Global Structure of RNA Determined with Residual Dipolar Couplings

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It is often possible to accurately predict the secondary structure of RNAs using phylogenetic or thermodynamic approaches;[1] however, it is not yet possible to predict RNA tertiary or global structure. RNA duplexes with standard Watson−Crick base pairs are usually well modeled by regular A-form geometry, thus a global RNA structure can be generated by determining the orientation of helical stems in RNA. This report describes a NMR method for determining the global helical structure of RNA in solution. A set of $^1H-^{15}N$ residual dipolar couplings were measured in partially oriented samples of ^{15}N-labeled $tRNA^{Val}$ and used to determine the relative orientation of the helical arms in tRNA. The results demonstrate that it is possible to precisely determine the global orientation of RNA helices in solution from a relatively small set of dipolar couplings.

Residual dipolar couplings have recently become an important tool for solution structural studies of macromolecules.[2−4] In nonisotropic solution,[5] the dipolar couplings do not average to zero and therefore provide valuable information on the distance and orientation between NMR active nuclei. Dipolar couplings between covalently bonded nuclei are becoming routinely used to determine the relative orientation of $^1H-^{15}N$ and $^1H-^{13}C$ bond vectors in a molecule.[2−5] The orientational information contained in the dipolar couplings is converted into angular constraints and used in the structure refinement.[3,6] These constraints contain unique structural information characterizing long-range order,[2−4] which complements the strictly local structural information obtained from standard NOE-distance and *J*-coupling torsion angle constraints. In a multidomain molecule, the dipolar couplings measured for each domain can be used to determine the relative orientation of the various domains,[4,7,8] as will be shown here for tRNA.

tRNA was chosen as a model system to probe how well the global orientation of helical domains can be determined from a set of readily measured residual dipolar couplings. Uniformly ^{15}N-labeled and fully modified *E. coli* $tRNA^{Val}$ (Figure 1) was obtained by growing cells on minimal medium containing $^{15}NH_4Cl$ and purified as previously described.[9] The imino proton and nitrogen resonances were assigned from 2D NOESY and $^1H-^{15}N$ HSQC.

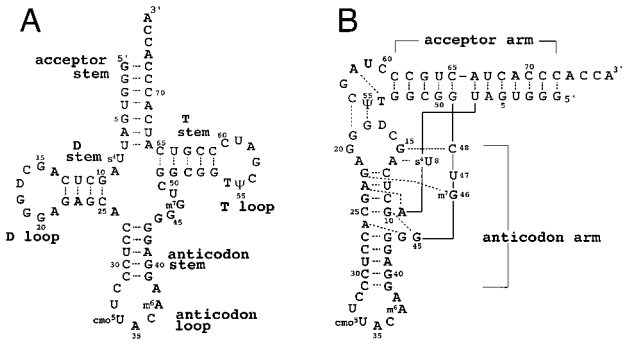

Figure 1. Nucleotide sequence and secondary structure for *E. coli* $tRNA^{Val}$. Solid lines connect covalently bonded nucleotides and dashed lines represent hydrogen bond interactions between bases: (A) cloverleaf representation and (B) L-shape representation. The acceptor and anticodon arms are shown.

The proton assignments agree with previous studies on *E. coli* $tRNA^{Val}$.[10] The tRNA was partially oriented by addition of 10 mg/mL of filamentous phage Pf1.[5b]

The $^1H-^{15}N$ residual dipolar couplings were measured for base paired imino groups using DSSE-HSQC and DSSE-TROSY experiments.[11] A total of 13 couplings were obtained for the acceptor arm, 11 couplings for the anticodon arm, and 3 couplings for the T- and D-loops. The dipolar couplings ranged from −25.3 to +25.1 Hz and had errors of ±1.5 Hz. As seen in Table S1 there is a large variation of the dipolar couplings in a given helical arm. This means that the 27 H−N bond vectors are sampling a wide range of orientations and therefore provide unique information for generating the global structure of tRNA.

