Oligonucleotide Studies. Part IV. Proton Magnetic Resonance Spectra of Three Dinucleotides, ApGp, CpGp, and UpGp, in D_2O*

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Oligonucleotides are regarded as useful models for understanding the conformational structure and conformational stabilization factors of synthetic polymers as well as naturally occurring polynucleotides in solution. For this reason, a number of papers have been published recently on studies of oligonucleotides by means of ORD (Warshaw et al., 1965; Warshaw and Tinoco, Jr., 1965; Cantor and Tinoco, Jr., 1965; Vournakis et al., 1966; Poland et al., 1966; Brahms et al., 1966; Warshaw and Tinoco, Jr., 1966; Inoue et al., 1967), UV (Leng and Felsenfeld, 1966; Naylor and Gilham, 1966), and phosphorescence (Guéron et al., 1966). We have also commenced the study of oligonucleotides obtained mainly from ribonuclease T_1 digests on which systematic study remains unaccomplished. In a previous paper we have reported a method of fractionation of di- and trinucleotides, according to sequence, which is suitable for a large scale preparation of these nucleotides (Aoyagi and Inoue, 1967), and a detailed account of the ORD spectra of these trinucleotides has also been reported (Inoue et al., 1967a; Inoue et al., 1967b). However, to the authors' knowledge, no proton magnetic resonance study of oligonucleotides has so far been reported in the literature, but only one on thymidine dinucleoside phosphate has appeared (Chan et al., 1966). Thus, in this communication we present a new finding on

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molecular geometry of the dinucleotides which has not been available through any of the other methods.

Three dinucleotides, ApGp, CpGp and UpGp, are obtained from RNase T_1 digestion and the details of the preparation were the same as those reported in a previous paper (Aoyagi and Inoue, 1967). Analytical data for the base composition as a purity criterion are summarized in Table 1.

Table 1. Base composition ratio of dinucleotides

	G	A	C	U
ApGp	1.00	1.07	a	a
CpGp	1.00	a	0.93	a
UpGp	1.00	a	a	1.01

a : undetectable or less than 0.05.

NMR spectra were obtained on a Varian HA 100 NMR spectrometer at 31.5°. Dinucleotides obtained as white fluffy residues in a form of ammonium salt were dissolved in D_2O for observation after a twice repeated process of dissolving in D_2O and freeze-drying to exchange protolytic hydrogens with deuterium. Concentration of sample solutions is approximately 0.2 M. The chemical shifts are expressed in terms of $-\delta$ in ppm as shifts from tetramethyl silane. In Fig. 1 the low field part of 100 Mc proton resonance spectra are reproduced, and all signals are due to protons attached to carbon atoms of base residues. Assignments of each signal are also included in Fig. 1.

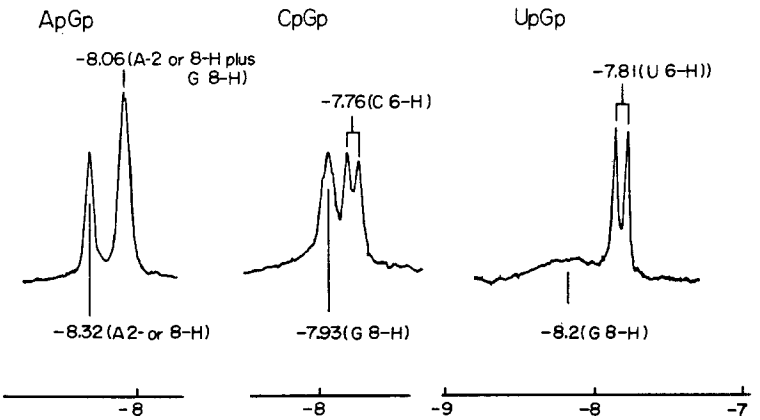

Fig. 1. Proton magnetic resonance part spectra of dinucleotides, ApGp, CpGp and UpGp, in D_2O, ppm from TMS, 100 Mc.

The ORD and hypochromicity criteria of the base-base interaction in dinucleotides have shown that ApGp exists in the stacked conformation at pH 7 and CpGp also exists, to a lesser extent, in the stacked form, whereas UpGp is present in random conformation at this pH. Although the ORD or hypochromism is useful in obtaining information about the gross molecular geometry of oligonucleotides, neither of them gives further detailed knowledge about conformational structure, e. g., information of internal rotation about a certain bond, and rate and equilibrium processes between a stacked and an unstacked conformation. However, this information can be obtained directly from appropriate application of an NMR technique including ^{1}H and ^{31}P resonance. Thus, it should be noted that the NMR spectral study gives evidence for the presence of a preferred conformation(s) in the unstacked form of a dinucleotide which may not be a literally random conformation as has been deduced from the ORD or hypochromicity measurement.

