Improving the Accuracy of NMR Structures of RNA by Means of Conformational Database Potentials of Mean Force as Assessed by Complete Dipolar Coupling Cross-Validation

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Abstract: The description of the nonbonded contact terms used in simulated annealing refinement can have a major impact on nucleic acid structures generated from NMR data. Using complete dipolar coupling cross-validation, we demonstrate that substantial improvements in coordinate accuracy of NMR structures of RNA can be obtained by making use of two conformational database potentials of mean force: a nucleic acid torsion angle database potential consisting of various multidimensional torsion angle correlations; and an RNA specific base-base positioning potential that provides a simple geometric, statistically based, description of sequential and nonsequential base-base interactions. The former is based on 416 nucleic acid crystal structures solved at a resolution of ≅2 Å and an R-factor ≅25%; the latter is based on 131 RNA crystal structures solved at a resolution of ≅3 Å and an R-factor of ≅25%, and includes both the large and small subunits of the ribosome. The application of these two database potentials is illustrated for the structure refinement of an RNA aptamer/theophylline complex for which extensive NOE and residual dipolar coupling data have been measured in solution.

Introduction

NMR structure determination involves seeking the minimum of a target function comprising terms for the experimental NMR restraints, covalent geometry, and nonbonded contacts.1 The description of the nonbonded contacts can have a significant impact on the accuracy of a NMR structure determination,2 particularly in the case of nucleic acids where the density of short interproton distances is rather limited.3 On the basis of the results of cross-validation against independent NMR observables (interproton distance restraints derived from nuclear Overhauser enhancement measurements and dipolar couplings), we recently showed that significant improvements in the accuracy of NMR structures of DNA can be obtained by including both torsion angle and base-positioning database potentials of mean force in the description of the nonbonded interactions.3 These statistical potentials, which are derived from high resolution crystal structures, seek to bias sampling during simulated annealing refinement to physically reasonable regions of conformational space within the range of possibilities that are consistent with the experimental NMR restraints.4,5 Although double stranded DNA can adopt a number of distinct conformations (e.g., A, B, or Z-DNA), interstrand hydrogen-bonding is usually limited to Watson-Crick base pairing, no tertiary interactions are present, and interresidue contacts are generally limited to nucleotides and base pairs adjacent in the linear sequence.6 Although the local conformation of RNA is typically A-type, RNA can adopt much more complex structures than DNA, including not only a variety of non-Watson-Crick interstrand hydrogen-bonding interactions, but also long-range internucleotide tertiary interactions between nonsequential nucleotides or base pairs.7-9 As a consequence, the design of the base-base positioning potential employed successfully for DNA3 in which interactions were limited to linearly sequential intra- and interstrand base-base contacts is not appropriate for RNA. In this paper, we describe a base-base positioning potential of mean force specifically designed for RNA, and demonstrate using complete dipolar coupling cross-validation10 that the use

References

Table 1. Breakdown of Databases Used to Create the Torsion Angle and Base—Base Positioning Potentials of Mean Force

<table>
<thead>
<tr>
<th>A. nucleic acid torsion angle database potential (resolution ≤ 2 Å, R factor ≤ 25%)*</th>
<th>no. of structure</th>
<th>no. of valid residue pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA structures</td>
<td>64</td>
<td>A-RNA 24</td>
</tr>
<tr>
<td>protein-RNA</td>
<td>2</td>
<td>B-RNA 61</td>
</tr>
<tr>
<td>drug-RNA</td>
<td>8</td>
<td>Z-RNA 42</td>
</tr>
<tr>
<td>unusual RNA</td>
<td>19</td>
<td>drug→DNA 89</td>
</tr>
<tr>
<td>DNA structures</td>
<td>11</td>
<td>protein-DNA 66</td>
</tr>
<tr>
<td>A-DNA</td>
<td>332</td>
<td>unusual DNA 22</td>
</tr>
<tr>
<td>B-DNA</td>
<td>52</td>
<td>DNA/RNA hybrids 20</td>
</tr>
<tr>
<td>Total</td>
<td>416</td>
<td>Total: 131</td>
</tr>
</tbody>
</table>

