^{31}P Chemical Shift Anisotropy as an Aid in Determining Nucleic Acid Structure in Liquid Crystals

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The low density of protons in nucleic acids and the paucity of long-range NOE restraints make NMR structure determination a notoriously difficult problem.[1] Measurement of residual dipolar couplings for nucleic acids dissolved in a dilute aqueous liquid crystalline medium can alleviate this problem,[2−5] but does not provide direct information on the phosphodiester linkages connecting the nucleotides. Recent experiments that measure ^{1}H−^{31}P dipolar couplings aim to address this problem, but are experimentally challenging because of the relatively long ^{1}H−^{31}P distances and concomitantly small dipolar couplings.[6,7] Changes in chemical shift between isotropic and aligned samples are caused by chemical shift anisotropy (CSA) and also contain valuable structural information.[8] Here we demonstrate that the large CSA of ^{31}P can be used effectively to constrain the orientation of the phosphodiester groups relative to the molecular alignment tensor, improving structural accuracy as judged by cross validation.

The change in chemical shift for a given ^{31}P upon switching from the isotropic to the liquid crystalline phase is given by:

$$\Delta\delta = \sum_{i=x,y,z} \sum_{j=x,y,z} A_{jj} \cos^2 \theta_{ij} \delta_{ii} \qquad (1)$$

where θ_{ij} is the angle between the δ_{ii} principal axis of the traceless CSA tensor and the A_{jj} principal axis of the diagonalized traceless molecular alignment tensor.[9] Little precise information on the ^{31}P CSA tensor of oligonucleotides is available. However, chemically this phosphate is very similar to that in diethyl phosphate, for which single-crystal NMR studies yielded a very precise CSA tensor.[10,11] Small deviations from symmetry were reported for the orientation of the CSA principal axis frame relative to the O3−P−O4 and O1−P−O2 planes of symmetry. However, the direction of these asymmetries depends on the naming of the chemically equivalent O1 and O2 oxygens in diethyl phosphate. Therefore, in our study we assume δ_{11} to be parallel to O3′$_{i-1}$−O5′ and δ_{22} to bisect O1P−P−O2P (Figure 1). The isotropic ^{31}P shifts span only a narrow range of several ppm, which includes the diethyl phosphate resonance. This suggests that the assumption of a uniform CSA tensor for backbone phosphates in oligonucleotides is entirely reasonable.

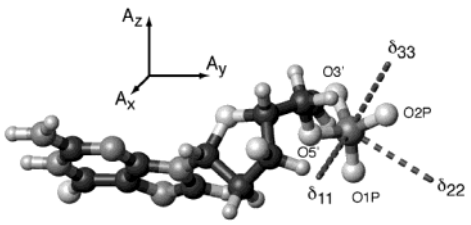

Figure 1. Definition of the ^{31}P CSA tensor orientation, illustrated for nucleotide G4 in d(CGCGAATTCGCG)$_2$, with the helix axis pointing vertical. δ_{11} is orthogonal to the O1P−P−O2P plane and δ_{22} bisects the O1P−P−O2P angle. The traceless δ tensor used here is opposite in sign relative to the chemical shielding tensor, and its values are $\delta_{11} = +80$ ppm, $\delta_{22} = +20$ ppm, and $\delta_{33} = -100$ ppm.[10,11] These values are within 3 ppm from those obtained from a systematic search, optimizing the fit between observed $\Delta\delta$ values and those predicted by the NMR structure calculated without the CSA term, but including $D_{H3'P}$, and fixing the tensor orientation relative to the phosphates as described above.

The method is demonstrated for two samples containing 0.6 mM d(CGCGAATTCGCG)$_2$ in 99.5% D_2O, 50 mM NaCl, 2 mM EDTA, and 10 mM phosphate at pH 6.8 (uncorrected meter reading). The second sample additionally contained 20 mg/mL Pf1, which causes nematic liquid crystalline order.[2] Both samples were washed repeatedly in Centricon cells to ensure identical buffer composition. 2D H3′−^{31}P spectra were recorded at 600 MHz by using a selective HSQC experiment, where the ^{1}H 180° pulses were of the selective RE-BURP type,[12] and no ^{31}P decoupling was used during ^{1}H acquisition. The alignment tensor in Pf1 was determined by fitting $^{1}D_{CH}$ and $^{1}D_{NH}$ dipolar couplings, measured for a separate ^{13}C/^{15}N-labeled sample, to the oligonucleotide structure, determined in a liquid crystalline bicelle medium.[5]

