OLIGONUCLEOTIDE CONFORMATIONS

2. Optical studies on 8-bromoguanylyl-3′, 5′-8-bromoguanosine*

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1. Introduction

There are many detailed studies in the literature on dinucleotide phosphate conformations in solution [1]. But if the conformations of dinucleoside phosphates containing U, C and A are essentially clear, the structure of dinucleoside phosphates containing G is far from being elucidated. Because of differences in their optical properties it was proposed that there is no, or different, stacking in some of these compounds, and, for example, in GpU the bases seem to be perpendicular [2].

The particular behavior of guanosine is sometimes conditioned by the facility of *syn–anti* transition, for example upon protonation [3]. So the compounds in which the guanosine residue is fixed in one of the conformations (for example *syn*) are of special interest. The study of these will facilitate the correlation of optical properties with conformation.

It is well known from the literature [4, 5] that the presence of a bulky substituent like bromine in the 8-position of guanosine is sufficient to cause guanosine to be in the *syn* conformation. This is why the conformation in solution of BrGpBrG is of interest. In this work the synthesis of BrGpBrG was developed and CD, UV and MCD spectra were investigated.

* For part I, see [2].

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2. Material and methods

GpG was synthesized by condensation of G>p with Guo [6] ***. It was additionally purified by paper electrophoresis. Russel's viper venom phosphodiesterase was purchased from Calbiochem, ribonuclease T_1 (from Takadiastase) from Worthington. The buffer solutions came from Merck.

2.1. *Paper chromatography and electrophoresis*

The solvent system (A) for descending paper chromatography was 2-propanol–conc. ammonium hydroxide–water (7:1:2, v/v/v). Paper electrophoresis was carried out in 0.05 M triethyl ammonium bicarbonate (pH 8.5) for 1.5 hr at a potential of 45–50 V/cm. For both chromatography and electrophoresis Whatmann 3MM paper washed with 1 N HCl was used.

2.2. *Russel's viper venom phosphodiesterase digestion*

The mixture of 0.2 μmole of BrGpBrG was incubated with 0.1 ml of 0.05 M Tris-HCl buffer, pH 9, containing 10 units of enzyme. Incubation was gen-

*** Abbreviations used: CD: circular dichroism; MCD, magnetic circular dichroism; Guo: guanosine; BrGuo: 8-bromoguanosine, BrGpBrG: 8-bromoguanylyl-3′5′-8-bromoguanosine. GpG: guanylyl-3′5′-guanosine. G>p: 2′3′-cyclic guanosine phosphate. Enzymes: Ribonuclease T_1 (ribonucleotide guanine nucleotide-2′-transferase): EC 2.7.7.26; Venom phosphodiesterase (orthophosphoric diester phosphohydrolase): EC 3.1.4.1.

erally maintained for 12 hr at 37°.

2.3. *Ribonuclease T_1 digestion*

The mixture of approx. 0.2 μmole of BrGpBrG was incubated with 100 units of enzyme in 0.05 M Tris-HCl buffer, pH 7.5, containing 0.002 M EDTA (for 17 hr at 37°).

2.4. *Optical measurements*

The solvents used for optical measurements were 0.01 M phosphate buffer, pH 6.9, for experiments at room temp. and 0.01 M phosphate buffer containing 0.1 M NaCl for CD temperature-dependent measurements. The concentration of BrGpBrG was usually about 10^{-4} M nucleotide residues, and 1 cm silica Hellma cuvettes were used. For the CD concentration dependence 0.1 cm, 0.5 cm, 1 cm and 2 cm silica Hellma cells were used to maintain the same optical density (about 1) with different concentrations of BrGpBrG.

Ultraviolet absorption spectra were run on a Cary 14 spectrophotometer. The concentrations were obtained from the absorbance of the samples using the molar extinction coefficients of BrGuo $\epsilon_{261}=15700$ [7]. The CD spectra were measured on a Russel Jouan II dichrograph. A thermostated cell compartment flushed with nitrogen was used for the temperature variation experiments. Temperature was controlled with a platinum thermistor connected with a MECI recorder. MCD measurements were carried out in a Jouan II dichrograph with a superconducting magnet which gives a magnetic field of 67,700 gauss.

