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A Maximum Likelihood Method for Determining D_a^{PQ} and R for Sets of Dipolar Coupling Data

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The algorithms available today that use dipolar coupling data for macromolecular structure determination require the independent determination of two parameters, D_a^{PQ} and R. Methods exist for obtaining these parameters when the set of dipolar couplings available is large and the orientations of the interatomic vectors on which they report is isotropically distributed. These methods are less satisfactory when the set is small and anisotropic. Described here is a maximum likelihood method that extracts accurate values for D_a^{PQ} and R from small, anisotropic data sets. Also demonstrated is a procedure for estimating the errors associated with the values of D_a^{PQ} and R obtained and for incorporating these errors into refinement protocols.

Key Words: **NMR; rhombicity; dipolar coupling; maximum likelihood.**

INTRODUCTION

Dipolar coupling data are potentially of great use to NMR spectroscopists since they contain long range information (as opposed to NOE and scalar couplings). Since, however, the dipolar coupling of an isotropically tumbling molecule averages to zero, useful dipolar coupling data was, until recently, only available for the small number of paramagnetic proteins (*1*), and protein–DNA complexes (*2*) that align spontaneously in strong magnetic fields. The recent introduction of liquid crystal media that induce tunable levels of physical alignment, such as phospholipid mixtures (*3*), filimentous phage (*4, 5*), and purple membranes (*6*), should allow dipolar coupling data to be collected from essentially all nucleic acids and proteins.

The dipolar coupling between two nuclei is given by

$$D_{PQ}(\theta, \phi) = D_a^{PQ}[(3\cos^2\theta - 1) + 1.5R\sin^2\theta\cos 2\phi], \quad [1]$$

where D_a^{PQ} subsumes the gyromagnetic ratios of the two nuclei, the order parameter, the dependence on the distance between nuclei, etc., and θ and ϕ are the polar angles specifying the orientation of the internuclear vector in the principal axis system of the alignment tensor.

The programs currently available for solution structure determination (*2, 7–9*) can use the orientational information contained in dipolar couplings only after the variables D_a^{PQ} and R in Eq. [1] have been independently determined. For proteins this can be accomplished by measuring a large number of couplings, normalizing by type of nuclei and bond length, and plotting the data as a histogram (*8*). Assuming that the internuclear vectors between coupled nuclei are randomly oriented in space, D_a^{PQ} and R will be related to the extrema (D_{11} and D_{33}) and mode (D_{22}) of the histogram as follows:

$$D_{11} = -2D_a^{PQ} \quad [2]$$

$$D_{22} = D_a^{PQ}(1 - 1.5R) \quad [3]$$

$$D_{33} = D_a^{PQ}(1 + 1.5R). \quad [4]$$

Clearly, the accuracy with which D_a^{PQ} and R are determined depends on the accuracy of one's estimates of the values of the mode and two extrema, which depends on the number of couplings observed and the degree of anisotropy in the orientations of the corresponding internuclear vectors.

Spectroscopists interested in using dipolar data naturally measure as many dipolar couplings as possible, both to increase the number of constraints available for structure determination and to improve the accuracy with which the coupling histogram is determined. However, it is not always possible to measure large numbers of couplings (see, e.g., (*10, 11*)), and in such cases it is generally impossible to accurately determine D_a^{PQ} and R using the histogram methodology. In these cases, rounds of structural refinement are carried out with a number of different input values of D_a^{PQ} and R, and the lowest energy structures which emerge are selected (*9, 10*). This work-around is undesirable for two reasons. First, it can be extremely expensive computationally, since a round of refinement must be undertaken for

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every pair of D_a^{PQ} and R values tested. Second, it is difficult to deconvolute the effects that the structural model, coupling data, and parameter values will have on one another in such a process, and it is certainly not unthinkable that the true parameter values might not yield the lowest energy final structures. Thus an effective, structure independent method for determining D_a^{PQ} and R when only a modest number of couplings is available would be desirable.

It should also be noted that the quality of one's estimates of D_a^{PQ} and R will affect the accuracy of structures computed using dipolar data. It is important that the method used to determine these parameters also estimate the errors associated with them, so that they can be taken into account during structure refinement.

Below we describe a maximum likelihood method for determining D_a^{PQ} and R from a set of couplings of any size that yields rigorous error estimates for both parameters. We also propose a method for using these error estimates in CNS (*7*), a popular program for determining solution structures that includes no provision for taking such errors into account.