Figure 2 shows a superposition of the HSQC spectra recorded in isotropic and liquid crystalline phases. Clearly, substantial ^{31}P chemical shift changes are induced by the partial orientation of the dodecamer. H3′ shifts remain unchanged, as expected for protons with small CSA. Remarkably, both upfield and downfield changes in ^{31}P are observed relative to the isotropic shift values, indicating that there is considerable variability in the orientations of the individual phosphate groups relative to the helical axis of this B-form DNA dodecamer. The maximum possible range of $\Delta\delta$ values is bracketed by the cases where the δ_{11} and δ_{33} axes of the CSA tensor are parallel to the *z* axis of the alignment tensor that coincides with the helix axis, and would yield $\Delta\delta$ values of +69 and −84 ppb, respectively. Experimentally, the observed $\Delta\delta$ range covers from +48 to −33 ppb.

Figure 3A shows the correlation between measured and predicted $\Delta\delta$ ^{31}P values for a NMR solution structure, calculated as described previously,[5] including very loose backbone angular restraints to improve convergence,[5] and additionally using the recently determined H3′−P dipolar couplings[7] as input restraints. With a Pearson's correlation coefficient $R_P = 0.96$ and an rmsd of 11.7 ppb, agreement is good, but the difference between measured and predicted $\Delta\delta$ ^{31}P values greatly exceeds the uncertainty in the measurement (±1.5 ppb). This is not surprising when considering that the C−H, N−H, and H−H dipolar couplings, used to determine the NMR structure, exert no direct effect on the orientation of the phosphodiester groups. Clearly, the single H3′−P dipolar coupling is insufficient to define rotations about the ϵ, ζ, α, and β torsion angles. Agreement with crystallographically determined structures is notably worse (R_P

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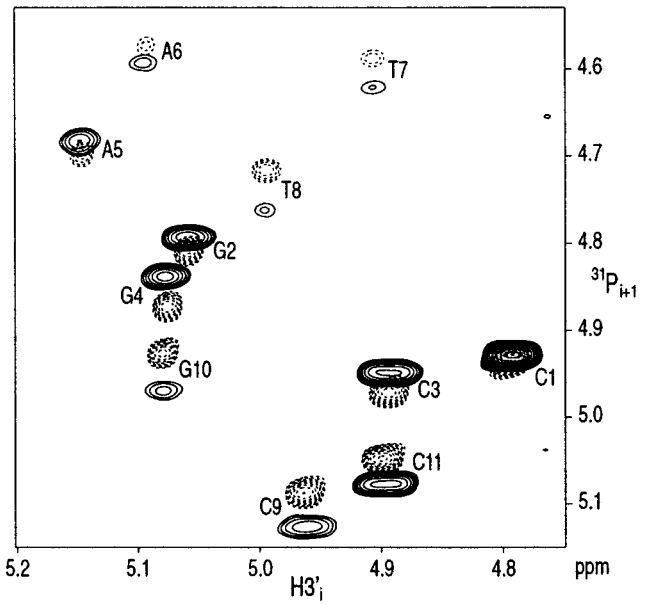

Figure 2. Overlay of the selective ^{1}H3′-^{31}P HSQC spectra of 0.6 mM d(CGCGAATTCGCG)$_2$, recorded at 600 MHz ^{1}H frequency in the absence (dashed contours) and presence (solid contours) of 20 mg/mL Pf1. Spectra result from HSQC spectra with acquisiton times of 200 (t_1) and 128 ms (t_2), apodized with a 48°-shifted sine bell (t_1) and a 72°-shifted quared sine bell (t_2). Total measuring time was 1 h per spectrum.

