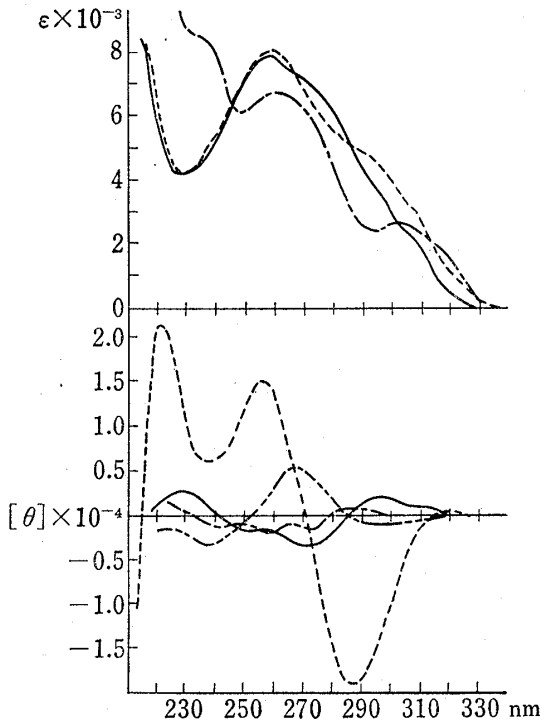

Fig. 5. UV Absorption and CD Spectra of FUG

Conditions are the same as described in Fig. 4. UV absorption spectra
------: at pH 2, ——: at pH 7, ———: at pH 12
CD spectra
------: at 0°, ——; at 25°, ———: at 60°, ————: the monomer mixture at room temperature

19) Y. Inoue, S. Aoyagi, and K. Nakanishi, *J. Am. Chem. Soc.* **89**, 5701 (1967).
20) C.R. Cantor and I. Tinoco, Jr, *J. Mol. Biol.*, **13**, 65 (1965).

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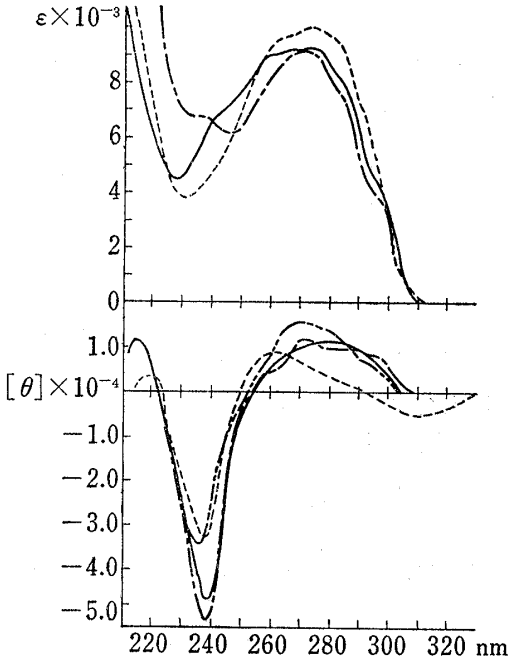

Fig. 6. UV Absorption and CD Spectra of ^{s}AUG

Conditions are the same as described in Fig. 4.
UV absorption spectra
------: at pH 2, ——: at pH 7, ———: at pH 12
CD spectra
------: at 0°, ——: at 25°, ———: at 60°, ————: the monomer mixture at room temperature

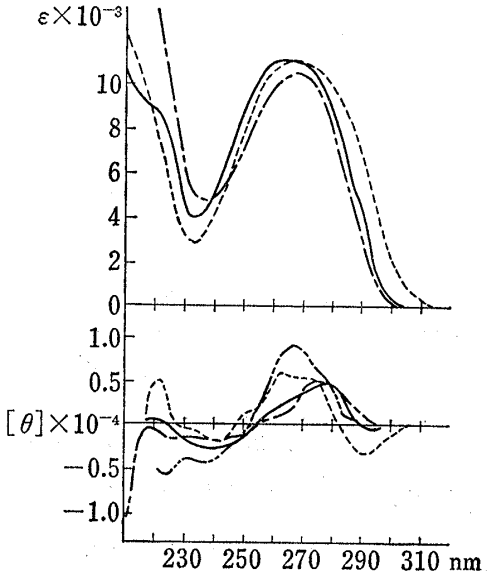

Fig. 7. UV Absorption and CD Spectra of A^{s}UG

Conditions are the same as described in Fig. 4.
UV absorption spectra
------: pH 2, ——: pH 7, ———: pH 12
CD spectra
------: O°, ——: 25°, ———: 60°, ————: the monomer mixture at room temperature