B. RNA specific base-base positioning potential (resolution ≤ 3 Å, R factor ≤ 25%)*

<table>
<thead>
<tr>
<th>no. of structures</th>
<th>no. of valid residue pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-RNA</td>
<td>30</td>
</tr>
<tr>
<td>tRNA</td>
<td>9</td>
</tr>
<tr>
<td>protein-RNA</td>
<td>43</td>
</tr>
<tr>
<td>ribosomal protein-RNA</td>
<td>6</td>
</tr>
<tr>
<td>drug-RNA</td>
<td>21</td>
</tr>
<tr>
<td>unusual RNA</td>
<td>22</td>
</tr>
<tr>
<td>Total: 131</td>
<td>105350</td>
</tr>
</tbody>
</table>

* The torsion angle database potential comprises 26 2D surfaces: αl, αl2, αl3, αl4, αl5, αl6, αl7, αl8, βl, βl2, βl3, βl4, βl5, βl6, βl7, βl8, γl, γl2, γl3, γl4, γl5, γl6, γl7, γl8, δl, δl2, δl3, δl4, δl5, δl6, δl7, δl8, and εl. 2 3D surfaces: γl/γl2, γl3, γl4, γl5, γl6, γl7, γl8, δl, δl3, δl4, δl5, δl6, δl7, and εl. 1 4D surface: γl/γl2/γl3/γl4.

of these database potential coupled with a torsion angle database potential of mean force leads to a considerable improvement in the accuracy of the NMR structures of an RNA aptamer/theophylline complex for which extensive nuclear Overhauser enhancement (NOE) and residual dipolar coupling data have previously been measured in solution.11,12

Methods

Database Potentials. The database potentials are derived from the structures present in the Nucleic Acid Database13 as of March 2001. The torsion angle and base-base positioning potentials are distributed with Xplor-NIH.14

The DELPHIC torsion angle database potential of mean force, $E_{\text{delphi}}$, consists of a set of multidimensional potential surfaces derived from high-resolution crystal structures describing various torsion angle correlations in two-, three-, and four-dimensions (Table 1A).15 The raw potentials are fitted by sums of multidimensional quartic bell functions as described previously,15 and $E_{\text{delphi}}$ is given by

$$E_{\text{delphi}} = k_{\text{delphi}} \sum_{i=1}^{N} E_{\text{delphi}}(i)$$

where $k_{\text{delphi}}$ is a unitless force constant, $N$ is the number of DELPHIC torsion restraints (i.e., the number of torsion angle potential surfaces), and $E_{\text{delphi}}(i)$ is the sum of the quartic bell functions fitted to the potential surface appropriate for a particular set of torsion angle correlations. In the case of a two-dimensional surface correlating torsion angles $\alpha$ and $\beta$, for example, $E_{\text{delphi}}(i)$ is of the form

$$E_{\text{delphi}}(i) = \sum_{j=1}^{Q} \text{torsionQuart}(i,j)$$

where $Q$ is the number of quartic bells used to fit the raw DELPHIC torsion angle potential of mean force, and

$$\text{torsionQuart}(i,j) = \text{height}(j) \cdot \alpha \text{Frac}(i,j)^2 \cdot \beta \text{Frac}(i,j)^2$$

where $\text{height}(j)$ is the height of a particular fitted quartic bell function $j$ (in kcal/mol$^{-1}$), and

$$\alpha \text{Frac}(i,j) = \frac{[\alpha_{\text{width}}(j)^2 - \Delta \alpha(i,j)^2]}{\alpha_{\text{width}}(j)^2}$$

and

$$\beta \text{Frac}(i,j) = \frac{[\beta_{\text{width}}(j)^2 - \Delta \beta(i,j)^2]}{\beta_{\text{width}}(j)^2}$$

where $\alpha_{\text{width}}(j)$ and $\beta_{\text{width}}(j)$ are the widths of the fitted quartic bell function $j$ along the $\alpha$ and $\beta$ axes, and $\Delta \alpha(i,j)$ and $\Delta \beta(i,j)$ are the minimal angular distances from the center of the fitted quartic bell function $j$ (along each axis) to the current values of the torsion angles from DELPHIC torsion restraint $i$.