Two approaches have recently been proposed for using dipolar couplings to determine the relative orientations of domains in proteins. One method independently calculates the alignment frame for each domain, and then reorients one domain so that it has the same alignment frame as the other domain.[7] A second method rotates one domain relative to the other to find the global conformation that gives the best fit between measured and calculated dipolar couplings.[8] In both approaches a molecular alignment tensor is determined by fitting the experimental dipolar couplings to the equation[2]

$$D_{NH} = D_0^{NH} \boldsymbol{S} A_a[(3\cos^2\theta - 1) + {}^3/_2 R \sin^2\theta \cos(2\phi)]$$

where $D_0^{NH} = -({}^1/_2\pi)(\mu_0/8\pi^2)h\gamma_H\gamma_N\langle r^{-3}_{HN}\rangle$, $\boldsymbol{S}$ is the generalized order parameter ($\boldsymbol{S}$ was assumed to be the same for all the imino NH vectors, which is a reasonable assumption for base pairs in RNA helices), A_a and R are the axial component and the rhombicity of the molecular alignment tensor, and θ and ϕ are the polar angles specifying the orientation of the bond vector with respect to the molecular alignment frame.

Since there is no X-ray structure for *E. coli* $tRNA^{Val}$, a 3D model for it was generated starting from the crystal structure of yeast $tRNA^{Phe}$ (Figure 2), by performing the appropriate base replacements (the two tRNAs share 68% sequence identity), adding hydrogens, and minimizing the structure.[12] The program CONFORMIST[8] was then used to determine components of the alignment tensor that give the best fit of the measured and calculated dipolar couplings. Each arm of the L-shaped tRNA molecule was initially considered as a separate domain. An

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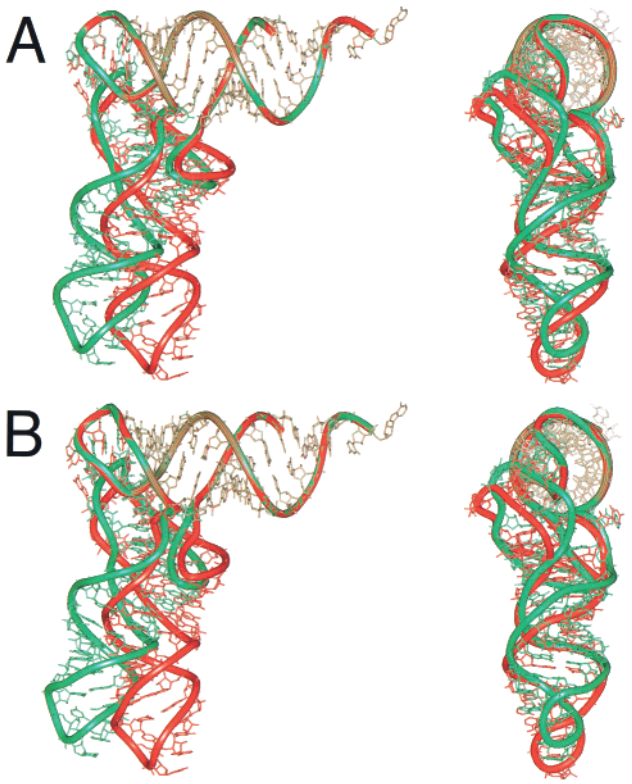

Figure 2. Comparison of the model structure of $tRNA^{Val}$ (red) with the global structure determined here with residual dipolar couplings. (A) Global structure resulting from domain orientation using the X-ray structure-based model for the helical arms (green). (B) Global structure resulting from domain orientation using an A-form model for the helical arms (green).