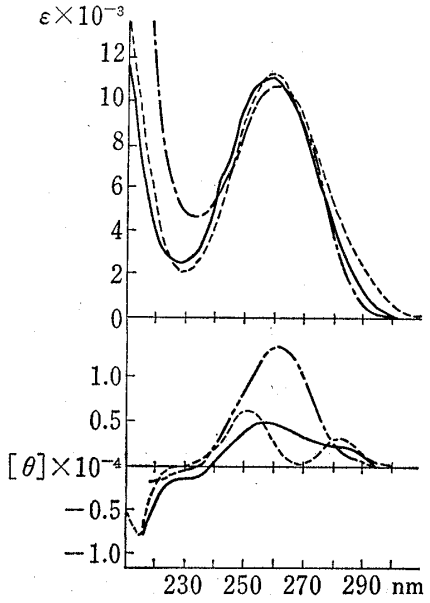

Fig. 8. UV Absorption and CD Spectra of °AUG

Conditions are the same as described in Fig. 4.
UV absorption sepctra
------: pH 2, ——: pH 7, ———: pH 12
CD spectra
------: 0°, ——: 25°, ————: the monomer mixture at room temperature

CD and UV spectra of AUG analogs

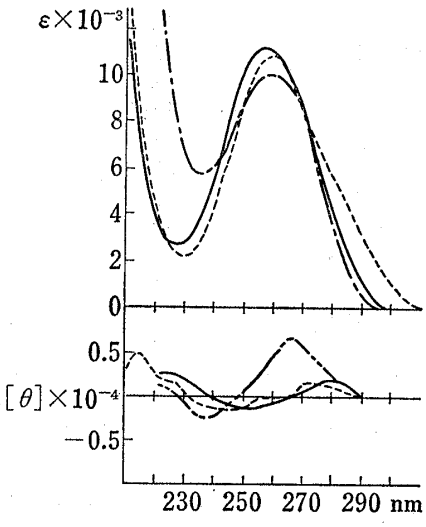

Fig. 9. UV Absorption and CD Spectra of A°UG

Conditions are the same as described in Fig. 4.
UV absorption spectra
------: pH2, ——: pH 7, ———: pH 12
CD spectra
------: 0°, ——: 25°, ————: the monomer mixture at room temperature

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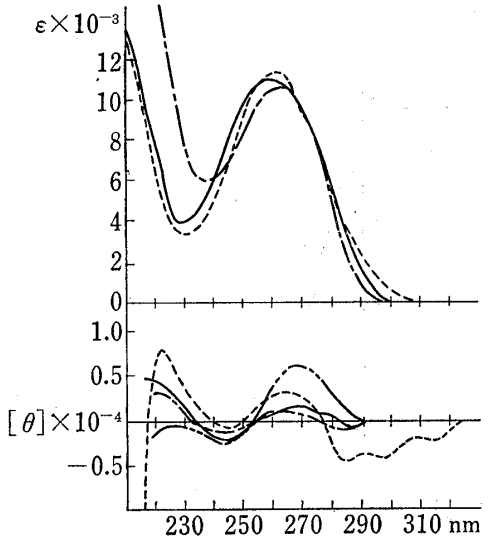

Fig. 10. UV Absorption and CD Spectra of Br-AUG

Conditious are the same as described in Fig. 4.
UV absorption spectra
------: pH 2, ——: pH 7, ———: pH 12
CD spectra
------: 0°, ——: 25°, ————: the monomer mixture at room temperature

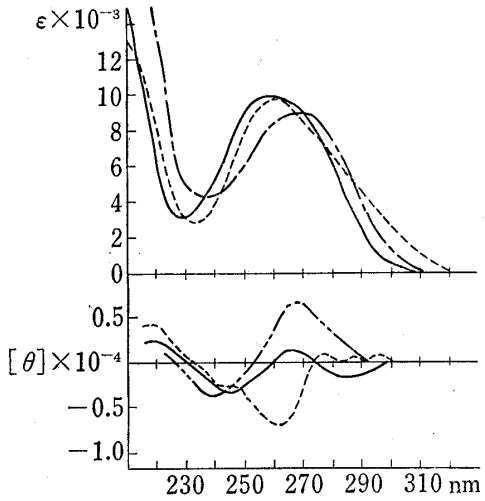

Fig. 11. UV Absorption and CD Spectra of HO-AUG

Conditions are the same as described in Fig. 4.
UV absorption spectra
------: pH 2, ——: pH 7, ———: pH 12
CD spectra
------: 0°, ——: 25°, ————: the monomer mixture at room temperature

