Tetrahedron Letters No.37, pp. 3575-3577, 1967. Pergamon Press Ltd. Printed in Great Britain.

OLIGONUCLEOTIDE STUDIES. PART II.
OPTICAL ROTATORY DISPERSION OF TWO PAIRS OF SEQUENCE ISOMERS OF TRINUCLEOTIDES [1)]

Y. Inoue, S. Aoyagi and K. Nakanishi

Department of Chemistry, Tohoku University, Sendai, Japan.

(Received in Japan 13 June 1967)

Optical rotatory dispersion (ORD) is understood as the most powerful physical method in the characterization of the conformational structures of oligo- and polynucleotides of different composition and sequence. For this reason, in recent years, ORD data have been reported for several oligonucleotides [2-7)]. However, these studies have been restricted to the observation of the oligonucleotides obtained either from bovine pancreatic ribonuclease I (EC 2. 7. 7. 16) digestion of ribonucleic acids (RNA) or from a partial degradation of synthetic polynucleotides. Recently, we have succeeded in the isolation in pure form of seven trinucleotides (ApApGp, ApCpGp, ApUpGp, CpApGp, CpCpGp, UpApGp, and UpUpGp) [8)] out of nine possible trinucleotides [9)] produced on Taka-Diastase ribonuclease T_1 (EC 2. 7. 7. 26) digestion of high molecular weight RNA [1)].

The purpose of this communication is to report the results of an ORD [10)] study of the two pairs of sequence isomers, ApCpGp and CpApGp, and ApUpGp and UpApGp. The fact that no ORD work has been reported previously in the structural characterization of trinucleotides having the guanylic acid moiety as the terminal residue represents a missing link in study, to date, of oligonucleotides as models of nucleic acids. Although the absorption spectra are, in general, not sensitive to base-sequence, a detailed analysis of these spectra together with hyperchromicity measurements makes it possible to determine nucleotide sequence of di- and trinucleotides [1)]. However, the ORD criteria for the identification of sequence isomers are more convenient as can be clearly seen in Fig. 1.

Since it is evident from the literature [4, 7)] that a 3' terminal phosphate does not substan-

tially change the conformation of a trinucleoside diphosphate, the absolute value of maximum difference in molecular rotation between trinucleotides, say, ApCpGp, and its component monomers, A + pC + pG, for wavelengths longer than 230 mμ, viz., $\left|[M]_t - [M]_m\right|$, was employed as ORD criterion of stacking (see also ref. 4). The results at pH 7 are included with hyperchromicity data in Table I.

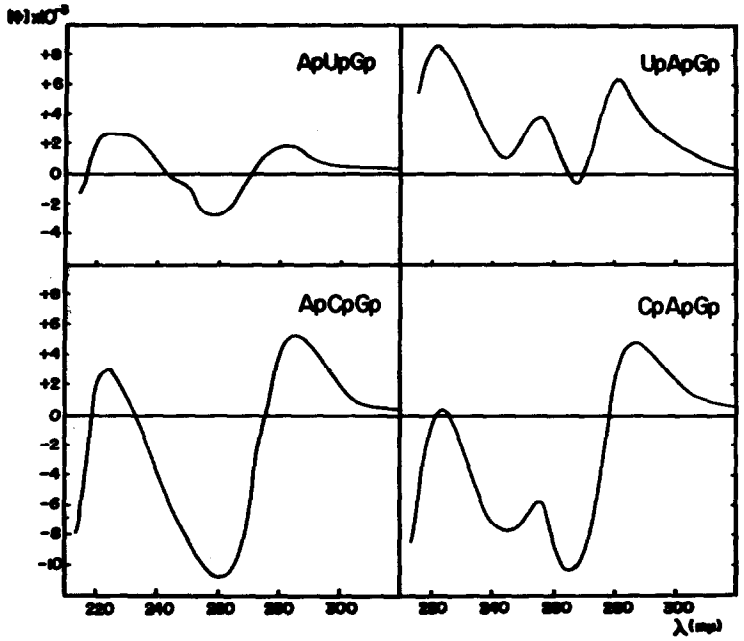

FIG. 1. Ultraviolet rotatory dispersion of ApCpGp, CpApGp, ApUpGp, and UpApGp at pH 7 and the ionic strength 0.1 at 20°.

