A Method for Direct Determination of Helical Parameters in Nucleic Acids Using Residual Dipolar Couplings

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A double-helix is the most common structural element of nucleic acids. Its geometrical configuration is determined to a large extent by the base-pairing and base-stacking interactions along with the phosphodiester backbone conformation and sugar puckers. These interactions control the relative orientations and positions of purine and pyrimidine bases. The mutual orientation of the bases reflects not only their local sequential arrangements but also the overall locus of the helical structure. A large number of nucleic acids structural studies, following the determination of the first high-resolution DNA structures,[1−4] have established that DNA (and RNA) helices display an unanticipated variability and flexibility in their helical structure. The specific description of the base-stacking and base-pairing interactions in the successive steps of a nucleic acid fragment is in general defined using a set of helical parameters.[5−7] These parameters, however, are not accessible directly from X-ray or NMR experimental data, and only analysis of the refined structure can be used to derive their values. Here we present a novel approach, which to the best of our knowledge represents the first method for determination of helical parameters directly from experimental data. The METHADON (**ME**thod for de**T**ermination of **H**elic**A**l parameters using **D**ipolar c**O**upli**N**gs) procedure is based on the Saupe order matrix analysis of NMR residual dipolar couplings and takes advantage of the restricted mobility and rigid (and well-defined) geometry of purine and pyrimidine bases. The method is demonstrated on the DNA dodecamer d(CGCGAATTCGCG) (Drew-Dickerson dodecamer) using the recently determined high-resolution (1.4 Å) X-ray crystal structure[8] (NDB code: BDL084).

In principle, the dipolar couplings are a valuable source of distance and angular restraints for NMR studies of macromolecules.[9−11] The dipolar coupling between nuclei *n* and *m* can be expressed as

$$D_{nm} = -\frac{2\mu_0\hbar}{(4\pi)^2}\gamma_n\gamma_m\frac{1}{r_{nm}^{3}}\sum_{i,j=\{x,y,z\}} S_{ij}\cos\varphi_i^{nm}\cos\varphi_j^{nm}$$

where μ_0 is the permittivity of free space, $\hbar$ is Planck's constant divided by $2/\pi$, γ_n and γ_m are the magnetogyric ratios of coupled nuclei, r_{nm} is the internuclear distance, S_{ij} are the elements of the Saupe order matrix defining the time-dependent orientation of a molecular coordinate frame with respect to the direction of the external magnetic field, and cos φ^{nm} are the time-independent direction cosines relating the *nm*-th dipolar interaction vector to the molecular coordinate frame.[12] The coordinate frame, in which the Saupe order matrix is diagonal, is referred to as the principal order frame. The Saupe order square matrix [3 × 3] is traceless and symmetrical and contains only five independent elements. Their values, and hence the molecular orientational properties, can be evaluated from just five independent measurements. If the molecular structure is composed of rigid fragments, for which the lengths and time independent direction cosines cos φ^{nm} of at least five dipolar vectors are known, the measured residual dipolar couplings can be used to determine their principal order frames. The bases of the nucleic acids represent one of the best examples of this situation. The method for the determination of rotational helical parameters from residual dipolar data is based on the following considerations: (i) purine and pyrimidine bases have a perfectly rigid (neglecting amino groups) and well-defined geometry independent of the local and global conformation of the molecule; (ii) the angle between bases is invariant with respect to the translation; and (iii) the direction cosines of dipolar vectors in the local principal order frame are constant. In addition, it is assumed that (i) at least five residual dipolar couplings per base between nuclei with fixed distances can be measured in an oriented medium; (ii) the motion of the nonterminal nucleic acid bases is restricted due to the base-stacking and base-pairing interactions; and (iii) the considered base-pairs are stable enough (on the NMR time scale) to have a common principal order frame.

The METHADON approach consists of two steps. In the first one, the relative orientation of two bases is evaluated using the known base geometry and residual dipolar data. In the second step, the helical parameters are extracted using a standard definition and commonly available software (vide infra).

To determine their relative orientation, two bases are placed into the Common Cartesian Coordinate System (CCCS). The starting orientation can be completely random. The residual dipolar couplings (at least five for each base) and the known base geometry are used to generate the principal order frame for each base in the CCCS. This is performed using AMBER 6.0 by minimizing the difference $\sum(D_{calc} - D_{exp})^2$. The mutual orientation of the two bases, for which the helical parameters are being extracted, is subsequently evaluated by a three-dimensional orthogonal transformation (rotation in this case), which makes the principal order frames of the bases in question identical. Next, the orthogonal matrix representing such a transformation is used to modify the Cartesian coordinates of one of the two bases to produce their correct mutual orientation. These coordinates can then be used to extract the helical parameters using the standard definitions.[6,13]

In this context it is important to emphasize that the rotational helical parameters, which can be determined by the METHADON procedure, do not depend on the spatial displacement of purine

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and pyrimidine bases in the 3D structure. Therefore, it is possible to extract their values without the knowledge of the distance between the bases (or fragments, in general) in the molecule. Since the method uses only the angular information contained in the residual dipolar couplings, the translational helical parameters cannot be determined using the procedure described above.