Table III. Circular Dichroic Data for AUG Analogs[a)]

Compound	λ_{max} (nm)	$[\theta]_{max}$[b)] $\times 10^{-3}$	λ_{min} (nm)	$[\theta]_{min}$[b)] $\times 10^{-3}$
AUG	218	4.1	250	4.0
	272.5	1.3		
FUG	228	4.1	253	−1.8
	298	2.4	273	−3.6
	310	1.1		
^{s}AUG	215	12.0	238	−47.0
	281	11.4		
A^{s}UG	218.5	7.0	242	−2.9
	278	4.9	295	−0.5
^{o}AUG	255	4.8		
A^{o}UG	225	2.9	250	−1.2
	278	1.9	292	−0.4
Br-AUG	220	4.7	244	−3.2
	271	1.8		
HO-AUG	218	2.5	244	−3.8
	267	1.3	286	−2.0

a) Spectra were taken at 25° in 0.1M KF, 0.05M phosphate buffer (pH 7.0).
b) Expressed in a per residue value.

ApUpG are very similar to the average of the monomer spectra. It means that ApUpG does not have an ordered stacking structure under this condition.

This result is in good agreement with that reported by Inoue, *et al.*[19)] and Gray, *et al.*[21)] As discussed by Gray, *et al.* the CD spectrum of ApUpG is quite different from that predicted from the spectra of ApU and UpG. These dimers are thought to be in stacked conformation

21) D.M. Gray, I. Tinoco, Jr., and M.J. Chamberlin, *Biopolymers*, **11**, 1235 (1972).

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because they give very different spectra from the average of monomer spectra.[22] Therefore, ApUpG does not contain such base stacking interaction as in the component dimers.

As to the ApUpG analogs studied, all trimers showed comparable or smaller CD bands when compared with the average of the monomer spectra at 25°. FpUpG (Fig. 5) shows rather smaller but quite different CD bands when compared with the average of the monomer spectra. ^{s}ApUpG (Fig. 6) gives very similar shape and magnitude of CD spectrum to the average of the monomer spectra. In other trimers containing cyclonucleosides (Fig. 7—9), the magnitude is much smaller than the average of the monomer spectra and the shape is somewhat different. Some similarity is observed between the difference spectra of A^{s}pUpG and A^{o}pUpG with respect to the average of the monomer spectra. In the case of Br–ApUpG and HO–ApUpG (Fig. 10—11), they give CD bands of much smaller magnitude. The patterns of their CD spectra containing a small negative bands at the long wavelength region are similar to each other. It may be noted that the CD pattern of Br–ApUpG also resembles that of ApUpG. CD spectra at 60° were measured with some of the trimers, ApUpG, FpUpG, ^{s}ApUpG, A^{s}pUpG and Br–ApUpG, but only a little difference was observed except for FpUpG.

On the other hand, we found that an usual CD spectrum, a negative band at 290 nm ($[\theta]-10.1\times10^{3}$) and two positive bands at 263 and 222 nm ($[\theta]$ 6.1×10^{3} and 11.3×10^{3}, respectively), were observed at 0° when the frozen sample of ApUpG was thawed gradually and the temperature of the solution was never raised to room temperature before measurement. Other trimers also gave quite different CD patterns from those at 25° under the same condition. FpUpG gives almost the same CD pattern, a negative band at 286 nm ($[\theta]-13.6\times10^{3}$ and 21.9×10^{3}, respectively) as that of ApUpG. The magnitude of these bands are about twice as large as that of ApUpG. One of the reasons for the large magnitude may be that the concentration of FpUpG sample was higher that of ApUpG because the absorbance of these samples were about the same (1.5 OD units) at λ_{max} of each compound and λ_{max} of FpUpG is much smaller than that of ApUpG. The same type of CD patterns, negative band in the long wavelength region and positive bands in the medium and short wavelength region, are also observed in ^{s}ApUpG, A^{s}pUpG and even in BrApUpG, though the magnitudes are not so large as those of ApUpG and FpUpG. As for ^{o}ApUpG and HO–ApUpG, their CD spectra at 0° are different from those 25° but no apparent similarlity to the pattern of ApUpG is observed.