TABLE I. Hyperchromicity and $\left|[M]_t - [M]_m\right|$ at pH 7

Substance	Hyperchromicity[a] at 260 mμ, h(%)	$\left\|[M]_t - [M]_m\right\|$[b] $\times 10^{-4}$
ApCpGp	15.7	2.70 (261 mμ)
CpApGp	15.3	2.49 (268 mμ)
ApUpGp	10.3	0.83 (280 mμ)
UpApGp	13.2	2.24 (257 mμ)

[a] $h = \dfrac{A_{Xp} + A_{Yp} + A_{Zp} - A_{XpYpZp}}{A_{XpYpZp}} \times 100 = 100\left(\dfrac{\sum \varepsilon_m}{\varepsilon_t} - 1\right)$

[b] Interaction Parameter = $\left|[M]_t - [M]_m\right| = \left|[M]_{XpYpZp} - ([M]_X + [M]_{pY} + [M]_{pZ})\right|_{max}$

Stacking of the nearest-bases in the pair of sequence isomers, ApCpGp and CpApGp, is more or less comparable, since both the hyperchromicity and $|[M]_t - [M]_m|$ value are much the same, whereas in another pair of the isomers, ApUpGp and UpApGp, the former is less stacked at pH 7 than the latter. This finding may be compared to the previous observation by Ts'o et al. [11], i.e., an insertion of uridyl residue into the purines exerts destacking effect. Thus, for the two pairs of sequence isomers, the stacking decreases in the order of ApCpGp $\gtrsim$ CpApGp $>$ UpApGp $>$ ApUpGp.

A full account of the ORD study of di- and trinucleotides obtained from ribonuclease T_1 will soon be reported elsewhere.

Acknowledgements

We wish to thank Professor F. Egami for his interest and encouragement throughout this work. We would also like to thank Sankyo Co., Ltd., Tokyo for the provision of purified ribonuclease T_1.

REFERENCES

1) Part I in this series is by S. Aoyagi and Y. Inoue, J. Biol. Chem., in press.

2) M. M. Warshaw, C. A. Bush and I. Tinoco, Jr., Biochem. Biophys. Res. Comm., 18, 633 (1965).

3) M. M. Warshaw and I. Tinoco, Jr., J. Mol. Biol., 13, 54 (1965).

4) C. R. Cantor and I. Tinoco, Jr., J. Mol. Biol., 13, 65 (1965).

5) J. Brahms, A. M. Michelson and K. E. Van Holde, J. Mol. Biol., 15, 467 (1966).

6) J. N. Vournakis, H. A. Scheraga, G. W. Rushizky and H. A. Sober, Biopolymers, 4, 33 (1966).

7) M. M. Warshaw and I. Tinoco, Jr., J. Mol. Biol., 20, 29 (1966).

8) Abbreviations used: ApApGp, adenylyl-(3'-5')-adenylyl-(3'-5')-guanosine-3'-phosphate ApCpGp, adenylyl-(3'-5')-cytidylyl-guanosine-3'-phosphate; ApUpGp, adenylyl-(3'-5')-uridylyl-(3'-5')-guanosine-3'-phosphate; etc.

9) A pair of sequence isomers, CpUpGp and UpCpGp, are not yet resolved.

10) ORD measurements were made on a JASCO recording spectropolarimeter model ORD/UV-5. Optical rotations used in this communication are defined as $[\phi]=\frac{[M]}{3}=\frac{1}{3}\cdot[\alpha]\cdot\frac{\text{Molecular weight}}{100}$, where $[\phi]$, $[M]$, and $[\alpha]$ are molecular rotation per residue, molecular rotation, and specific rotation, respectively.

11) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, J. Am. Chem. Soc., 87, 5241 (1965).