pH INDUCED CHANGES IN OPTICAL ACTIVITY OF GUANINE NUCLEOSIDES

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Optical rotatory dispersion and circular dichroism have been used to investigate the protonation of guanosine and some of its analogues. An inversion of the principal Cotton effect and the dichroic band is observed below the acid pK. It is suggested that a conformational change from the *anti* form above the pK to the *syn* form below the pK occurs. The reasons why this change should occur only in guanosine and not in adenosine are discussed.

1. Introduction

From the data in the literature [1–6] it appears that the *anti* conformation (fig. 1) of natural pyrimidine nucleosides is characterized by a single positive Cotton effect, while pyrimidine nucleosides fixed in the *syn* conformation have a negative Cotton effect. In contrast, the natural purine nucleosides show a negative Cotton effect [1,3,7]. Recently, many contradictory results have appeared on the correlation of the sign of the Cotton effect with the conformation of purine nucleosides [8-11]. In many cases either substitution of sulfur on one of the sugar carbons or cyclisation between the purine and a sugar OH have been used to make model compounds. Such methods are equivocal since they introduce additional asymmetries absent in the natural compound. X-ray diffraction studies on nucleosides [12] have pointed to the nearly exclusive presence of the *anti* conformation. Two exceptions are known: deoxyguanosine which is *syn* in a mixed crystal with 5-bromodeoxycytidine [13] and 3′, 5′-cyclic AMP [14] which can coexist in both conformations.

A further point of confusion is the question of what is *syn* and what is *anti*. Using the angle ϕ_{CN} defined by Donohue and Trueblood [15] the *anti* conformation corresponds to $\phi_{CN} = -30^{o}$ and the *syn* conformation to $\phi_{CN} = +150^{o}$. This is confirmed by calculations taking in account Van der Waals contacts [12] and energetical considerations [16]. While in pyrimidine nucleosides the rotation around the glycosidic linkage is restricted, purine nucleosides have much more freedom. Two NMR studies [17,18] support the *anti* conformation in all natural nucleosides in neutral medium and down to pH 2. Recent work [19] on the pH dependent changes of the ORD of DNA induced us to investigate at low pH the behaviour of guanosine and related compounds which are free of additional steric restraints.

* Boursier du SCPRI (Ministère de la Santé) (1964–5) et du CEA (1965–8).

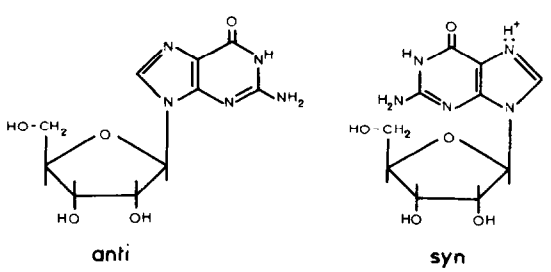

Fig. 1. *Anti* and *Syn* conformation of guanosine.

2. Material and methods

Guanosine (rG), deoxyguanosine (dG) and 2, 6-diaminopurine riboside (DArPu) were commercial