In the spectrum of ApGp an H_8 signal at - 8. 06 ppm of the guanine residue is overlapped with an absorption due to either the H_2 or H_8 proton of the adenine base. In the case of CpGp and UpGp the corresponding proton signals appear at - 7. 93 and approximately - 8. 2 ppm, though partially overlapped, separately from peaks due to the nearest neighboring bases. Signals due to the base portion of the 5'-terminal nucleotide exhibit increased narrowness in the order ApGp > CpGp > UpGp while those assignable to the H_8 proton of the guanine part exhibit a marked broadening. The former phenomenon of the small but noticeable narrowing of absorption signals may be due to cleavage of the stacked conformation, which in turn leads to a disruption of the dipole - dipole interaction between the nearest bases, and to an increase in the rotational motion of the 5'-terminal base moiety. However, to understand the latter observation, a plausible explanation lies in the appearance of the hindered rotation about the bond between the anomeric carbon and the N_9 of the 3'-terminal guanine in the dinucleotide for which the stacked conformation is less favored at room temperature. Thus, the signal at approximately - 8. 2 ppm in the spectrum of UpGp exhibits remarkable broadening ($\Delta\nu_{\frac{1}{2}}$, about 54 cps) whereas the peak at - 7. 81, assignable to the H_6 proton of the uracil part, is quite sharp, indicating that in this dinucleotide liberation

from stacking at room temperature produces a favored conformation in which the uracil group is rotating freely about its glycosidic linkage whereas the rotation is strongly restricted for the guanine base about its glycosidic bond. In a dinucleotide where a strong base-base interaction is operating, both of the 3'- and 5'-terminal bases are completely restricted in their rotation about their glycosidic linkages, and such a molecule exhibits the proton magnetic resonance spectra as can be seen in ApGp. CpGp seems to be the intermediate case between ApGp and UpGp. The results obtained in this study are summarized pictorially as shown in Fig. 2.

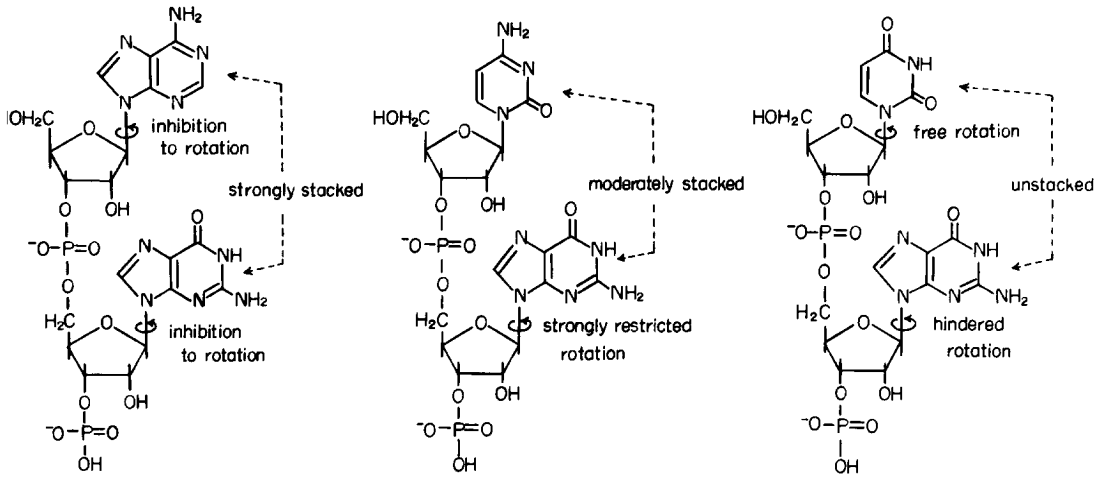

Fig. 2. Schematic representation of molecular geometry of ApGp, CpGp and UpGp (Based upon the ORD and NMR data).

The present finding of the hindered rotation of the 3'-base moiety in the unstacked dinucleotide, UpGp, seems to be specific to the molecule of this sequence, since thymidine dinucleoside phosphate, TpT, (Chan et al., 1966) and poly U (McTague et al., 1964; McDonald et al., 1964) exhibit rather sharp proton resonance signals at room temperature although these nucleotides are believed to exist in the unstacked form at this temperature. Thus, the restricted rotation of the 3'-terminal base found for UpGp should be ascribed to bulk of guanine base.

The present finding of marked broadening of the signal due to hindered rotation of a base in oligonucleotides should be regarded as important in

understanding NMR spectra of other oligonucleotides containing ——UpGp or ——UpGpUp—— and especially naturally occurring polynucleotides. Earlier, the interpretation of the broadening of NMR signals of RNA resided upon the complete non-resolution due to inability to attain averaging of the local fields produced around protons within ordered segments by dipole-dipole interactions (McTague et al., 1964; McDonald et al., 1965; Inoue and Nakanishi, 1966). However, the present results indicate that the presence of the unstacked base sequence as shown above even in a loop portion of RNA molecules causes a marked broadening or complete non-resolution of the signal due to guanine moieties, and the slow rotation should be accelerated with increasing temperature.

A quantitative aspect of the present study will be reported elsewhere.

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