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Problems with, and Alternatives to, the NMR *R* Factor

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The determination of the structures of large molecules in solution has relied to a considerable extent on the information present in proton nuclear Overhauser effect, NOE, data which can be obtained using one-, two-, or higher-dimensional experiments (*1–3*). The NOE is a through-space effect and can arise via a direct magnetization transfer between two protons or through a diffusion pathway. If a particular NOE arises via a direct transfer between two protons, then, at short mixing times, the intensity of the NOE cross peak as a function of the mixing time is approximately $\sigma_{ij}\tau_m$, where τ_m is the mixing time used in the experiment and σ_{ij} is the cross-relaxation rate between the protons. If a particular NOE arises both from a direct transfer and as diffusion transfers then the intensity of the NOE cross peak at short mixing times is $\sigma_{ij}\tau_m + \sum \sigma_{ik}\sigma_{kj}\tau_m^2$ plus higher-order terms.

The method commonly used for comparing the NOE intensities calculated from a model with those experimentally determined as a function of the mixing time was proposed by Gonzalez *et al.* (*4*) and others (*5–7*). Following a method developed for crystallography the "NMR *R* factor" was proposed, which is given by

$$R(\tau_m) = \sum_{ij} |E_{ij}(\tau_m) - T_{ij}(\tau_m)| / \sum_{ij} E_{ij}(\tau_m),$$

where $E_{ij}(\tau_m)$ is the experimental intensity of the cross peak between protons i and j at mixing time τ_m and $T_{ij}(\tau_m)$ is the theoretical intensity. The *R* factor for the entire mixing time series is given by

$$R = \sum_{m} \sum_{ij} |E_{ij}(\tau_m) - T_{ij}(\tau_m)| / \sum_{m} \sum_{ij} E_{ij}(\tau_m).$$

There are certain features of this method which suggest that it is not the most appropriate one for evaluating the fit between theoretical models and NMR data.

The *R* value comparison contains a somewhat hidden bias. For example, if an experimental NOE has a value of 3 units and the theoretical data a value of 1 unit, then the *R* value is $\frac{2}{3}$. If the experimental data has a value of 1 unit and the theoretical prediction a value of 3, then the calculated *R* value is 2. It is not obvious to us how a statistical test for NOE data having this property can be justified. When the difference between the theoretical and experimental values is the same, the error should be the same. The result of the analysis should not depend on whether the experimental value is larger or smaller than the theoretical. This property of the *R* value may lead to systematic errors in structure determinations.

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It is also noted that the signal-to-noise in NOE experiments is related to the mixing time since the NOE intensities tend to increase with increasing mixing time. The relatively lower signal-to-noise ratio in data obtained at the shorter mixing times may tend to accentuate the bias in the R-value-based methods.

In our investigations of evaluating models for the dynamical structure of DNA based on NOE data we have found that the R values may not offer sufficient discrimination. The R value method can generally discriminate between quite different structures such as A- and B-form DNA. However, as is shown below, the R value method can, in certain cases, give equally good fits of experimental data to structures as different as A- and B-form DNA. For these and the reasons mentioned below it is not clear that this method is appropriate for investigations of the structure of DNA or of proteins for that matter.

The bias in the R factor can be removed by using a different method for calculating the comparison of the theoretical and experimental data. A simple extension can give

$$Q(\tau_m) = \sum_{ij} |E_{ij}(\tau_m) - T_{ij}(\tau_m)| / \{\sum_{ij} E_{ij}(\tau_m) + \sum_{ij} T_{ij}(\tau_m)\}.$$

The Q factors will not depend on whether the experimental or theoretical value is the larger. Comparison of the Q and R factors for various theoretical models should indicate the importance of the bias in the R factors. It is expected that the Q and R values for entire models will tend to be somewhat similar but that distinct differences will be obtained for specific portions of the models.