3. Results

3.1. *Synthesis of BrGpBrG*

Several methods for direct bromination of Guo and its derivatives have been reported [7–10]. In order to avoid possible oxidation of the guanine base [8] in water, or even in *N*, *N*-dimethyl formamide [9, 10], the bromination of GpG was performed in formamide according to the procedure of Michelson et al. [8] which seems to maximally exclude side products. BrGpBrG was obtained in 75% yield. The product was purified by paper chromatography (in solvent A). The chromatography was repeated because of the slow mobility of BrGpBrG in this solvent, and then followed by electrophoresis. (R_f of BrGpBrG in solvent A is 0.23. GpG moves more slowly. The R_{pG} for BrGpBrG is 0.35.)

The structure of BrGpBrG was confirmed by enzymatic digestion with venom phosphodiesterase and ribonuclease T_1. In the first case BrG and *p*BrG were obtained, and, in the second, BrGp and BrG in equimolar quantities.

It was observed that reduction of incubation time in the RNAase T_1 reaction at conditions which are sufficient for complete digestion of GpG produced measurable quantities of BrGp. For this reason the incubation was extended overnight. This may indicate that the steric constraints of the *syn* BrG are not the most favorable for the two reactions of RNAase T_1.

3.2. *Optical properties of BrGpBrG*

In fig. 1 are shown the absorbance, CD and MCD spectra of BrGpBrG. Several points should be noted. The absorbance spectrum is quite similar in its shape and molar extinction to that of BrGuo [5], a shoulder around 285 nm indicating an additional band. The MCD spectra confirm the wavelength positions at 285 and 255 nm, corresponding to the B_{2u} and B_{1u} band,

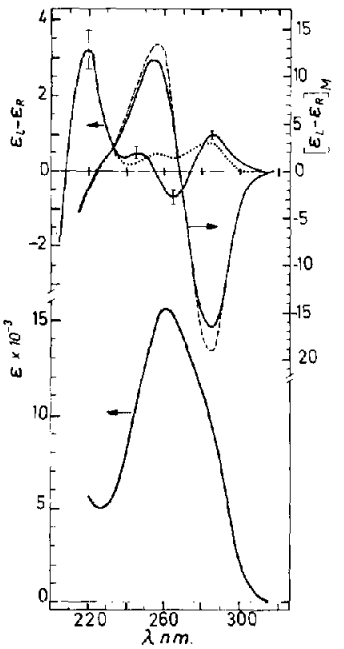

Fig. 1. CD, MCD (top) and absorption spectra (bottom) of BrGpBrG (—) and BrGuo (- - -) in 0.01 M sodium phosphate buffer pH 7.

respectively. The MCD spectra are very similar to that of Guo [12] and its analogues [13], indicating that the polarization of the transition moments is essentially unchanged in the 8-Br-guanine chromophore compared with guanine.

The CD spectra show a more complex behavior and are not comparable with those of 8-BrGuo [5], nor with those of the gels formed by BrGuo. An interaction between the two bases is therefore involved. The shape of the CD spectrum indicates that both the B_{2u} and the B_{1u} bands may be implicated in the coupling between the two bases. It should be noted, however, that the ellipticities are rather low and that coupling between the transitions may not be very intense.

3.2.1. Temperature dependence of CD spectra

It was therefore decided to investigate the temperature dependence of CD spectra of BrGpBrG. Two series of experiments were performed, one in 0.1 M NaCl, 0.01 M phosphate buffer, pH 6.9, and another in 4.5 M LiCl. In fig. 2a the variation of the CD spectra with temperature is shown. Neat isosbestic points are observed which indicate that the two-state model [14] may be applicable. In fig. 2b the amplitude between the first two bands ($\Delta\epsilon_{285} - \Delta\epsilon_{265}$) is plotted against the temperature [15]. It is quite clear from this figure that the stacking of BrGpBrG is far from optimal at 0°, and not complete even at −40°. The transition is very broad and the thermodynamic parameters which can be computed from these data are quite low ($\Delta H^\circ = -2.8$ kcal/mole, $\Delta S^\circ = -10.0$ e.v., $\Delta G^\circ_{25^\circ} = +0.2$ kcal/mole) [16]. These values as well as the low ellipticity values of BrGpBrG are indicative of low stacking.

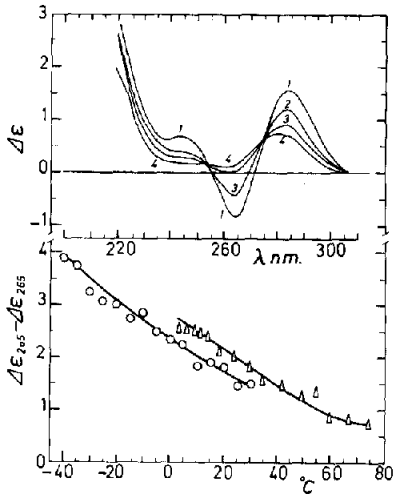

Fig. 2. Top: Temperature dependence of CD spectra of BrGpBrG in 0.01 M sodium phosphate buffer pH 7: 1: 3°; 2:30°; 3: 60°; 4: 75°. Bottom: Variation of $\Delta\epsilon_{285} - \Delta\epsilon_{265}$ with temperature in 5 M LiCl (o-o-o) and 0.01 M sodium phosphate buffer, pH7 (Δ-Δ-Δ).

