

Assessment of Errors Involved in the Determination of Interproton Distance Ratios and Distances by Means of One- and Two-Dimensional NOE Measurements

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There is little doubt that the nuclear Overhauser effect is potentially the most powerful tool for the investigation of the solution structures of biological macromolecules since it can be used both to demonstrate the proximity of two protons in space and to determine their separation (1-10). Given the interconvertibility of intramolecular distances, torsion angles, and Cartesian coordinates, providing the chirality of the structure is known (11-14), a large interproton distance data set can in principle be used either to solve ab initio the complete three-dimensional structure of a molecule in solution using a distance-geometry algorithm (11, 14) or to refine an initial trial model using either constrained least-squares minimization procedures or molecular dynamics calculations incorporating the distance data in the form of pseudopotentials. In the case of small proteins, it is usually sufficient to obtain a qualitative assessment of distance, as has been demonstrated in the case of lipid bound glucagon (15) and scorpion insectotoxin I₅A (16). In the case of oligonucleotides a qualitative assessment of distance is sufficient to distinguish the three classes of DNA conformations, namely right-handed A and B DNA and left-handed Z DNA, but is not adequate for further refinement (8, 17-20). This requires distance determinations to an accuracy of $\sim \pm 0.2 \text{ \AA}$ (9, 21, 22). Considering large molecules for which $\omega\tau_c \gg 1$ (the spin-diffusion limit), such accuracy can in principle be obtained by measuring the initial slope of the time development of the NOE. This is because the initial slope of the NOE, N_{ij} , is simply given by (23, 24)

$$\left. \frac{dN_{ij}}{dt} \right|_{t=0} = \sigma_{ij} \quad [1]$$

where σ_{ij} is the cross-relaxation rate between protons i and j given by (25, 26)

$$\sigma_{ij} = \frac{\gamma^4 \hbar^2}{10r_{ij}^6} \left(\tau_{\text{eff}} - \frac{6\tau_{\text{eff}}}{1 + 4\omega^2\tau_{\text{eff}}^2} \right) \quad [2]$$

where γ and \hbar have their usual meanings, τ_{eff} is the effective correlation time of

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the i - j interproton vector, and r_{ij} is the distance between protons i and j . (Note the sign convention of Kalk and Berendsen (26) is used.) Distance ratios, or distances if one distance is already known, can then be obtained from

$$(r_{ij}/r_{kl}) = (\sigma_{kl}/\sigma_{ij})^{1/6} \quad [3]$$

providing the effective correlation times of the i - j and k - l interproton vectors are the same. In practice, initial slope measurements are not entirely trivial. First, the magnitudes of the NOEs at very short times are small; this inevitably poses a signal-to-noise ratio problem. Second, the measured NOE at short times may not reflect the true magnitude of the NOE. Thus, in the one-dimensional experiment involving the saturation of resonance j for a time t followed by the observation of the intensities of the other proton resonances, it may not be feasible, owing to resonance overlap, to use a sufficiently high power approximating to instantaneous saturation. Similarly, in the two-dimensional experiment, it may not be possible to completely remove the contribution from zero quantum coherence transfer to the intensity of a cross peak involving two coupled spins (27). Third, it may not be possible, for reasons of length of measuring time, to obtain a sufficient number of points covering the time course of the NOE with adequate signal-to-noise ratios. Finally, the measured initial slope for the NOE between protons i and j will not reflect the true initial slope if both $\sigma_{ij} \ll \sigma_{ik}$ and $\sigma_{ij} \ll \sigma_{jk}$ where k represents a third proton. Thus, from the practical viewpoint of determining interproton distance ratios and distances it is important to ask two questions: (1) How many time points for the NOE time course does one really need to obtain; and (2) What is the time dependence of the magnitude of the error Δr_{kl} introduced into the determination of the unknown interproton distance r_{kl} when r_{kl} is calculated from the approximate relationship