A more satisfactory approach than either Q or R, from a statistical point of view, is to use a root-mean-square calculation which is given by

$$\mathrm{RMS}(\tau_m) = [\sum_{ij} \{E_{ij}(\tau_m) - T_{ij}(\tau_m)\}^2 / \{\sum_{ij} E_{ij}(\tau_m)^2 + \sum_{ij} T_{ij}(\tau_m)^2\}]^{1/2}.$$

The RMS values have the advantage of being dependent on a point-by-point comparison of the experimental and theoretical results and depend on the differences between the experimental and theoretical values and not on which is larger. It is noted that the computational demands for determining R, Q, and RMS are quite small and will not be important in choosing between them.

To evaluate the R, Q, and RMS approaches we have applied these methods of analysis to a variety of models for the structure of a duplex DNA by means of comparing the predicted with the experimental NOE data. Two-dimensional NOE data were obtained using a Varian Unity 500 MHz spectrometer for the mixing times of 25, 50, 75, 100, 150, and 200 ms on a 3 mM sample of the palindromic dodecamer DNA duplex of 5′($C_1G_2C_3G_4A_5A_6T_7T_8C_9G_{10}C_{11}G_{12}$)3′ paired with the self-complementary 5′($C_{13}G_{14}C_{15}G_{16}A_{17}A_{18}T_{19}T_{20}C_{21}G_{22}C_{23}G_{24}$)3′ in 0.1 M NaCl at pH 7.0 and a temperature of 30°C. The peak assignments in the dodecamer spectra were made by sequential methods and found to be consistent with those previously reported (*8, 9*). A total of 217 inter- and intraresidue proton–proton NOE correlations were obtained for each strand of the symmetric duplex. The NMR results clearly show that the structure of the dodecamer in solution falls into the B-form family.

The molecular dynamics simulations involve the dodecamer duplex, 1927 water molecules, and 22 Na^+ counterions carried out in two different forms: the ES model, using a canonical B-form initial structure and the GROMOS 86 force field, and the

WC model, essentially the ES model including harmonic distance restraints between the atoms involved in Watson–Crick base-pair hydrogen bonding. The procedures for performing these molecular dynamics simulations have been described (*10*) as have the general features of the use of these models to predict the experimental data (*11, 12*). In addition, the experimental data were compared with those predicted using the canonical A- and B-form structures of this DNA as well as that predicted by the X-ray structure (*13, 14*). The static models, the A- and B-form structures, and the X-ray structure were used to predict the experimental data taking orientation effects into account and the dynamical models were used to predict the experimental data taking the orientation and internal motion into account by the methods that we have previously described (*11, 12*).

The *R* values for A- and B-form DNA as well as for the X-ray structure are shown in Fig. 1. Also shown in Fig. 1 are the *Q* and RMS values for these three models for