Another indication of low stacking in BrGpBrG is the relatively small spectral difference between the dinucleotide and the monomer (fig.1). The difference, i.e. the interaction, spectrum would yield two small positive bands around 290 and 295 nm and a slightly larger negative band centered at 265 nm. This could be explained as two overlapping exciton bands of opposite sign, the amplitudes of which certainly would not be comparable with those of other bromodinucleotides like ApA or CpC [1].

Temperature hyperchromicity of BrGpBrG between 0° and 75° was about 4%, again indicating very little stacking. In the MCD spectra (fig. 3) a difference of about 10–15% is observed at 285 and 255 nm between BrGp and BrGpBrG. These values are comparable with those observed for the differences between ApA and Ap (21% and 6% at 271 and 252 nm, respectively), while there is little difference between UpU and Up [17].

In view of the well known gel formation of Guo and its analogues [18], including BrGuo [5], the concentration dependence of BrGpBrG was followed between 10^{-3} and 10^{-5} M in a 0.01 M phosphate buffer at room temp. The CD spectra were the same within experimental error and no indication of aggregation was found in this concentration range. This does not exclude the possibility that aggregation could take place under different experimental conditions (e.g., dinucleotide concentration, temperature, ionic strength).

4. Discussion

The study of BrGpBrG was initiated in order to obtain information on the forces involved in oligonucleotide conformations. It was, therefore, tempting to study a *syn–syn* dinucleotide, particularly in view of the recent work of Michelson et al. [8] on poly

(BrG). It should be noted that the CD spectra of poly (BrG) and BrGpBrG are very similar. Michelson et al. [8] had concluded that their data were consistent with a random coil conformation of poly (BrG). This conclusion may be a bit strong, but certainly comes close to our results. The absence of hyperchromicity, the low CD spectra and the very broad melting curve clearly point out that BrGpBrG is poorly stacked at room temp. The relatively large MCD differences, on the other hand, are indicative of interactions between the bases, although hyperchromicity and MCD changes are by no means parallel phenomena, as has been shown by Maestre et al. [17].

During the course of this work Yuki and Yoshida [19] reported the synthesis and optical properties of BrGuo di- and trinucleotide. We find their results at variance with those reported in this communication as well as with the data of Michelson et al. [8] on poly (BrG). Neither the large hyperchromicities nor the optical activities reported by Yuki and Yoshida [19] agree with the data reported here. It should be noted that their optical rotatory dispersion spectra of BrGuo also disagree with those in the literature [4, 20, 21], both in sign and size.

The data in fig. 1 as well as the CD spectrum of poly (BrG) [8] indicate a positive, long-wavelength exiton band, although the bases are necessarily in the *syn* conformation [4]. BrGuo gels also show a positive exciton band [5]. Bush and Tinoco [23] in their computations of optical properties of oligonucleotides assumed their conformation to be that of the B-form of DNA, i.e., a right-handed helix with 36° turn angle and the nucleosides in the *anti*-conformation. The sign of the turn angle between the bases enters into their computations as well as does the orientation of the transition moments of the bases. In the degenerate case, i.e., in homodinucleotides, this term will disappear.

It should be noted that inversion of one base from *anti* to *syn*, as in GpA [8], or the reversion from a right-handed to a left-handed helix, will change the sine of the turn angle and, therefore, also sign. On the other hand, maintaining a right-hand helix with both bases in the *syn* conformation, as in the present case, will still yield a positive turn angle between the transition moments and, therefore, a positive exciton band, which is in agreement with the present observations.

The reason why BrGpBrG is poorly stacked could be searched in the large size of the bromine atoms (diameter 4 Å) which is larger than the stacking distance between free bases. Model building studies indicate that the bromine atom of the 3′-bound nucleoside could seriously interfere with the base of the 5′-bound nucleoside. In the crystal structure of 8-Br-Guo, similar close contacts between bromine and the neighboring base are observed. It is thus to be expected that intense packing (stacking) of BrGpBrG will take place only at very particular conditions, while in solution the steric hindrance will interfere.

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