$$r_{kl} \sim r_{ij}[N_{ij}(t)/N_{kl}(t)]^{1/6} \sim r_{ij}[a_{ij}(t)/a_{kl}(t)]^{1/6} \quad [4]$$

as opposed to the exact expression [3], where r_{ij} is a known fixed internal reference distance, $N_{ij}(t)$ and $N_{kl}(t)$ are the NOEs observed between protons i and j and between protons k and l , respectively, in the one-dimensional selective saturation experiment at time t , and $a_{ij}(t)$ and $a_{kl}(t)$ are the corresponding cross-peak intensities in the two-dimensional NOE experiment.

To answer these two questions we consider the three-spin system illustrated in Fig. 1. This system provides a perfectly adequate description of a multiple-spin system as demonstrated by previous calculations for both the one- (24, 25, 28) and two- (29) dimensional NOE experiments. The z magnetization of these three spins is governed by three coupled ordinary differential expressions (25) which can be conveniently expressed in matrix form:

$$\begin{bmatrix} \dot{I} \\ \dot{J} \\ \dot{S} \end{bmatrix} = \begin{bmatrix} -\rho_I & \sigma_{IJ} & \sigma_{IS} \\ \sigma_{IJ} & -\rho_J & \sigma_{SJ} \\ \sigma_{IS} & \sigma_{SJ} & -\rho_S \end{bmatrix} \begin{bmatrix} I - I_0 \\ J - J_0 \\ S - S_0 \end{bmatrix}. \quad [5]$$

I is the magnetization of proton I at time t , I_0 is the equilibrium magnetization of proton I prior to the perturbation which permits exchange through cross-relaxation to occur, ρ_I is the total spin-lattice relaxation rate of proton I given by $\sum_{J \neq I} \sigma_{IJ} + R_{1I}$ (where R_{1I} is the external relaxation rate), and similarly for protons J and S .

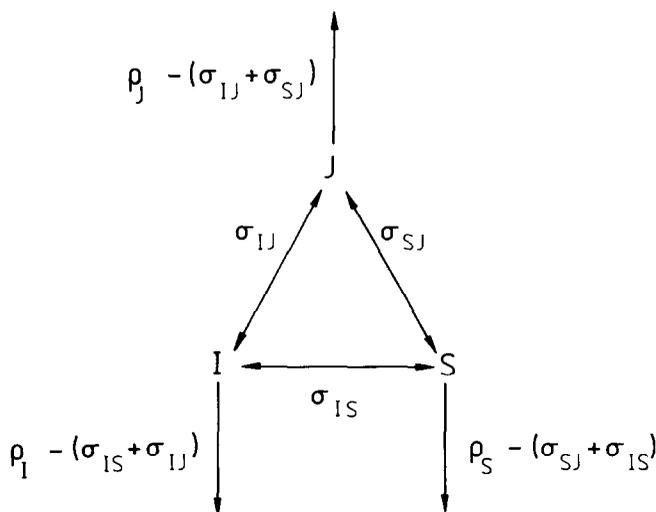


FIG. 1. Three-spin system used for the simulations presented in Fig. 2.

For the one-dimensional experiment we consider the case where resonance J is selectively saturated by a second radiofrequency field for a time t followed by the observation of the intensities of the other resonances. Thus, we have $I_0 = J_0 = S_0 = 1$, and at $t = 0$, $I = S = 1$, and $J = 0$. The magnitude of the NOE, N_{JI} , observed on resonance I following saturation of resonance J is simply $(I(t) - I_0)/I_0$, and similarly for the NOE, N_{JS} , observed on resonance S .