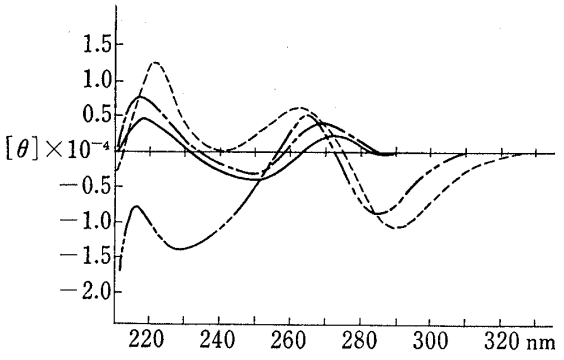

Fig. 12. CD Spectra of AUG under Various Conditions

The solvent contained KF (as indicated) and 0.05M phosphate (pH 7.0).
——: 0.1M KF at 25°, ------: 0.1M KF at 0° (the temperature was never raised to room temperature before measurement)
– – – –: 0.1M KF at 0° (temperature was once raised to room temperature before measurement, ——— ———: 5M KF at room temperature

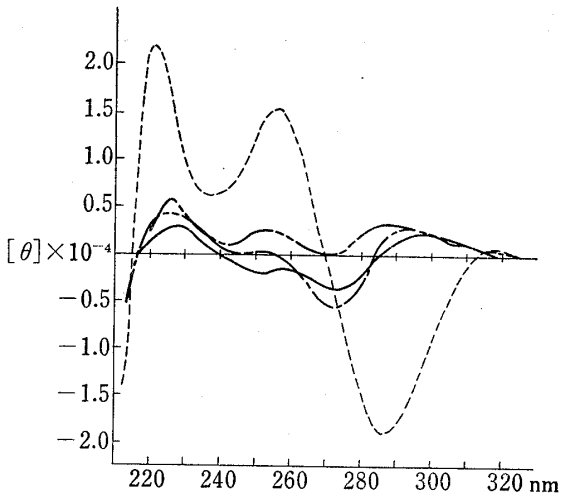

Fig. 13. CD Spectra of FUG under Various Conditions

Legends are the same as described in Fig. 12.

22) M.M. Warshaw and C.R. Cantor, *Biopolymers*, **9**, 1079 (1970).

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When the temperature of the sample was once raised to 25° and the CD spectrum was measured at 0°, ApUpG and FpUpG showed almost same spectra as those found at 25° (Fig. 12). In the presence of 5M KF, ApUpG showed the same type of anomalous CD spectra even at 25°, as that measured at 0° in the earlier experiment though FpUpG failed to give the characteristic spectrum under the same condition. So the anomalous spectra in the earlier experiments at 0° may be due to non-cooperative aggregation. Brahms, *et al.*[23] have reported that GpGpUp forms aggregates in 0.1M KF, 0.01M Tris buffer (pH 7.5) at around 0° but aggregation is not reported previously to the best of our knowledge.

Conclusion

From CD studies it can be concluded that ApUpG and its analogs do not have stable, orderly stacked conformation at room temperature, though they show significant hyperchromism in UV absorption on digestion to component monomers. FpUpG which has the same activity as ApUpG in the binding experiment, exhibited the same characteristic CD bands presumably of an aggregate as those of ApUpG under certain conditions. So, FpUpG could take quite similar conformation to that of ApUpG even at room temperature. FpUpG gives small but quite different CD spectra at 25° from the average of monomer spectra. The big difference in CD spectra at 25° could caused by the change of torsion angle about glycosidic linkage of the F residue from *syn-anti* boundary in the monomer to anti region (in the trimer), because the sign of CD band around 300 nm from FpUpG is positive whereas the monomer F has negative CD around this region where F has its UV absorption maximum (295 nm) in neutral solution. A^{s}pUpG, which shows about 30% activity in the binding experiment compared with ApUpG, may have only a little fraction of stacked conformation according to its CD spectrum. A^{s}pUpG, which shows about 20% activity of ApUpG, may have more stacked conformation than ^{s}ApUpG. The conformation of A^{s}pUpG and A^{o}pUpG may be different from those of ^{s}ApUpG and ^{o}ApUpG, but show only a little or no activity in the experiments. Br–ApUpG and HO–ApUpG exhibit similar CD pattern at 25° but Br–ApUpG shows no activity and HO–ApUpG shows a little activity in the binding experiments. The torsion angle of HO–A is more easily changeable than that of Br–A, though they are staying in *sny* conformation in the normal condition.

For forming a complex with the anticodon of the tRNA, the torsion angle of the first letter A residue in ApUpG may be very important and χ value should be very small in positive (*anti*) region. It may be concluded that all ApUpG analogs studied have only weak stacking interactions at room temperature. The tendency to form aggregates at low temperature is also noteworthy.

23) J. Brahms, A.M. Aubertin, G. Dirheimer, and M. Grunberg-Manago, *Biochemistry*, **8**, 3269 (1969).

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