The raw DELPHIC base-base positioning potentials of mean force are likewise fitted by sums of multidimensional quartic bell functions, and the energy for the DELPHIC base-base positioning potential, $E_{\text{delpos}}$, is given by

$$E_{\text{delpos}} = k_{\text{delpos}} \sum_{i=1}^{N} E_{\text{delpos}}(i)$$

where $k_{\text{delpos}}$ is a unitless force constant, $N$ is the number of DELPHIC positional restraints (i.e., the number of base-base positional potential surfaces) and $E_{\text{delpos}}(i)$ is the sum of the quartic bell functions fitted to the potential surface type appropriate for the four orienting atoms of restraint $i$

$$E_{\text{delpos}}(i) = \sum_{j=1}^{Q} \text{positionQuart}(i,j)$$

where $Q$ is the number of quartic bells used to fit the raw DELPHIC positioning potential of mean force, and

$$\text{positionQuart}(i,j) = \text{height}(j) \cdot x \text{Frac}(i,j)^2 \cdot y \text{Frac}(i,j)^2 \cdot z \text{Frac}(i,j)^2$$

where $\text{height}(j)$ is the height of a particular fitted quartic bell function $j$ (in kcal/mol$^{-1}$), and

\[ x\text{frac}(i,j) = \begin{cases} \frac{x_{\text{width}}(j)^2 - \Delta x(i,j)^2}{\left|x_{\text{width}}(j)^2\right|} \\ 0 \end{cases} \text{ if } |\Delta x(i,j)| < x_{\text{width}}(j) \]

\[ y\text{frac}(i,j) = \begin{cases} \frac{y_{\text{width}}(j)^2 - \Delta y(i,j)^2}{\left|y_{\text{width}}(j)^2\right|} \\ 0 \end{cases} \text{ if } |\Delta y(i,j)| < y_{\text{width}}(j) \]

\[ z\text{frac}(i,j) = \begin{cases} \frac{z_{\text{width}}(j)^2 - \Delta z(i,j)^2}{\left|z_{\text{width}}(j)^2\right|} \\ 0 \end{cases} \text{ if } |\Delta z(i,j)| < z_{\text{width}}(j) \]

where \( x_{\text{width}}(j), y_{\text{width}}(j), \) and \( z_{\text{width}}(j) \) are the widths (in Å) of the fitted quartic bell function \( j \) along the local x, y, and z axes, respectively, and

\[ \Delta x(i,j) = x_{\text{Pos}}(i) - x_{\text{Cen}}(j) \]

\[ \Delta y(i,j) = y_{\text{Pos}}(i) - y_{\text{Cen}}(j) \]

\[ \Delta z(i,j) = z_{\text{Pos}}(i) - z_{\text{Cen}}(j) \]

where \( x_{\text{Cen}}(j), y_{\text{Cen}}(j), \) and \( z_{\text{Cen}}(j) \) are the coordinates of the center of the quartic bell function \( j \), and \( x_{\text{Pos}}(i), y_{\text{Pos}}(i), \) and \( z_{\text{Pos}}(i) \) are the local, standardized coordinates of the oriented atom \( i \) of restraint \( i \), which are defined using the global coordinates of the orienting atoms I, J, K (of the first base), and the oriented atom I (of the second base) of DELPHIC position restraint \( i \), as described in ref 3.

Simulated Annealing. All simulated annealing calculations were carried out in torsion angle space\(^{16}\) using the NMR molecular structure determination package Xplor-NIH.\(^{14}\) In addition to terms for the nonbonded interactions, the target function comprises quadratic squarewell potentials for the distance and torsion angle restraints,\(^{7}\) a harmonic potential for the dipolar couplings,\(^{18}\) and a harmonic potential for Watson–Crick base pair planarity restraints to prevent undue buckling while allowing propeller twisting to occur\(^{7}\). Three main sets of calculations were carried out using three different descriptions of the nonbonded interactions: (i) a quartic van der Waals repulsion term;\(^{17}\) (ii) A 6–12 Lennard–Jones van der Waals and electrostatic term from the all-hydrogen CHARMM nucleic acid empirical energy function;\(^{19}\) (iii) A quartic van der Waals repulsion term together with the torsion angle\(^{18}\) and base–base positioning\(^{2}\) database potentials of mean force designed for nucleic acids and RNA, respectively. The resulting structures are referred to as (R), (LJ), and (R + Db), respectively. In addition, a fourth set of calculations, yielding structures (LJ + Db), was also carried out in which the 6–12 Lennard–Jones and electrostatic potentials were combined with the torsion angle and base-base positioning database potentials.

