Determination of the backbone torsion angle $\epsilon$ in nucleic acids

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SUMMARY

The multiplet structure of cross peaks in double-quantum-filtered COSY NMR spectra is analysed for those resonances that include passive heteronuclear couplings. Interestingly, the cross peak involving the sugar-ring protons H2' and H3' in nucleic acids display an E.COSY-type appearance exclusively when the backbone torsion angle $\epsilon$ (C4'-C3'-O3'-P) adopts a gauche(-) conformation. This observation allows an unambiguous analysis of the conformation around $\epsilon$, without the knowledge of $^3J_{C,P}$ coupling constants.

INTRODUCTION

Efficient methods for studying structures of nucleic acids using NMR methods are well established (Van de Ven and Hilbers, 1988; Wijmenga et al., 1993). They generally require the measurement of NOEs, which are interpreted using physical relaxation models to account for spin diffusion. Furthermore, coupling constants defining the sugar-ring conformation (Altona, 1982) are evaluated as additional restraints. However, homo- and heteronuclear coupling constants reflecting the conformation of the phosphate backbone have been largely neglected, although they are of particular interest for structures different from A- or B-type helices, such as hairpins, bulges, mismatches or certain nucleic acid analogues designed for antisense DNA application.

In this paper, we report a simple method to distinguish between the gauche(+), trans and gauche(−) conformations around the backbone torsion angle $\epsilon$ (C4'-C3'-O3'-P). It will be demonstrated below that this information is easily extracted from a conventional double-quantum-
Fig. 1. Karplus curve representing the relationship between the $^3J_{\text{H3P}}$ coupling constant and the backbone torsion angle $\epsilon$ (C4'-C3'-O3'-P) parameterised by Lankhorst et al. (1984): $^3J_{\text{H3P}} = 15.3 \cos^2 (\epsilon + 120) - 6.2 \cos (\epsilon + 120) + 1.5$. The sterically allowed regions for this torsion angle, trans and gauche(−), are indicated, as well as the corresponding conformations. The bonds which connect the H2', H3' and P spins are indicated in bold.

filtered COSY experiment (2QF-COSY) (Piantini et al., 1982) by considering the appearance of the H2'-H3' cross peak.

METHODS

Unambiguous determination of the torsion angle $\epsilon$ requires the knowledge of three coupling constants, i.e., $^3J_{\text{H3P}}$, $^3J_{\text{C2P}}$, and $^3J_{\text{C4P}}$ (Blommers et al., 1988). Due to the degeneracies in the Karplus curve (Lankhorst et al., 1984) presented in Fig. 1, $\epsilon$ may not be restricted to a single value when only the $^3J_{\text{H3P}}$ value is known. In the gauche(+) conformation the phosphate lies below the sugar ring, leading to severe van der Waals repulsion, and hence this conformation has never been observed so far. However, in modified nucleoside analogues the gauche(+) conformation may well be sterically allowed and would be conveniently recognised from the large $^3J_{\text{H3P}}$ coupling around 23 Hz. Unfortunately, for the trans and gauche(−) conformations this coupling is of similar magnitude.

The backbone of nucleic acids is not necessarily rigid and conformational transitions may be expected between the trans and gauche(−) conformations. The observed coupling constants then display a time-averaged value, which may be described as:

$$J_{\text{obs}} = x_{\text{trans}} J_{\text{trans}} + (1 - x_{\text{trans}}) J_{\text{ gauche}}$$

where $x_{\text{trans}}$ is the molar fraction of the trans conformation and $J_{\text{trans}}$ and $J_{\text{ gauche}}$ the $^3J_{\text{H3P}}$ coupling constants for the trans and gauche(−) conformations, respectively. Since $J_{\text{trans}}$ and $J_{\text{ gauche}}$ have similar values, neither the molar fraction nor the torsion angle value of the rotamers can be obtained from the $^3J_{\text{H3P}}$ coupling constant solely.