^{13}C-NMR ASSIGNMENT, STRUCTURE, AND DYNAMICS OF DEOXYOLIGONUCLEOTIDES.

Nilo Zanatta*, Philip N. Borer+, and George C. Levy+

*Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.

+Chemistry Department, Syracuse University, Syracuse, NY, USA.

Introduction

Recently more attention has been devoted to the unique spectral properties of ^{13}C-NMR to study nucleic acids. Some of the important features of ^{13}C-NMR in oligonucleotide studies have been the issue of recent publications[1-5].

The main difficulty in studying oligonucleotides by ^{13}C-NMR has been the need to use a large amount of sample to compensate for the low natural abundance along with low magnetogyric ratio of the ^{13}C nucleus which make this technique relatively insensitive. However, recent improvements in NMR instrumentation and advances in oligonucleotide synthesis[6,7] make ^{13}C-NMR studies on these molecules tractable.

In this study, high resolution ^{13}C-NMR spectra, T_1 relaxation times and NOEs were measured for duplex of the self-complementary oligo-DNAs: d(CG)$_3$ and d(GGTATACC). The target of this study is to develop a systematic ^{13}C-NMR spectral assignment and to investigate the structure and dynamics of these two sequences by this techniques.

Base Carbons

Secure assignments of the base carbons in oligonucleotides are relatively simple in ^{13}C-NMR as contrasted to ^{1}H-NMR. This is a consequence of the wide dispersion of the carbon signals producing unique resonance positions for most of the carbons in the four mononucleotides. The direct comparison of $[d(GGTATACC)]_2$ with $[d(CG)_3]_2$ (Fig. 1), is also very useful in distinguishing Ado and Thd signals from Cyd and Guo. Finally, the temperature dependence of the chemical shift (the melting profiles) was very useful in making assignments.

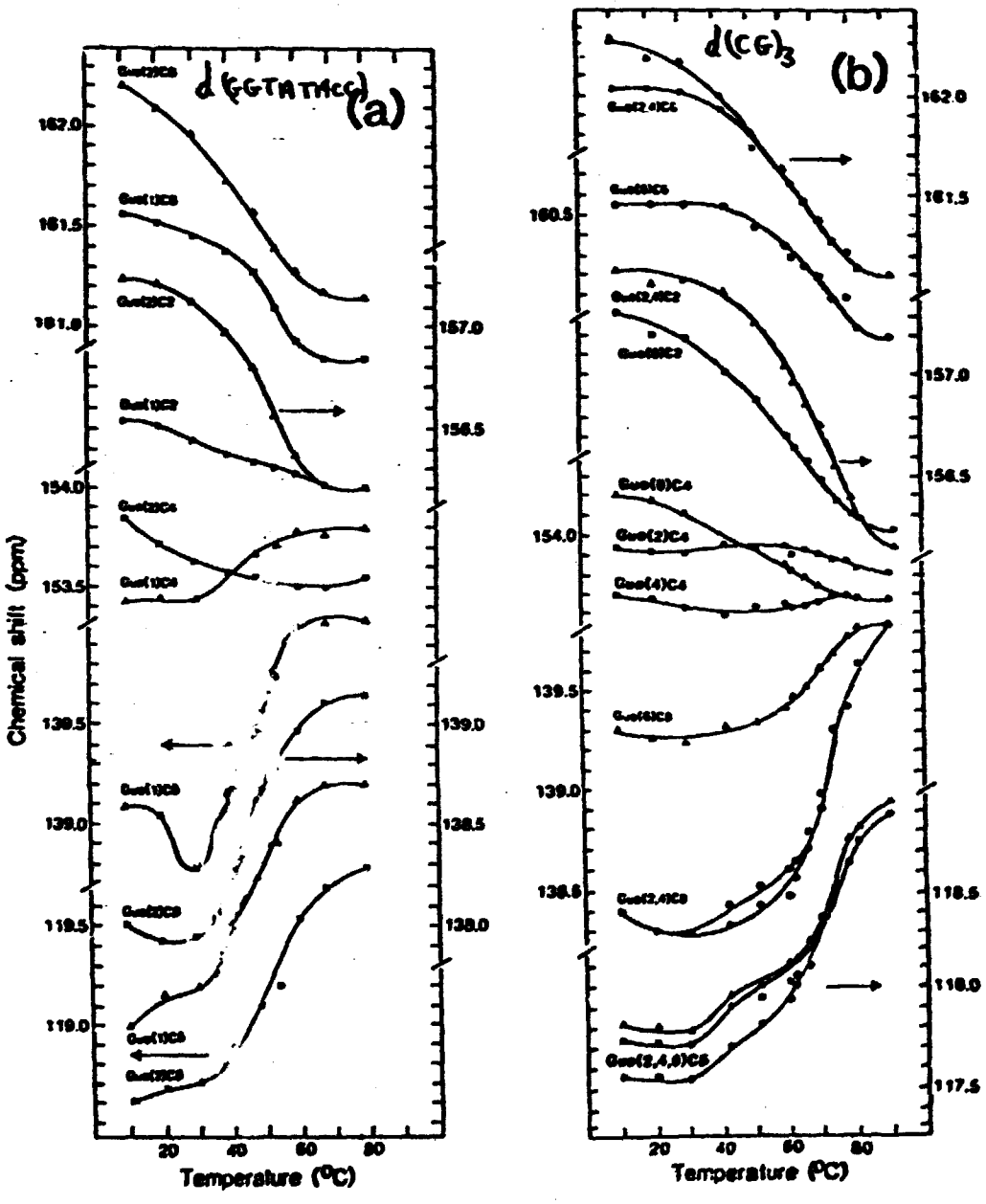
Carbon signals of a given type with strong temperature dependence tend to have parallel, or at least similar melting profiles. Profiles with shallow shapes also tend to be found for certain carbon types, although their shape are more variable. The following principles were stablished to assign the ^{13}C-NMR base carbons signals:

1) Base carbons on internal nucleotides of a given type will have similar melting profiles: (a) carbons with strong positive sloping profiles (deshielded upon duplex dissociation) will be more shielded than the base carbons on the external residues, due to base stacking effects[8,9]; (b) base carbons on internal residues with strong negative profiles will be less shielded than the base carbons on external residues due to hydrogen-bonding effects.

2) The order of base stacking effects is: Ado>Guo>Cyd>Thd[8,9].

3) Base carbons on external nucleotides show smaller chemical shift changes with temperature than the base carbons on internal nucleotides due to fraying at the termini.

4) The melting transition is usually wider for base carbons on terminal nucleotides.


d(GGTATACC)
(a)
d(CG)3
(b)
Chemical shift (ppm)
Temperature (°C)
Temperature (°C)

Sugar Carbons

^{13}C-NMR spectra of the sugar carbons present four distinct and separated regions which are C1'-C4', C2', C3' and C5'. Each region absorbs in a range of chemical shift of only a few ppm which make the assignments difficult due extensive overlap of the resonance lines. Tentative assignment of each sugar carbon resonance line was obtained by direct comparison between the two sequences and with mononucleotides, and by analyzing the temperature dependence of the chemical shift.

Analysis of the ^{13}C-NMR variable temperature data of $d(CG)_3$ and d(GGTATACC) duplexes, provided the following conclusions: (a) changes in chemical shift begin earlier in the sugar than in the bases, indicating that these ^{13}C magnetic shielding respond primarily to motion of the deoxyribose at low temperatures. (b) The melting curves of the 1'- and 2'-carbons are very dependent on the nature of the bases. (c) The difference on the melting curves of the C2' and C3' region among the hexamer and the octamer indicate that there may be a different population distribution for the sugar conformation between these two sequences. (d) The convergence of the 3'-,4'-, and 5'-carbons at high temperature suggests that the chemical shift of these carbons are mainly dependent on conformational changes of the phosphate backbone structure. However, the divergent character of the melting profiles indicates that there may be some influence of the bases on the ^{13}C chemical shifts of these atoms.

Relaxation Study

Investigation of the conformational flexibilities and internal motion in double-stranded DNA has recently been studied by multinuclear (^{1}H, ^{31}P, and ^{13}C) NMR. Several groups have reported that relatively short (100-300 np), double-stranded DNA molecules undergo

rapid internal motion with correlation times of a few nanoseconds [10-17]. Consistent trends were also observed in mononucleotides[18] and short deoxyoligonucleotides[1].

With the intention of understanding a little more about DNA dynamic processes, a systematic carbon-13 T_1 and NOE measurement was performed on two small oligonucleotides: $[d(GGTATACC)]_2$ and $[d(CG)_3]_2$. The relaxation data were analyzed with a computer program "MOLDYN"[19], to model the overall and internal motion of these molecules.

From the relaxation studies we have done on both hexamer and octamer deoxyoligonucleotide duplexes we draw the following conclusions: (a) at 30°C the two duplexes tumble "isotropically" with an overall frequency of motion around 1.3 nsec for the hexamer and 3.7 nsec for the octamer. (b) The internal motion of the bases is on roughly the same time scale as the overall motion. (c) The internal motion of the sugar occurs about one order of magnitude faster than the overall motion. (d) The terminal sugars move much faster than the core sugars. (e) The cone angle produced by the model are roughly 30° for the sugars, indicating relatively large amplitude of motion.

References

1. P.N. Borer, N. Zanatta, T.A. Holak, G.C; Levy, J.H. van Boom, and A.H.J. Wang J. Biomol. Struct. and Dyn. 1, 1373 (1984).
2. M.P. Stone, S.A. Winkle, and P.N. Borer J. Biomol. Struct. Dyn. (in press).
3. P.P. Lankhorst, C. Erkelens, C.A.G. Haasnoot, and C. Altona, Nucleic Acid Res. 11, 7215 (1983).
4. P.P. Lankhorst, C.A.G. Haasnoot, C. Erkelens, and C. Altona, J. Biomol. Struct. Dyn. 1, 1387 (1984).