The quartic van der Waals repulsion term, \( \epsilon_{\text{rep}} \), is given by\(^{17}\)

\[ \epsilon_{\text{rep}} = \begin{cases} 0 \\ k_{\text{vdw}}(s_{\text{vdw}} - r_{\text{min}})^2 r^2 \\ k_{\text{vdw}}(s_{\text{vdw}}^2 r_{\text{min}}^2 - r^2) r^2 \end{cases} \]

where \( k_{\text{vdw}} \) is a force constant; \( r \) the distance between a pair of atoms; \( r_{\text{vdw}} \), the corresponding sum of the van der Waals radii between the two atoms of the pair; and \( s_{\text{vdw}} \) a van der Waals radius scale factor (whose optimal value is 0.78) to account for the absence of an attractive component to the potential.

The simulated annealing protocol is similar to that previously described for DNA and comprises three steps: (i) 10 ps of dynamics at 3000 K, in which all nonbonded interactions involving either the quartic van der Waals repulsion term or the Lennard-Jones and electrostatic terms are turned off with the exception of those between C1′ atoms; (ii) 119 cycles of 0.2 ps each in which all nonbonded interactions are turned on, the temperature is slowly reduced from 3000 to 25 K, and the force constants for the various terms in the target function are gradually increased to their final values; and (iii) a few cycles of torsion angle minimization. The final values of the force constants are as follows: 1 kcal mol\(^{-1}\) Å\(^{-2}\) for the dipolar couplings, 30 kcal mol\(^{-1}\) Å\(^{-2}\) for the distance restraints, 200 kcal mol\(^{-1}\) rad\(^{-2}\) for the torsion angle restraints, 20 kcal mol\(^{-1}\) Å\(^{-2}\) for the planarity restraints, except for the end base-pair (G1–C33) where a force constant of 80 kcal mol\(^{-1}\) Å\(^{-2}\) was used; 4 kcal mol\(^{-1}\) Å\(^{-4}\) for the quartic van der Waals repulsion term with a scale factor of 0.78 for the van der Waals radii; 1 for the torsion angle database potential; and 0.3 for the base-base positioning database potential. In the case of the calculations with the Lennard–Jones and electrostatic terms, the parameters for these two potentials are left unchanged during the entire course of the calculation. (Note that a 1/\( r^6 \) screening function is employed for the electrostatics,\(^{20}\) and the net charge on the phosphate group is reduced to \(-0.32\) e\(^2\);\(^{21}\) nonbonded interactions are switched off between 9.5 and 10.5 Å using a cubic switching function, and pairs up to 11.5 Å are included in the nonbonded list).

**Results and Discussion**

**Torsion Angle Database Potential.** The torsion angle database potential of mean force comprises a set of multidimensional potential surfaces (26 2D, 8 3D, and 1 4D) describing various torsion angle correlations (see footnote a to Table 1). The raw multidimensional potential surfaces are derived from 416 crystal structures of nucleic acids (64 RNA and 332 DNA) solved at ≤2 Å resolution with an R-factor of ≤25%. The breakdown of structures is shown in Table 1A. The raw potential surfaces, each of which comprise an average of 3207 ± 386 examples, are then fitted by a sum of multidimensional quartic functions,\(^{15a, 18} \) and these fitted functions are incorporated as a pseudo-potential into the target function for refinement.\(^{18} \) There are more DNA structures than RNA ones, but this does not pose a problem since there are numerous representatives in the DNA database whose local backbone structure is similar to RNA. Only structures solved at a resolution of ≤2 Å resolution were employed, since the sugar–phosphate backbone torsion angles, sugar pucker and glycosidic bond torsion angles can only be determined accurately from high-resolution crystallographic data.

**RNA Base–Base Positioning Potential.** The base-base positioning potential of mean force is derived from 131 RNA crystal structures solved at a resolution of ≤3 Å and an R-factor ≤25% (Table 1B). These database includes both the 2.4 Å resolution structure of the large 50S ribosomal subunit\(^{22} \) and the 3 Å resolution structure of the small 30S ribosomal subunit,\(^{23} \) which make up ∼39% and ∼18%, respectively, of all the base-base interactions in the database. Because the bases comprise large rigid planar groups, their positions can still be relatively accurately determined even at comparatively low resolution. The overall position of each base is defined by the coordinates of three orienting atoms (I, J, K) that have been translated and