The approach was tested on the theoretically calculated set of residual dipolar couplings from the high-resolution X-ray structure of d(CGCGAATTCGCG)[8] using the AMBER 6.0 program.[14] Five residual dipolar couplings per base were obtained using fixed values for the Cartesian components of the Saupe order matrix ($S_{xx} = -110.253$, $S_{yy} = -40.451$, $S_{xy} = 2.520$, $S_{xz} = 30.737$, $S_{yz} = 70.831$) and geometries of the purine and pyrimidine bases taken from Shui et al.[8] Since at least 6 (for purines) and 8 (for pyrimidines) of these couplings are accessible by NMR measurements of one- and two-bond $^1H-^{13}C$, $^1H-^{15}N$, $^{13}C-^{13}C$, and $^{13}C-^{15}N$ coupling constants in isotopicaly labeled nucleic acids in weakly orienting media,[15] application of this method is experimentally feasible. The METHADON procedure was implemented in the MAPLE 5.4 software package (Waterloo Maple Inc., Canada). The helical parameters were calculated with the CURVES program[6,13] using a nonlinear helical axis. The geometry of bases in the Drew−Dickerson dodecamer was taken from the crystal structure.[8]

By applying the METHADON method six rotational helical parameters were calculated along the helix of the Drew−Dickerson dodecamer: buckle, propeller twist, opening, tilt, roll, and twist (Figure 1a). In the first step, the reproducibility of the helical parameters from the theoretical dipolar data was tested. In this case, the rotational helical parameters derived using the METHADON procedure were in absolute agreement with the reference data set. To mimic the experimental errors, ⟨−8%,+3%⟩ random variation was introduced into the theoretical dipolar data set. The error limits were chosen to simultaneously simulate both random ±3% and systematic −5% errors. The choice of error limits is rather arbitrary since the systematic error strongly depends on the method selected for the measurements of residual dipolar couplings. The results and helical parameters derived from the crystal structure are shown in Figure 1b. As shown, the rotational helical parameters derived using the METHADON procedure clearly fit the overall trends of the reference data. The deviations from reference helical parameters arise from the uncertainty in the dipolar coupling values. The errors in the dipolar couplings are transferred into the determination of principal order frame orientations, which are finally reflected in the values of the helical parameters. Although the random and systematic errors are partially compensated for by the minimization of the difference $\sum(D_{obs} - D_{calc})^2$ during the individual principal order frame calculations, the small cumulative errors may influence the values of helical parameters.

The most pronounced differences between the original and reproduced values of helical parameters are observed for the opening parameter. As has been shown recently by Tjandra et al.,[16] the base-pair opening is quite sensitive to the accuracy of the alignment tensor determination. Our results demonstrate that the opening parameter exhibits similar sensitivity to the accuracy of the principal order frame determination. Therefore, the opening parameter cannot be interpreted quantitatively.

The results presented here demonstrate that the helical parameters can be in principle determined directly from the residual dipolar coupling constants without a prior knowledge of the refined structure. While the quality of the helical parameters determined from the refined structures is influenced by the resolution of the structure and by the empirical potentials used in the refinement procedure, the quality of the helical parameters derived via the METHADON approach is influenced mainly by the accuracy of the residual dipolar couplings measurements. Since only the dipolar couplings and geometry of the bases are required as input information, the procedure should provide a relatively fast method for characterization of the helical parameters in an unknown structure. Moreover, these are some of the parameters that are most difficult to determine accurately using only NOE methods, since there are relatively few base-base NOEs in DNA and RNA helices. Thus, the implementation of this procedure along with the base-base and base-sugar NOEs and dihedral angle restraints currently used to determine nucleic acid helical structures should greatly improve the quality of the calculated structures. Finally, by measuring the distance-dependent residual dipolar couplings the METHADON approach can be extended to incorporate the translation parameters as well.

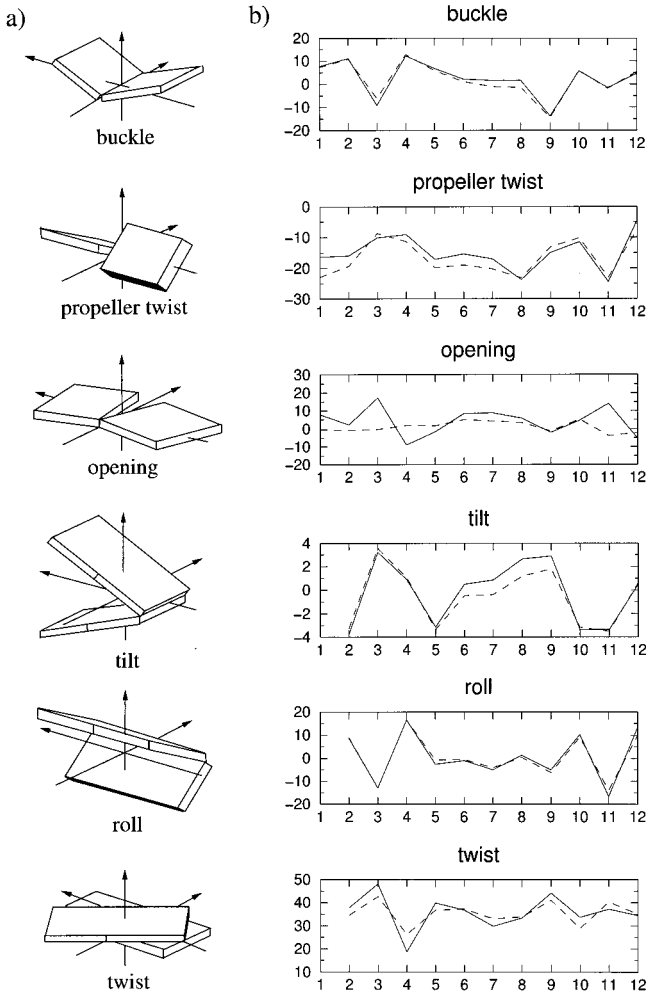

Figure 1. (a) Schematics of the helical parameters buckle, propeller twist, opening, tilt, roll, and twist. (b) Comparison of the rotational helical parameters derived from the d(CGCGAATTCGCG) X-ray structure (dashed line) and from the structure derived using the METHADON approach (solid line).

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