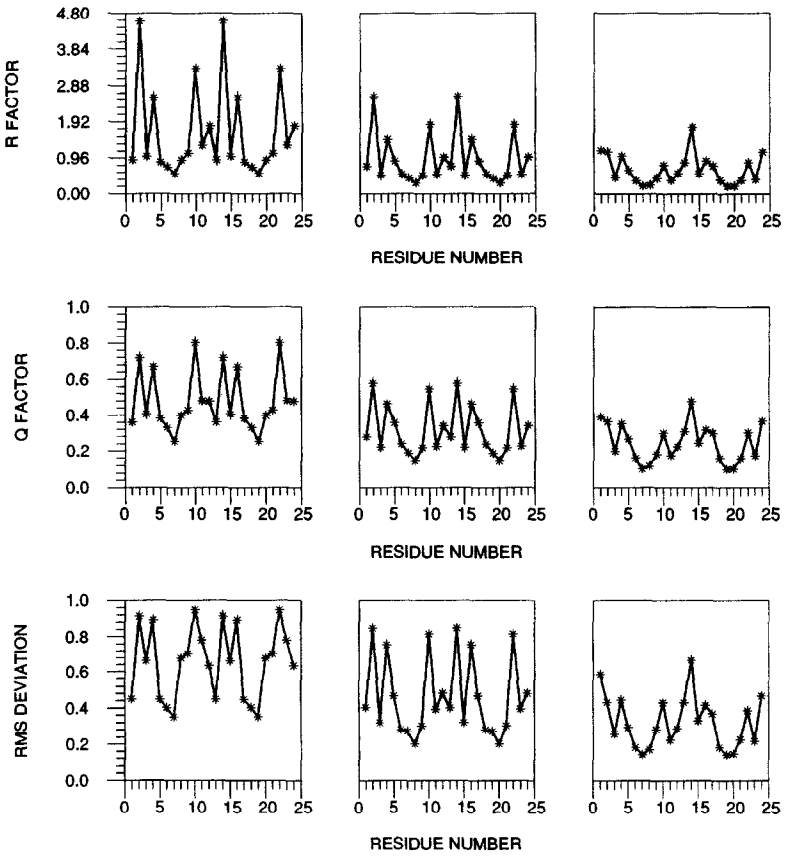

FIG. 1. The values of the *R* factor, *Q* factor, and RMS deviation calculated for theoretical and experimental NOE results for the dodecamer d(CGCGAATTCGCG)$_2$. The theoretical results are for the canonical form of A-DNA (left) and B-DNA (middle) and the X-ray structure (right). Results are determined for each residue in the duplex.

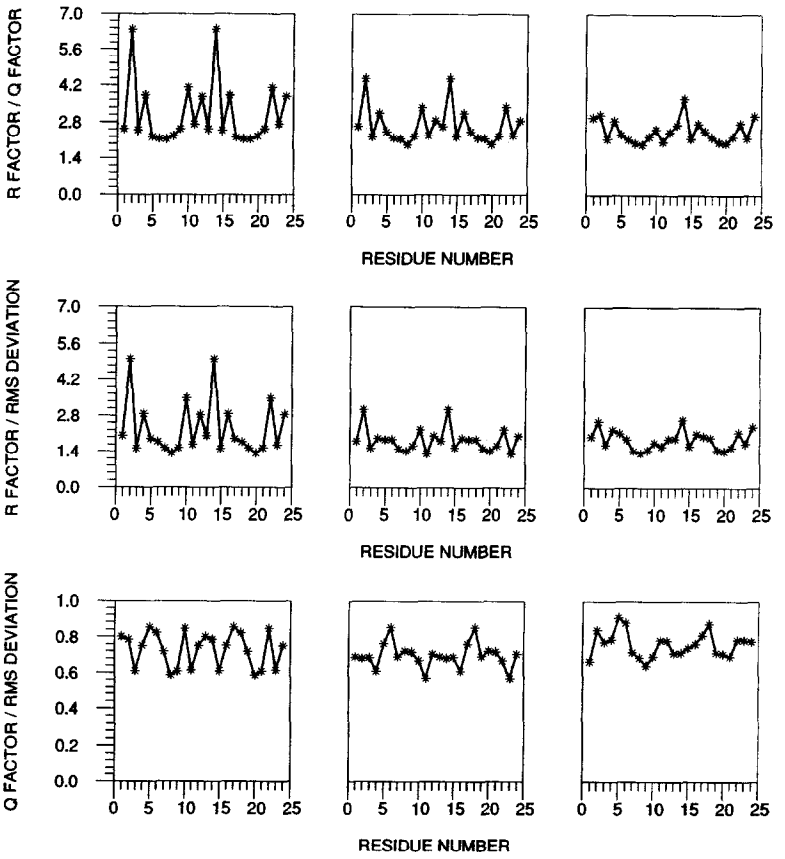

FIG. 2. The values of the R/Q factor ratio, R factor/RMS value ratio, and Q factor/RMS deviations ratio calculated for the theoretical and experimental NOE results for the canonical form of A-DNA (left) and B-DNA (middle) and the X-ray structure (right). Results are determined for each residue in the duplex.

the DNA. By all three measures the X-ray structure gives a better fit to the experimental data than does the B-form structure, which gives a better fit than the A-form structure.