\[ \text{Clore and Kuszewski} \]

rotated into a standard geometry; the relative geometry of a second base with respect to the first base is defined by the Cartesian coordinates of its three oriented atoms (I, J, K) to which the same rotations and translations have been applied. The orienting atoms I, J, and K are N7, N6/O6, and N3 for A/G bases; and C6, N4/O4, and O2 for C/U bases. Thus, the orientation of the second base relative to the first is described by three separate 3D surfaces. The RNA base-base positional potential comprises two components: a set of 96 \((3 + 3) \times 4^2\) 3D surfaces representing sequential (i, i+1) base–base interactions, and 48 \(3 \times 4^2\) 3D surfaces representing all nonsequential intra- and interstrand base–base interactions. The standard coordinate space over which the base–base positioning potentials are calculated comprises a 20 Å per side cube with atom J of the first base at the origin, atom I along the negative x axis and atom K in the xy plane. The average number of examples per 3D surface is 445 ± 157 for the sequential database, and 4732 ± 1608 for the nonsequential one. As in the case of the torsion angle database potential, the raw 3D surfaces are fitted by a sum of three-dimensional quartic functions that are then used in the target function for refinement.

Both the torsion and the base–base positioning potentials deal solely with interactions that are close in space. Consequently, the effects of crystal packing on the global structure of nucleic acids do not in any way decrease the utility of these database potentials in NMR structure determination because the databases are sufficiently large to include all conformations that are likely to exist in solution.

**Description of RNA System used to Assess the Impact of the Database Potentials.** To assess the impact of the torsion angle and base–base positioning potential on the accuracy of RNA structures determined by NMR, we made use of previously acquired experimental NMR data on an RNA aptamer/theophylline complex solved by Pardi and colleagues. This NMR structure has several features that make it ideally suited for the present study. First, the RNA/theophylline complex represents one of the few RNA structures that have been solved on the basis of both extensive NOE-derived interproton distance constraints and 13C–1H residual dipolar couplings, thereby permitting the use of dipolar coupling cross-validation as an independent means of assessing accuracy. Second the RNA/theophylline complex contains a range of RNA structural motifs which provide a rigorous test of the database potentials. In addition to the presence of regular A-RNA type stems, the RNA/theophylline complex features non-Watson–Crick base-pairing, the presence of three base triples, a base-zipper, and interstrand stacking motifs, and an S-turn in the backbone containing a reversed sugar.

Because the “true” solution structure is unknown, accuracy can only be judged by indirect means. The simplest approach, which has been extensively employed in work on proteins, is to compare the calculated NMR structures to an existing high-resolution crystal structure. The agreement between observed and calculated values of NMR observables (such as dipolar couplings, chemical shift anisotropy, chemical shifts and J couplings) is typically excellent for high resolution protein crystal structures, and usually significantly better than for the corresponding NMR structures refined in the absence of these observables. One can therefore conclude that, in general, structures of proteins in the crystal and in solution are very similar, and hence protein crystal structures usually provide a good reference point for judging accuracy. For nucleic acids, however, the situation is far more complex, since it is well-known that crystal packing forces can have a significant impact on global structure. For example, the palindromic Dickerson DNA dodecamer is asymmetric and kinked in the crystal, but symmetric and essentially straight in solution. Moreover, in the case of RNA, there are no examples for which both a high-resolution crystal structure has been determined and extensive NMR measurements, including residual dipolar couplings, are available. An alternative approach using cross-validation to assess accuracy must therefore be employed.

**Complete Dipolar Coupling Cross-Validation.** Cross-validation is a statistical method in which the structure calculation is carried out omitting a subset of the data (the test set) while refining against the remaining data (the working set). The quality of the fit and, consequently, the accuracy of the calculated structures are cross-validated by the agreement between the structures and the test set. Thus, cross-validation allows one to determine how well the data in the test set are predicted by structures calculated on the basis of the working data set, and a more accurate structure will predict the test data set better than a less accurate one. The cross-validated free-R-factor is routinely employed in macromolecular crystallography and is directly correlated with a model’s phase accuracy. Residual dipolar couplings measured in dilute liquid crystalline media are ideally suited for cross-validation: they provide both local and global orientational information; they can be accurately measured with known experimental error; and a dipolar coupling R-factor, \(R_{\text{dip}}\), which scales between 0% and 100% can be readily calculated (0% representing a perfect fit, and 100% a random orientation of internuclear vectors).