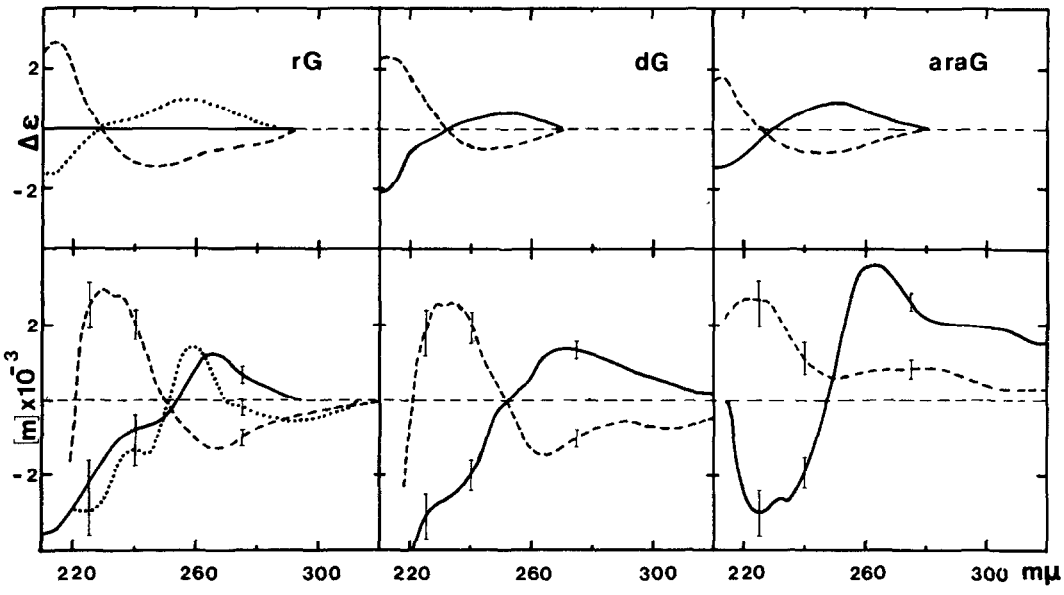

Fig. 2. CD (top) and ORD (bottom) of rG, dG and araG. ---- pH 7, —— pH 1.5, ... pH 0.5.

products (Nutritional Biochemicals, Cyclo Chemical Corporation, respectively). Guanine arabinoside (araG) was a gift of Dr. Privat de Garilhe (Institut Pasteur), 8-Iodo-guanosine (IrG), 8-Hydroxyguanosine (HOrG) and 8-Benzoxyguanosine (BOrG) were gifts of Prof. Robins (University of Utah), 7-methylguanosine (MrG) was prepared according to Jones and Robins [20].

Optical rotatory dispersion spectra (ORD) were recorded on a Cary 60 spectropolarimeter at very low scan speeds. In regions of low signal to noise ratio the pen response time was increased. Circular dichroism spectra (CD) were recorded on a Roussel-Jouan Dichrographe II . Ultraviolet absorption spectra were recorded on a digitalized Cary 14 spectrophotometer.

3. Results

Fig. 2 shows the ORD and CD spectra of rG, dG and araG at different pH values. The spectra of these compounds are qualitatively similar. There is a characteristic negative Cotton effect at pH 7 which is inverted at pH values about one unit below the pK. Even more clear cut are the CD spectra which are virtual mirror images. The lack of any negative rotation in the ORD of araG is surprising, and will be discussed in connection with our work on the optical properties of arabinosyl nucleosides [21].

If the rotation of these compounds is followed as a function of pH one can determine the pH at which the inversion takes place. As can be seen in fig. 3 it coincides with the pK, suggesting that the inversion of the Cotton effect depends on the protonation of the guanine residue which takes place on N-7 [22]. It was therefore of interest to study the behaviour of a compound substituted in N-7, such as MrG. It shows a positive Cotton effect at pH 5 while at pH 9 (above the pK) the Cotton effect becomes negative (fig. 4a). Apparently, not only the protonation is important, but also the charge distribution in the guanine residue.

If DArPu is titrated toward acid pH values, no significant changes in the ORD spectrum are observed till pH 1 (fig. 4b). If the pH is further lowered a small but appreciable inversion of the ORD and CD spectrum is observed, which corresponds to the second pk of the base [23], while the first protonation at pk = 5.1 is not accompanied by dramatic changes in optical activity. We conclude therefore that the second proton is probably attached to N-7,

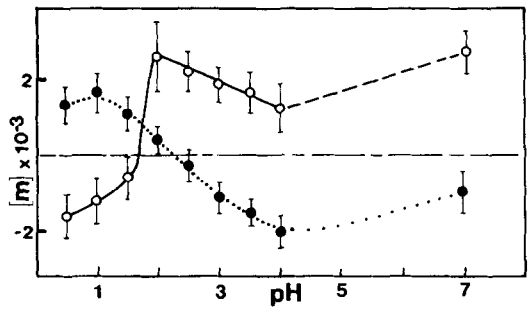

Fig. 3. Change of molar rotation of rG with pH. —— 235 mμ, ... 260 mμ.

while the first could possibly be attached to N-1.

Substitution in position 8 of the purine skeleton by a bulky group has an important effect on the optical activity of the compound. No inversion has been observed on three such compounds tested: IrG (fig. 4c), HOrG and BOrG. The CD and ORD spectra seemed to be lowered only in conc. HCl. It appears therefore that steric factors strongly influence the acid induced optical changes.