= 0.64, rmsd = 25 ppb for structure 1BNA;[13] R_P = 0.66, rmsd = 26 ppb for structure 355D [14]) (Supporting Information), even while overall these structures are quite similar to the NMR structure. This lower agreement results from the different environment in the crystallized samples, including several intermolecular contacts and a tightly coordinated Mg^{2+} ion, which appear responsible for two outliers in the correlation for 355D.[14]

To take full advantage of the experimental $\Delta\delta$ values they need to be used as input restraints in the structure calculation. To this extent, a subroutine that describes a term

$$E_{CSA} = k_{CSA}(\Delta\delta_{obs} - \Delta\delta_{pred})^2 \qquad (2)$$

was added to the energetic penalty function in the XPLOR-3.84 software system.[15] The force constant k_{CSA} was adjusted empirically to a value of 0.04 kcal/ppb^2, such that the agreement between observed ($\Delta\delta_{obs}$) and predicted ($\Delta\delta_{pred}$) values equals the estimated experimental uncertainty of 1.5 ppb. The total average increase in the other components (dipolar, NOE, etc.) of the penalty function (12 kcal) was smaller than the variation in these values between individual simulated annealing runs, indicating that the $\Delta\delta$ values are fully compatible with the other experimental parameters.

An independent way for assessing whether E_{CSA} improves structural accuracy compares the agreement between experimental H3′−P dipolar couplings and those predicted on the basis of structures calculated with and without the E_{CSA} term, and in both cases without $D_{H3'-P}$ restraints. Figure 3B shows a significantly better fit between experimental $D_{H3'-P}$ and the structure when E_{CSA} is included (rmsd = 0.31 Hz) than when it is not (rmsd = 0.39 Hz). Comparison of all backbone angles obtained with and without the E_{CSA} term shows that very small angular changes of the backbone angles (rms = 5°) are sufficient to make the structure agree with the experimental $\Delta\delta$ values. This confirms that ^{31}P $\Delta\delta$ values represent very sensitive structural information.

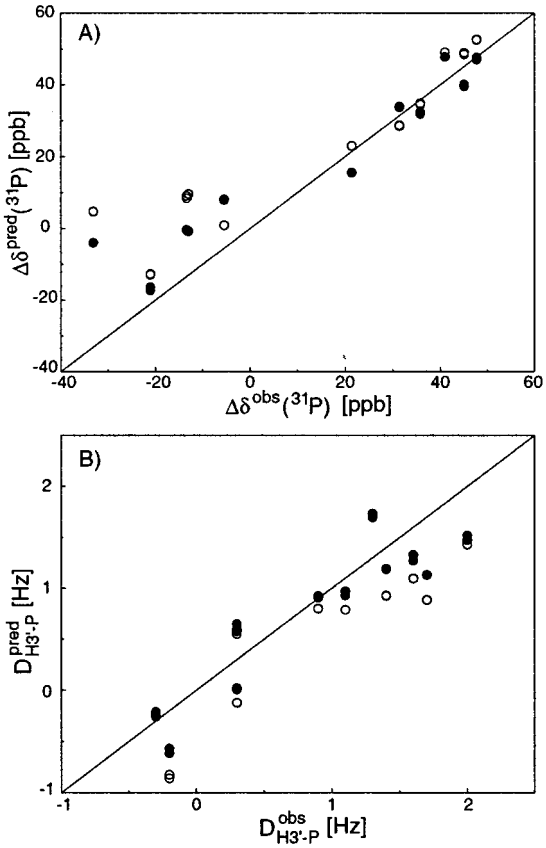

Figure 3. Comparison of observed and predicted parameters for d(CGCGAATTCGCG)$_2$. (A) $\Delta\delta$ ^{31}P values predicted on the basis of the original NMR structure (PDB code 1DUF) (open circles) or the structure calculated by also including $D_{H3'P}$ restraints (solid circles) versus experimental values. (B) $D_{H3'P}$ values predicted from the original NMR structure (open circles) or the structure with E_{CSA} included (solid circles) versus experimental $D_{H3'P}$ values. The alignment tensor has nearly the same orientation as previously reported in bicelles,[5] a larger magnitude (D_a^{CH} = 20 Hz), and a smaller rhombicity (0.05).

The ease with which $\Delta\delta$ ^{31}P values can be obtained and their importance in defining the phosphodiester backbone suggest that they will become widely used in oligonucleotide structure determination.

Supporting Information Available: Two figures comparing experimental $\Delta\delta$ ^{31}P values with values predicted on the basis of the crystal structures 1BNA and 355D (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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