The two-dimensional experiment consists of the basic sequence $90^\circ-t_1-90^\circ-\tau_m-90^\circ-t_2$ (30, 31). In the evolution period t_1 the spins are labeled according to their chemical shifts and in the mixing time τ_m (equivalent to the saturation time t in the one-dimensional experiment) exchange of magnetization between the spins occurs through cross-relaxation. The acquisition time is t_2 and the experiment is repeated for successive t_1 values. Two-dimensional Fourier transformation with respect to t_2 and t_1 then results in a two-dimensional spectrum. This consists of diagonal peaks representing the conventional one-dimensional spectrum and pairs of cross-peaks in symmetrical locations with respect to the diagonal peaks which arise from cross-relaxation. Bearing the considerations of symmetry in mind, the mathematics of the dynamics of the intensity a_{JJ} of the diagonal peak at location $(\omega_{1J}, \omega_{2J})$ and the intensities of the cross-peaks a_{JI} and a_{JS} at locations $(\omega_{1J}, \omega_{2I})$ and $(\omega_{1J}, \omega_{2S})$, respectively, are identical in form to the one-dimensional selective inversion experiment $180^\circ_s-t-90^\circ_{ns}$ with the selective 180° pulse applied to resonance J (31, 32). In the latter case, $I_0 = J_0 = S_0$ with initial conditions (at $t = 0$) $I = S = 1$ and $J = -1$. In the two-dimensional experiment, all that needs to be done to obtain the dynamics of $a_{JJ}(t)$, $a_{JI}(t)$ and $a_{JS}(t)$ is to modify Eq. [5] by setting $I_0 = J_0 = S_0 = 0$, replacing J by a_{JJ} , I by a_{JI} and S by a_{JS} , with initial conditions $a_{JI} = a_{JS} = 0$ and $a_{JJ} = 1$.

In the calculations presented in Fig. 2 we have integrated Eq. [5] numerically with conditions appropriate to the one- and two-dimensional experiments: $\omega\tau_{\text{eff}}$ is