The R factors for the A-form structure indicate that some regions of the DNA, mostly the AATT portion, give much better fits to the experimental data than do the other regions. A similar picture is obtained from the R factors for B-form DNA with the AATT portion giving the best fits. It is a curious feature of the R factors that the same region gives the best fit to two distinctly different structures. The R factor analysis for both the A- and B-form structures tends to indicate that some regions are well described by the model and others much worse described. This is mostly due to the bias in the R factors.

The Q factors for the A-form structure indicate that there is a more uniform poor fit of the data to this model than that obtained with the R factor. The same general feature is found for the B-form structure with the Q factors varying less from residue to residue than the R factors. Therefore, the Q factors indicate that both the A-form and the B-form structures give relatively poor fits to the experimental data at all

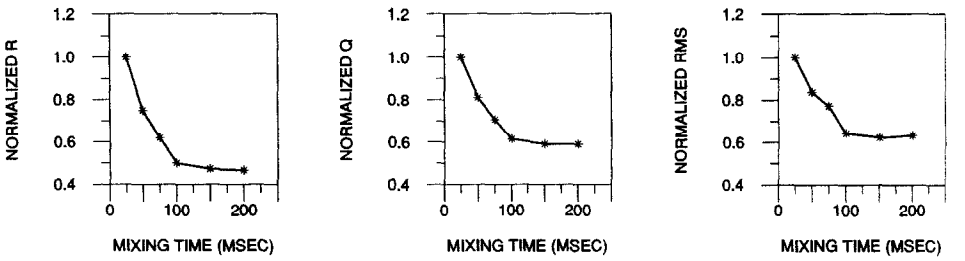

FIG. 3. The values of the R factor (left), Q factor (middle), and RMS (right) values calculated for the X-ray structure and experimental NOE results for the dodecamer d(CGCGAATTCGCG)$_2$. Results are determined for all residues and are shown as a function of experimental mixing times. All results are shown normalized to the 25 ms mixing time result.

residues whereas the R factors suggest that some regions give much better fits than other regions. The bias inherent in the R factor is less significant for the X-ray structure, in which the fit to experimental data is improved. As a result, the Q and R factors are more similar than is true for either A- and the B-form models.

The RMS values for the A- and B-form structures indicate that most of the residues give a relatively poor fit of the data to either of these models. For the X-ray structure, the RMS values follow a trend similar to that of the Q factors with a somewhat more even distribution of the quality of fit throughout the duplex.

These comparisons show that for models which do not fit the data very well the R values can be misleading in suggesting that some regions may actually be agreeing with the data. The Q values and RMS factors do not have as much of this feature. For good models the percentage difference between the experimental and theoretical values will be small for all comparisons and the Q and RMS methods will tend to offer quite similar pictures. However, when the percentage differences are large the RMS method will tend to accentuate the contributions of the largest differences and the RMS and Q methods may differ significantly.

The three approaches can also be contrasted with one another by examination of the ratio of their values as shown in Fig. 2. The R/Q ratio for a particular mixing time is given by

$$R(\tau_m)/Q(\tau_m) = 1 + \{\sum_{ij} T_{ij}(\tau_m)/\sum_{ij} E_{ij}(\tau_m)\}$$

and thus a value greater than 2.0 indicates reduced experimental intensities relative to the calculated intensities. This ratio fluctuates more for the A-form structure than for the B-form and the crystal structure. The ratio of R/RMS shows the same pattern. The consideration of these two ratios on a residue-by-residue basis indicates how the bias in the R factor can vary from site to site in a molecule. The ratio of Q/RMS shows less fluctuation and decreases somewhat in going from the worst model, the A-form structure, to the best of the three static models, the X-ray structure.