METHODS AND RESULTS

Given the inherently low quality of information about the location of the extrema and the mode of any distribution when the set of observed data is small, we set out to devise a technique that uses all available data to estimate D_a^{PQ} and R. A maximum likelihood approach (*12, 13*) proved most successful. The "likelihood function" for a set of N couplings being observed given a particular choice of D_a^{PQ} and R is

$$L\left(C_{1\ldots N}\middle|D_a^{PQ}, R\right) = \prod_{n=1}^{N} P_{D_a^{PQ},R}(C_n), \qquad [5]$$

where N is the total number of couplings, $P_{D_a^{PQ},R}$ is the probability density function for a particular choice of D_a^{PQ} and R, and C_n is the value (in Hz) of the nth coupling. Because an isotropically distributed set of dipolar coupling data will have the same shape as a chemical shift anisotropy (CSA) powder pattern, Grant's analytical expressions for the relative intensities of CSA powder patterns (*14*) are equivalent to probability density functions for the observed data ($C_1 \ldots C_n$) given particular values of D_a^{PQ} and R, i.e.,

$$P_{D_a^{PQ},R}(C_n) = \frac{\left(1+\left(\frac{1}{2}\right)^2 m^2 + \left(\frac{1\times 3}{2\times 4}\right)^2 m^4 + \left(\frac{1\times 3\times 5}{2\times 4\times 6}\right)^2 m^6 \ldots\right)}{2\sqrt{(D_{11}-D_{22})(C_n - D_{33})}},$$

$$m = \frac{(D_{11}-C_n)(D_{22}-D_{33})}{(C_n - D_{33})(D_{11}-D_{22})}, \quad \text{for } (D_{11} \le C_n < D_{22})$$

and

$$P_{D_a^{PQ},R}(C_n) = \frac{\left(1+\left(\frac{1}{2}\right)^2 m^2 + \left(\frac{1\times 3}{2\times 4}\right)^2 m^4 + \left(\frac{1\times 3\times 5}{2\times 4\times 6}\right)^2 m^6 \ldots\right)}{2\sqrt{(D_{11}-C_n)(D_{22}-D_{33})}},$$

$$m = \frac{(D_{11}-D_{22})(C_n - D_{33})}{(D_{11}-C_n)(D_{22}-D_{33})}, \quad \text{for } (D_{22} < C_n \le D_{33}), \qquad [6]$$

where D_{11}, D_{22}, and D_{33} are determined from D_a^{PQ} and R as indicated by Eqs. [2], [3], and [4]. A grid search through D_a^{PQ} and R is done to find the pair of parameters that maximizes the likelihood function, which is equivalent to selecting the pair of parameters that is most likely to have given rise to the couplings observed. For convenience we use a log likelihood function instead of equation [5], i.e.,

$$\log\left(L\left(C_{1\ldots N}\middle|D_a^{PQ}, R\right)\right) = \sum_{n=1}^{N} \log\left(P_{D_a^{PQ},R}(C_n)\right). \qquad [7]$$

The values of D_a^{PQ} and R that maximize Eq. [7] also maximize [5]. Extension of the method to sets of data containing errors requires that the powder pattern function be convoluted with a normal distribution with the desired standard deviation, as suggested by Grant and co-workers (*15*). This operation is conveniently performed by multiplying the Fourier transforms of the two functions, and then back Fourier transforming the product (*16*).

This approach was first tested using sets of computer-generated data that were random and completely isotropic. For each value of D_a^{PQ} and R, many data sets were obtained by generating random ϕ and sin weighted θ angles and, for each combination of angles, computing D_{PQ} (Eq. [1]). A grid search in D_a^{PQ} and R was then performed on each data set, calculating Eq. [7] at each step to determine the values of D_a^{PQ} and R most likely to have generated the data set. Table 1 shows the mean values of D_a^{PQ} and R found and their standard deviations for each input value of D_a^{PQ}, R, and data error, as a function of sample size. The quality of fits proved to be insensitive to the value of D_a^{PQ} (data not shown) and, as expected for a maximum likelihood approach, the larger the input data set, the more normal the distributions of estimated parameters (data not shown). Also, as one would expect, the larger the number of couplings available, the smaller the errors in one's parameters, and the larger the errors associated with one's coupling measurements, the larger the parameter errors that emerge. Clearly the maximum likelihood procedure does a good job of determining D_a^{PQ} and R with modest-sized sets of couplings ($\geq$50).