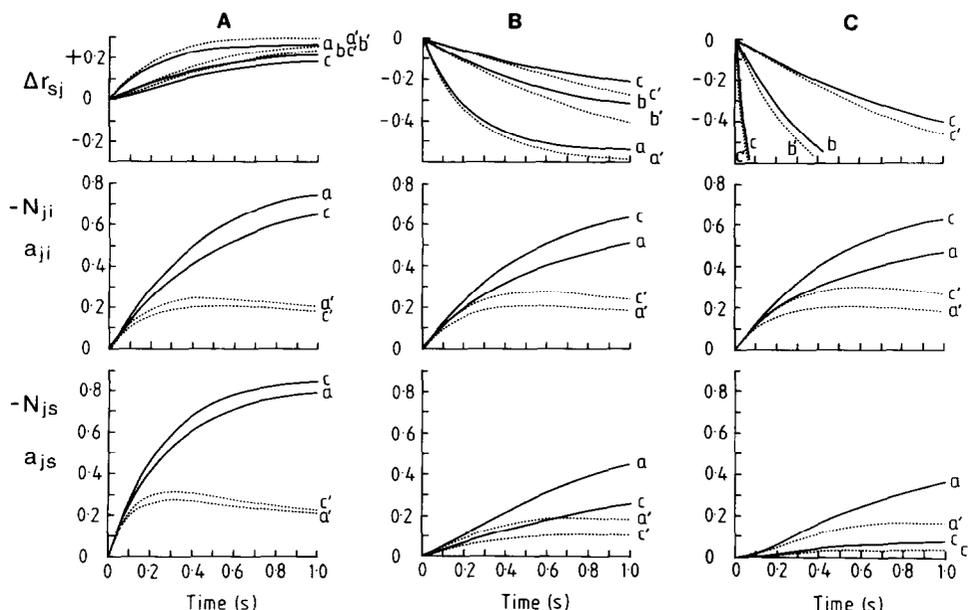


FIG. 2. Time dependence of (i) the error Δr_{SJ} (Å) in the estimate of the distance r_{SJ} calculated using the approximation [4] with r_{IJ} as the internal reference distance, (ii) the magnitudes of the NOEs, N_{JI} and N_{JS} , observed on resonances I and S , respectively, following irradiation of resonance J in the one-dimensional NOE experiment, and (iii) the cross-peak intensities a_{JI} and a_{JS} for the two-dimensional NOE experiment, for different values of r_{SJ} and r_{IS} . The continuous lines are the time courses for the one-dimensional experiment and the dashed lines those for the two-dimensional experiment. Δr_{SJ} is calculated using Eq. [6] for the one-dimensional experiment and Eq. [7] for the two-dimensional one. $\omega\tau_{\text{eff}}$ has a value of 15.7 (which corresponds to $\tau_{\text{eff}} = 5$ ns at a spectrometer frequency of 500 MHz), and the external relaxation rates ($\rho_i = \sum_{j \neq i} \sigma_{ij}$) have a value of 0.5 s^{-1} for the three protons, I , J , and S . The internal reference distance r_{IJ} has a value of 2.4 \AA which corresponds to a value of 1.47 s^{-1} for σ_{IJ} . r_{SJ} and σ_{SJ} have values of 2.1 \AA and 3.28 s^{-1} (A), 3.0 \AA and 0.39 s^{-1} (B), and 3.8 \AA and 0.093 s^{-1} (C). r_{IS} and σ_{IS} have values of 2.1 \AA and 3.28 s^{-1} (curves a and a'), 3.0 \AA and 0.39 s^{-1} (curves b and b'), and 4.6 \AA and 0.03 s^{-1} (curves c and c') (a, b, and c refer to the curves for the one-dimensional experiment, and a', b', and c' to those for the two-dimensional experiment.) Thus, the relationships between the cross-relaxation rates are as follows: (A) $\sigma_{SJ} > \sigma_{IJ}$ and $\sigma_{SJ} \geq \sigma_{IS}$; (B) $\sigma_{SJ} < \sigma_{IJ}$, $\sigma_{SJ} < \sigma_{IS}$ (curves a, a'), $\sigma_{SJ} \geq \sigma_{IS}$ (curves b, b', c, c'); (C) $\sigma_{SJ} < \sigma_{IJ}$, $\sigma_{SJ} < \sigma_{IS}$ (curves a, a', b, b') $\sigma_{SJ} > \sigma_{IS}$ (curves c, c').

set to 15.7 for the three interproton vectors which corresponds to a correlation time τ_{eff} of 5 ns at a spectrometer frequency of 500 MHz; the value of r_{IJ} , the reference distance, is 2.4 \AA which corresponds to a value of 1.47 s^{-1} for σ_{IJ} ; and all three external relaxation rates are set to 0.5 s^{-1} . The error Δr_{SJ} , in calculating r_{SJ} from the approximation [4] as a function of time is given by

$$\Delta r_{SJ}(t) = r_{IJ} \{ [N_{JI}(t)/N_{JS}(t)]^{1/6} - [\sigma_{IJ}/\sigma_{SJ}]^{1/6} \} \quad [6]$$

for the one-dimensional experiment and by

$$\Delta r_{SJ}(\tau_m) = r_{IJ} \{ [a_{JI}(\tau_m)/a_{JS}(\tau_m)]^{1/6} - [\sigma_{IJ}/\sigma_{SJ}]^{1/6} \} \quad [7]$$

for the two-dimensional experiment.

In Fig. 2, we examine the error Δr_{sj} , as well as the magnitudes of the NOEs (one-dimensional experiment) and cross-peaks (two-dimensional experiment) as a function of time (saturation or mixing time) for different values of r_{SJ} and r_{IS} . Four main features emerge from the time dependence:

(1) The initial slope of the time development of the one-dimensional NOEs is the same as that of the cross-peaks in the two-dimensional NOEs.