As mentioned above the NOE intensities are a function of the mixing time. Since the methods used here to calculate the NOEs take into account all magnetization-transfer pathways, there is no particular reason, other than signal-to-noise, to weight

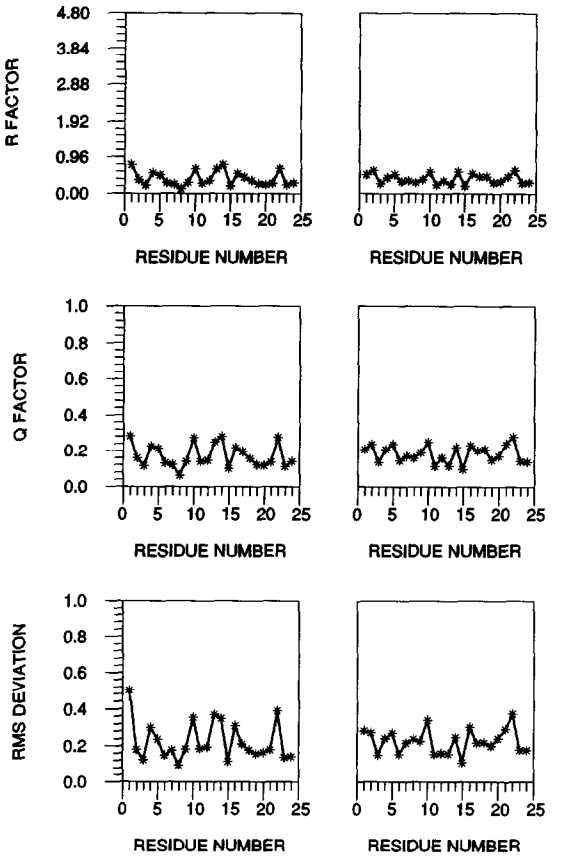

FIG. 4. The R factors, Q factors, and RMS values calculated for the WC (left) and ES (right) models and the experimental NOE results for the dodecamer $d(CGCGAATTCGCG)_2$. Results are calculated for each residue in the duplex.

the data at short mixing times more or less than the data at longer mixing times. The signal-to-noise feature can be partially overcome by allocating proportionately more experiment time to the short-mixing-time experiments.

The R, Q, and RMS values for the X-ray structure have been calculated for each of the mixing times and the results are shown in Fig. 3 normalized to the result at 25 ms. These results show that the R factors are significantly larger for the short-mixing-time data whereas the Q factors and RMS values show less dependence on mixing time. Thus, the R factors will tend to have relatively more unequal contributions from the data obtained at different mixing times than the Q factors and RMS values. Since a proper model should accurately predict the data at all mixing times this dependence of the R factors on mixing time may be considered an additional flaw arising from the bias in the R factors.

The three methods have also been applied to more accurate models for the dynamical structure of the duplex DNA. These models are generated from molecular dynamics simulations and include the contributions from internal motion as well as the aniso-

tropic motion of the duplex (*11, 12*). The *R*, *Q* factors and RMS values for two of these dynamical models for the duplex DNA are shown in Fig. 4. All three measures indicate that the WC and ES simulations fit the experimental data much better than the A- or B-form or crystal structure models. The overall quality of fit of the WC and ES models to the experimental data is similar to that detected by all three methods. However, the fit of data varies considerably from residue to residue for both models. These structural differences are detected in the *Q* factors and RMS values but suppressed to a large degree in the measure of the *R* factors. Thus, utilizing *R* factors as the only measurement of the quality of fit to experimental data prevents discrimination of similar structural models and the determination of a highly resolved structure.

The *Q* factor and RMS values offer similar residue-by-residue fits to the data for the WC and ES models, which is to be expected for good models. The largest difference between the *Q* factor and RMS values is for residue 1 in the WC model. It is likely that the WC model may not properly model the end of the helix and this may be reflected in the difference between the *Q* factor and the RMS value for this residue.

The *Q* factor and RMS values appear to be appropriate for evaluating the fit between experimental NOEs and those calculated from theoretical models. The bias in the *R* factor makes it unsuitable for this purpose. Since the *Q* factor and RMS values will be rather similar for good models there is not much to choose between them except that the RMS value is more readily justified from a statistical point of view.

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