4. Discussion

From the observations on ORD spectra of pyrimidine nucleosides [4,6] and purine nucleosides [6–8] it appears that changes in the sign of the principal Cotton effect in the region 220–250 mμ correspond primarily to two kinds of structural changes: a) change of the orientation of the glucosydic linkage (α versus β) or b) change of the conformation from the *anti* to the *syn* conformation or vice versa. Since the first of these two structural changes can be excluded the changes observed in fig. 2 can be explained by the conformational transition from *anti* to *syn*. The fact that the two extremes *anti* and *syn*, respectively, are probably not present exclusively nor predominantly, may explain the small amplitude of rotation observed in purine nucleosides which are much less restricted in their rotational freedom than the pyrimidine nucleosides, which show considerably higher rotation values. This change in Cotton effect is not observed in the other nucleosides studied [2] which maintain the same sign in the Cotton effect upon protonation.

It has now to be asked why only guanine nucleosides should show this change in conformation. It is

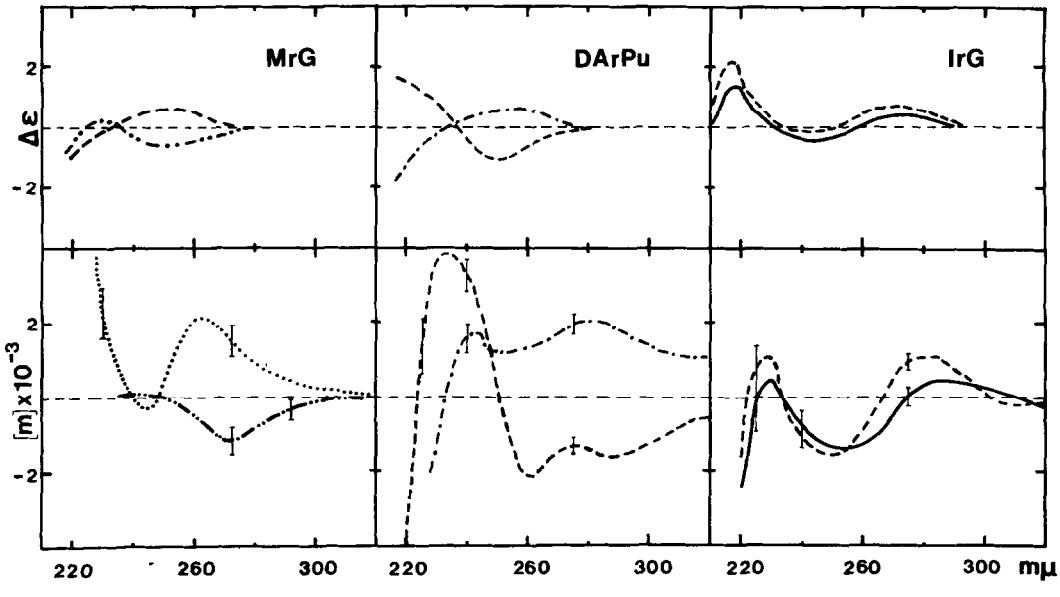

Fig. 4. CD (top) and ORD (bottom) of MrG, DArPu and IrG. –·– pH 9, --- pH 7, —— pH 1.5, –•– conc. HCl, ··· pH 5.

known that adenosine is protonated on N-1 [24] and guanosine on N-7 [22]. In the *anti* conformation a proton on N-1 of the purine skeleton is considerably further away from the OH-groups of the sugar. A proton on N-7 would be at about the same distance from the sugar hydroxyls in the *anti* or the *syn* conformation. Could a shielding effect of the purine skeleton in the *syn* conformation possibly be responsible for this effect? In this case, it should be expected that all N-7 substituted purine nucleosides which have rotational freedom around the glycosydic linkage would show a positive Cotton effect below pH 7.

Lack of an OH-group on the sugar which in acid medium would be at least partly charged should facilitate the possible inversion from *anti* to *syn*. This is the case as can be seen in fig. 2: dG shows complete inversion of optical activity already at pH 1.5, while rG at this pH shows only the midpoint of the inversion. These midpoints correspond to the pK values of these nucleosides. The question why araG which due to the configuration on C-2′ is sterically restricted in its rotation shows this phenomenon remains to be answered.

Although from the data presented here one cannot deduce the absolute configuration of the guanosine analogues in question, in the light of recent NMR data [17,18] we are inclined to favor the inversion from the *anti* conformation in neutral medium to the *syn* conformation below the acid pK for guanosine and other compounds showing this inversion (fig. 1). Such an inversion, would have strong repercussions on the stacking calculations of oligonucleotides and nucleic acids [25] which assume the *anti* conformation [26].

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