In the case of the RNA/theophylline complex it has been shown that the NOE-derived data is not sufficient to define the overall orientation of the two stems and that this can only be achieved by the incorporation of residual dipolar couplings. As a consequence, one cannot simply calculate a set of structures based solely on NOE data and expect the conformational database potentials to produce any significant improvement in the overall agreement between calculated and observed dipolar couplings. In addition, because each dipolar coupling only contains information relating to an individual interatomic vector, it is insufficient to carry out a set of calculations using only a single working set and test set, as is done in crystallography where each reflection contains information on the entire molecule. One therefore has to resort to complete dipolar coupling cross-validation to assess the impact of the various nonbonded terms on structure accuracy. To this end the dipolar couplings were divided into 10 pairs of working and test sets chosen at random, and comprising 70% and 30%, respectively.

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tively, of the data. The working sets are used in refinement, whereas the corresponding test sets are employed only for cross-validation, that is to calculate a dipolar coupling free $R$-factor, $R_{\text{dip}}(\text{free})$. 25 simulated annealing structures were calculated for each pair, resulting in a total of 250 structures per calculation.

**Results of Structure Calculations.** The results of the three sets of simulated annealing calculations are summarized in Figure 1 and Table 2. The agreement with the experimental restraints included in the target function (that is the distance and torsion angle restraints and the working set of dipolar couplings) is broadly comparable for all three ensembles of structures and is consistent with experimental error (Table 2). The $(R + Db)$ structures satisfy the torsion angle restraints somewhat better than the other structures which probably reflects a smoother path to the global minimum region of the target function as a consequence of the introduction of the torsion angle database potential. On the other hand, the dipolar coupling working $R$-factor, $R_{\text{dip}}(\text{work})$, is smallest for the $(R)$ structures.
Table 2. Structural Statistics\(^{\dagger}\)

<table>
<thead>
<tr>
<th></th>
<th>(\langle R \rangle)</th>
<th>(\langle L_j \rangle)</th>
<th>(\langle R_{\text{Db}} \rangle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipolar coupling R-factors (101)(^b)</td>
<td>(50.3 \pm 7.4)</td>
<td>(39.2 \pm 6.6)</td>
<td>(26.8 \pm 2.8)</td>
</tr>
<tr>
<td>(R_{\text{ap}}) (work) (%)</td>
<td>(5.4 \pm 0.6)</td>
<td>(6.2 \pm 0.6)</td>
<td>(8.6 \pm 0.5)</td>
</tr>
<tr>
<td>rms deviation from other experimental restraints</td>
<td>(0.095 \pm 0.010)</td>
<td>(0.076 \pm 0.008)</td>
<td>(0.089 \pm 0.003)</td>
</tr>
<tr>
<td>distances (275) (Å)(^c)</td>
<td>(1.0 \pm 0.11)</td>
<td>(0.09 \pm 0.12)</td>
<td>(0.01 \pm 0.04)</td>
</tr>
<tr>
<td>torsion angles (110) (°)(^d)</td>
<td>(1.81 \pm 0.34)</td>
<td>(1.37 \pm 0.26)</td>
<td>(0.65 \pm 0.18)</td>
</tr>
<tr>
<td>coordinate precision (Å)(^e)</td>
<td>(1.69 \pm 0.35)</td>
<td>(1.26 \pm 0.25)</td>
<td>(0.59 \pm 0.18)</td>
</tr>
<tr>
<td>all residues excluding C27</td>
<td>(15.56 \pm 0.37)</td>
<td>(14.63 \pm 0.27)</td>
<td>(14.95 \pm 0.23)</td>
</tr>
<tr>
<td>measures of end-to-end length</td>
<td>(47.2 \pm 2.1)</td>
<td>(43.4 \pm 1.6)</td>
<td>(44.7 \pm 1.0)</td>
</tr>
<tr>
<td>(r^{\text{CT1H-CT1S}}) (Å)</td>
<td>(51.8 \pm 1.9)</td>
<td>(48.1 \pm 1.2)</td>
<td>(47.7 \pm 0.9)</td>
</tr>
</tbody>
</table>