(2) The approximation [4] introduces only small errors ($\leq 0.2 \text{ \AA}$) in the estimate of r_{SJ} up to relatively long times ($\sim 0.4 \text{ s}$ in the present case) for both the one- and two-dimensional experiments, providing either $\sigma_{SJ} \geq \sigma_{IJ}$ or $\sigma_{SJ} \geq \sigma_{IS}$. This is despite the fact that the initial rate approximation ($\sigma_{SJ} \sim N_{JSt} \sim a_{JSt}$) breaks down at much shorter times ($\sim 0.05 \text{ s}$ in the present case).

(3) The errors in the two-dimensional experiment arising from the use of Eq. [4] are always larger at any given time t than those in the one-dimensional experiment. This is obvious from the nature of the time courses of the NOEs and cross-peak intensities: whereas the former increase in absolute magnitude to a maximum value of -1 , the latter first exhibit an increase in magnitude followed by a decrease back towards zero. A corollary of this behavior is that the magnitudes of the one-dimensional NOEs are larger than those of the corresponding cross-peaks in the time domain which is useful for determining distances from an experimental viewpoint (viz., 0.1 to 0.4 s for the conditions in the calculations). This clearly constitutes a potential advantage of the one-dimensional experiment over the two-dimensional one when quantitative information needs to be extracted from the experimental data.

(4) If the cross-relaxation rate of the S - J vector is greater than that of the reference I - J vector (Fig. 2A), Eq. [4] leads to an overestimation of r_{SJ} . If, on the other hand, the cross-relaxation rate of the S - J vector is less than that of the reference I - J vector (Figs. 2B and C), the value of r_{SJ} will be underestimated through the use of Eq. [4].

In practical terms, what do we conclude from these calculations? Any potential advantage of measuring the time development of the NOEs to extract cross-relaxation rates and calculate distances using Eq. [3], as opposed to measuring the NOEs at one or at the most two well chosen time points with excellent signal-to-noise ratios and calculating distances from Eq. [4], is insignificant when considerations of experimental errors are borne in mind. Thus, all that is required of the investigator is to determine the appropriate time domain for the NOE experiment. In the one-dimensional case, this is easily achieved by measuring the time courses of a few selected NOEs, in particular those involving protons a fixed distance apart which will later be used as internal reference distances. For example, considering proteins and nucleic acids, this would involve NOEs between two methylene protons on the same carbon atom which are separated by $\sim 1.8 \text{ \AA}$ or between two adjacent protons on an aromatic ring which are separated by $\sim 2.5 \text{ \AA}$. These fixed distances are known to a precision of better than $\pm 0.05 \text{ \AA}$ so that any uncertainty in their values will only make a minimal contribution to the error in the determination of the unknown interproton distances. Such a preliminary set of experiments also has the benefit of enabling one to determine the effective correlation times of the fixed

interproton distance vectors using Eq. [2]. With this information in hand, the appropriate time domain for experiments requiring excellent signal-to-noise ratios is easily chosen. In the one-dimensional case, one irradiation time is usually adequate. For the two-dimensional experiment, on the other hand, it is probably safer to carry out measurements with two mixing times.

One final point should be borne in mind when interpreting NOE data. Namely, the effective correlation time of the reference interproton vector may not be the same as that of the unknown interproton vector. This will necessarily result in further errors; given the r_{ij}^{-6} dependence of σ_{ij} , however, these will in general be fairly small. Nevertheless, certain practical considerations can help in this respect. Thus, it is clear that if different fixed distance interproton vectors have different effective correlation times, differential internal motion must be present. This is in fact the case for double stranded DNA oligonucleotides where the effective correlation time of the H2'-H2'' vector in the deoxyribose sugar ring is significantly smaller than that of the H5-H6 vector of the cytosine bases (33). The question then arises as to which reference distance should be used in calculating a particular unknown distance from the NOE data. Fortunately, this choice is not impossible and can be based on stereochemical considerations taking into account the expected ranges of the unknown interproton distances and the expected motions of the different interproton vectors (21). The latter can often be assessed from the analysis of crystallographic thermal factors, ^{13}C NMR relaxation studies and molecular dynamics calculations on related systems.

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