Table 1. Parameters of the Alignment Tensor for the Whole TRNA Molecule and Each Arm Determined with CONFORMIST

domain	A_a ($\times 10^3$)	R	R_c[a]	rmsd (Hz)
full $tRNA^{Val}$	1.22 ± 0.04[b]	0.60 ± 0.03	0.94	4.9
acceptor arm	1.21 ± 0.04	0.54 ± 0.06	0.95	5.2
anticodon arm	1.22 ± 0.10	0.62 ± 0.08	0.95	3.2

[a] Correlation factor. [b] The uncertainty in the parameters was estimated from test calculations with errors of ± 1.5 Hz added to a set of simulated data.

alignment tensor was calculated for each arm based on the residual dipolar couplings measured for that arm with the results given in Table 1. The axial component (A_a) and the rhombicity (R) of the alignment tensor are very similar for both arms and the full tRNA, consistent with the expected rigid structure between these domains in tRNA.[12] The data in Table 1 show that the local and global structures of the helical arms are well described by the conformation in the X-ray structure.

The next step was to determine the relative orientation of the two arms of $tRNA^{Val}$ using only local helical structural information from the X-ray structure. A conformational search was performed by rotating the acceptor arm while keeping the anticodon arm fixed, using the algorithm implemented in CONFORMIST.[8] The orientations that give the best fits between the predicted and measured dipolar couplings were analyzed. In general, four different solutions exist for orienting one domain relative to another, and these solutions are simply related to each other by 180° rotations of one domain about the *x*, *y*, or *z* axes of the alignment frame.[14] A method for removing the 4-fold degeneracy by combining dipolar couplings from two alignment media has recently been proposed.[15] However, this was not necessary for $tRNA^{Val}$ here because two orientations were not consistent with the covalent structure and a third was not consistent with the nuclear Overhauser effects observed between the D- and T-loops in this tRNA (data not shown).

Figure 2 shows the model for $tRNA^{Val}$ determined from the dipolar couplings, superimposed on $tRNA^{Val}$ derived from the X-ray structure. There is a slight difference in the angle between the helical arms for the two models. The inter-arm angles are 86° for the $tRNA^{Phe}$ crystal structure and $99 \pm 2°$ for the $tRNA^{Val}$ structure determined from the dipolar couplings. These angles where calculated using helical axes of the acceptor arm and the anticodon stem generated with CURVES 5.2.[16] The uncertainty of 2° for the angle is estimated on the basis of the experimental uncertainty of ± 1.5 Hz in the dipolar coupling. There is significant variation for the inter-arm angles in 3 X-ray structures of different tRNAs, ranging from 76 to 96°,[12,17] and the angle determined here for $tRNA^{Val}$ in solution is near the upper range of what has been observed by X-ray.

These results demonstrate that a small number of residual dipolar couplings can be used to determine the global structure of tRNA, if the local structure of the helical stems is known. However this method will be most valuable for RNAs whose 3D structure has not yet been solved. To test how well the dipolar couplings can be used to determine global structure of an RNA with a known secondary structure, we replaced the four stem regions in $tRNA^{Val}$ with A-form helices. A conformational search was performed to find the orientations of the anticodon and acceptor arms that give the best fit to the measured dipolar couplings. This structure is given in Figure 2B and has an inter-arm angle of $101 \pm 2°$. This is in excellent agreement with the angle determined with the helices from the X-ray structure and shows that A-form geometry represents a good model for Watson−Crick helical regions in RNA.

This communication reports the first application of residual dipolar couplings to the global structure determination of RNA. The global conformation of $tRNA^{Val}$ was precisely determined from a small number of $^1H-^{15}N$ dipolar couplings. This procedure can be used to determine the relative orientation of helical regions in RNAs, DNAs, or nucleic acid−protein or drug complexes. It has also been shown that dipolar couplings can be used to accurately determine DNA structure[18] and DNA bending[19] in solution, and together these methods should significantly improve solution structures of nucleic acids.

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Supporting Information Available: A figure with the HSQC spectra of $tRNA^{Val}$ and a table with ^1H and ^{15}N chemical shifts and dipolar couplings for $tRNA^{Val}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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