\(^{\dagger}\) The notation of the structures is as follows: \(\langle i \rangle\) is an ensemble of 250 simulated annealing structures calculated with complete dipolar coupling cross-validation (see footnote b). \(\bar{x}\) are the average coordinates derived from each ensemble; \(x_{ik}\) are the restrained regularized mean coordinates. The nonbonded terms for the three sets of structures are as follows: \(\langle R \rangle\), structures calculated with a quartic van der Waals quartic repulsion term; \(\langle L_j \rangle\), structures calculated with the Lennard–Jones van der Waals and electrostatic potentials using the all-hydrogen CHARMM TOPNAH1ER1 nucleic acid parameters; \(\langle R_{\text{Db}} \rangle\), structures calculated with the quartic van der Waals repulsion term together with the torsion angle and base-base positioning database potentials of mean force. The number of terms for the various experimental restraints are given in parentheses. \(^{\dagger}\) There are a total of 101 experimentally measured \(^{13}\)C–\(^1\)H dipolar couplings, comprising 55 dipolar couplings within the sugars (C1′–H1′, C2′–H2′, and C3′–H3′) and 46 within the bases (C8−H8, C6−H6, C5−H5, C2−H2).\(^{12}\) The dipolar couplings were divided into 10 pairs of working and test data sets chosen at random and partitioned in a ratio of 70% (working) to 30% (test). 25 simulated annealing structures were calculated for each pair, and the results represent the averages obtained for all 250 calculated structures.

Table 3. Atomic rms Differences Between the Regularized Mean Coordinates

<table>
<thead>
<tr>
<th></th>
<th>(\langle R + \text{Db} \rangle)</th>
<th>(\langle L_j \rangle)</th>
<th>(\langle R \rangle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R_{\text{ap}}) (work)</td>
<td>(0.064)</td>
<td>(0.052)</td>
<td>(0.042)</td>
</tr>
<tr>
<td>(R_{\text{ap}}) (free)</td>
<td>(0.050)</td>
<td>(0.037)</td>
<td>(0.030)</td>
</tr>
</tbody>
</table>

\(^{\dagger}\) The regularized mean coordinates are derived from the average coordinates of the 250 simulated annealing structures by restrained regularized minimization and include all the dipolar couplings. \(^{b}\) Values above the diagonal are for all residues, and below the diagonal exclude C27 which is poorly determined by the experimental NMR restraints.

Accuracy of NMR Structures of RNA

The inclusion, however, of the two database potentials in combination with the quartic van der Waals repulsion term reduces \(R_{\text{ap}}\) (free) much further: by a factor of \(~1.5\) relative to the Lennard–Jones and electrostatic terms, and \(~2\) relative to the quartic van der Waals repulsion term alone. Equally importantly, the distribution of \(R_{\text{ap}}\) (free) is \(~2.5\) to \(~3\) times narrower for the \(\langle R + \text{Db} \rangle\) structures than for the \(\langle R \rangle\) and \(\langle L_j \rangle\) structures (Figure 1). Thus \(R_{\text{ap}}\) (free) values range from \(~21\) to \(~34\)\% for the \(\langle R + \text{Db} \rangle\) structures, from \(~26\) to \(~67\)\% for the \(\langle L_j \rangle\) structures, and from \(~33\) to \(~69\)\% for the \(\langle R \rangle\) structures. One can therefore conclude that the inclusion of the torsion angle and base-base positioning database potentials result in a substantial increase in accuracy, as measured by complete dipolar coupling cross-validation.

What does an \(R_{\text{ap}}\) (free) value of \(~26.8 \pm 2.8\)\% observed for the \(\langle R + \text{Db} \rangle\) structures relate to in terms of structure quality? The simplest means of providing a qualitative answer to this question is to survey a variety of protein crystal structures for which N–H backbone dipolar couplings have been measured in our laboratory (G. M. C., unpublished data). The measurement error for normalized \(^{15}\)N–H and \(^{13}\)C–H dipolar couplings is comparable, so that the values of \(R_{\text{ap}}\) (free) for the RNA/theophylline complex can be directly compared to those of \(R_{\text{ap}}\) (free) for proteins. \(R_{\text{ap}}^{\text{SH}}\) is found to be correlated to crystallographic resolution, and ranges from \(~15\%\) to \(~27\%) for protein structures solved at resolutions of 1.5 to 2.5 Å. This suggests, that the ensemble of \(\langle R + \text{Db} \rangle\) structures calculated with the torsion angle and base-base positioning database potentials is...
approximately equivalent in accuracy to a 2.5 Å resolution protein crystal structure.

The left-hand panels of Figure 1 illustrate the conformational space sampled in the three ensembles of structures using an atomic density probability map representation.31 Best-fit superpositions of the three restrained regularized mean structures are shown in Figure 2a and b, and plots of coordinate precision as a function of residue number are displayed in Figure 2c. The overall topology and RNA fold of the three ensembles of structures are clearly the same. However, excluding C27 which is poorly determined by the experimental NMR restraints, the pairwise atomic RMS difference between the \((R + Db)\), \((LJ)\), and \((R + Db)\) restrained regularized mean structures ranges from ~1.7 to 2.1 Å. Thus, there are significant structural differences, both global and local, between the three ensembles of structures. This is also reflected in the overall dimensions of the structures, as measured by both the radius of gyration \((R_{\text{gyr}})\) and the two end-to-end distances, \(\langle r_{C1'-C15} \rangle\) and \(\langle r_{C1'-C15} \rangle\): the \((R)\) ensemble is expanded and the \((LJ)\) one slightly compressed relative to \((R + Db)\). It is also worth noting that, in this instance, the precision of the coordinates (Table 2 and Figure 2c) is directly correlated to \(R_{\text{dip}}\) (free) (Table 2 and Figure 1). The overall coordinate precision of the \((LJ)\) structures (1.4 ± 0.3 Å) is comparable to that reported for the structures calculated by Sibille et al.12 using all the dipolar couplings and the Lennard-Jones potential from the AMBER432 force field (1.5 ± 0.2 Å).

The \((R)\) ensemble is somewhat lower (1.7 ± 0.4 Å), whereas that of the \((R + Db)\) one is significantly higher (0.7 ± 0.2 Å).

A fourth set of calculations was also carried out combining the 6–12 Lennard–Jones and electrostatic potentials with the torsion angle and base–base potentials of mean force. The \(R_{\text{dip}}\) (free) and coordinate precision of the resulting ensemble of structures, \((LJ + Db)\), have values of 27.2 ± 3.1Å (with a range of 21–34%) and 0.6 ± 0.1 Å, respectively, which are almost identical to the corresponding values for the \((R + Db)\) structures (Table 2). In addition, the atomic RMS difference between the \((LJ + Db)\) and \((R + Db)\) mean coordinates is 0.6 Å which is comparable to the precision of both sets of coordinates. One can therefore conclude that the \((LJ + Db)\) and \((R + Db)\) ensembles are essentially the same within coordinate error. Thus, the introduction of the torsion angle and base-base positioning potentials of mean force removes artifactual and systematic distortions arising from conventional descriptions of the non-bonded interactions, either in terms of a simple repulsive potential to prevent atomic overlap or empirical 6–12 Lennard–Jones and electrostatic potentials.

**Concluding Remarks**

We have shown using complete dipolar coupling cross-validation that, even for an RNA data set comprising quite


extensive NOE-derived interproton distance restraints and dipolar couplings, the description of the nonbonded contacts used in the target function for simulated annealing has a large impact on both coordinate accuracy and precision, and local and global structure. A purely repulsive van der Waals term leads to expanded structures of lower precision and accuracy, because on entropic grounds, there are more expanded than compacted configurations that satisfy the experimental restraints. Lennard–Jones van der Waals and electrostatic terms result in some improvement in accuracy, relative to a purely repulsive van der Waals term, but tend to lead to structural compression, presumably because of the attractive component in the Lennard–Jones term. The addition of both torsion angle and base-base positioning potentials of mean force to the description of the nonbonded contacts (either van der Waals repulsion or Lennard–Jones plus electrostatics), however, leads to very substantial improvements in accuracy, as judged by a large decrease in $R_{\text{dip}}$ (free) which is accompanied by a concomitant increase in precision. Concomitantly, the introduction of the database potentials obviates systematic distortions associated with particular empirical descriptions of the nonbonded interactions. We therefore conclude that the routine use of the torsion angle and base-base positioning potentials should lead to significant improvements in the accuracy and quality of RNA structures generated from NMR data. In addition, these two database potentials may also be helpful in the refinement of low resolution (>3 Å) crystal structures, in modeling of RNA structures, and possibly in molecular dynamics studies of RNA as well.

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