As a further test of this procedure, it was used on sets of couplings computed from known protein structures. Dipolar couplings for various reasonable sets of internuclear vectors were predicted using the method of Zweckstetter and Bax (*17*). Given

TABLE 1
Application of the Maximum Likelihood Method to Isotropic Data

N	Target D_a^{PQ}	Target R	D_a^{PQ} (calc) error = 0.5 Hz	R (calc) error = 0.5 Hz	D_a^{PQ} (calc) error = 1 Hz	R (calc) error = 1 Hz
10	−12	0	-11.34 ± 1.47	0.065 ± 0.166	-11.28 ± 1.53	0.056 ± 0.147
50	−12	0	-12.01 ± 0.28	0.001 ± 0.006	-11.97 ± 0.38	0.001 ± 0.008
100	−12	0	-12.06 ± 0.17	0.000 ± 0.000	-12.02 ± 0.28	0.000 ± 0.000
500	−12	0	-12.07 ± 0.09	0.000 ± 0.000	-12.06 ± 0.13	0.000 ± 0.000
10	−12	0.2	-11.63 ± 2.13	0.148 ± 0.215	-11.60 ± 2.22	0.125 ± 0.214
50	−12	0.2	-12.06 ± 1.05	0.183 ± 0.091	-12.46 ± 1.23	0.125 ± 0.125
100	−12	0.2	-12.02 ± 0.40	0.185 ± 0.038	-12.28 ± 0.77	0.144 ± 0.093
500	−12	0.2	-12.03 ± 0.13	0.188 ± 0.013	-12.14 ± 0.37	0.170 ± 0.039
10	−12	0.4	-11.60 ± 2.41	0.299 ± 0.258	-11.66 ± 2.67	0.303 ± 0.268
50	−12	0.4	-11.81 ± 0.67	0.386 ± 0.072	-11.95 ± 0.72	0.376 ± 0.113
100	−12	0.4	-11.82 ± 0.38	0.404 ± 0.045	-11.90 ± 0.45	0.391 ± 0.056
500	−12	0.4	-12.01 ± 0.15	0.393 ± 0.019	-11.99 ± 0.18	0.386 ± 0.022

Note. For each combination of target values for D_a^{PQ} and R, 100 sets of random data of size N were generated as described in the text. A grid search in D_a^{PQ} and R was performed on each data set with D_a^{PQ} ranging from −2.5 to −20 Hz in 0.1 Hz steps and R ranging from 0 to 0.7 in steps of 0.01. The log likelihood score (see Eq. [7]) was calculated for each pair of D_a^{PQ} and R values and the most likely pair selected. Columns four and five give the mean most likely D_a^{PQ} and R values for the 100 data sets, ± the standard deviation of each value when an error of 0.5 Hz is added to the coupling data. Columns six and seven are equivalent to four and five, respectively, except that the data error is 1 Hz.

a protein or nucleic acid structure, this algorithm computes D_a^{PQ}, R, and the orientation of the alignment tensor based on a purely steric model for the interaction between the macromolecule and liquid crystals. These parameters, along with the structure of the macromolecule, allow dipolar couplings to be computed using Eq. [1]. Using this algorithm dipolar data were generated for four protein structures (*18–21*), chosen to represent a variety of shapes, from extended to essentially spherical. Our method was then used to see if the values of D_a^{PQ} and R used to generate these data sets could be recovered from them. The results of these experiments are shown in Table 2. The second and third columns give the input values for D_a^{PQ} and R estimated using the Zweckstetter and Bax algorithm. Our most likely D_a^{PQ} and R values calculated from the data, in columns five and six, compare quite favorably.

Table 2 also includes estimates of the errors associated with each parameter. These were obtained the same way as the errors determined for the artificial data sets in Table 1. One hundred random sets of coupling data of the same size as the "observed" data set were generated using our calculated most likely D_a^{PQ}

TABLE 2
Application of the Maximum Likelihood Method to Predicted Data from Known Structures

Structure	D_a^{PQ} (steric)	R (steric)	Error (Hz)	Set of couplings used (N)	D_a^{PQ} (predicted)	R (predicted)
tim	−18.2	0.312	0.5	N-H (247)	-18.4 ± 0.3	0.29 ± 0.02
calpain	−12.0	0.199	0.5	N-H (173)	-12.3 ± 0.2	0.18 ± 0.03
calmodulin	−15.1	0.503	0.5	N-H (148)	-15.1 ± 0.3	0.43 ± 0.03
jun	−20.3	0.195	0.5	N-H (43)	-20.5 ± 1.5	0.19 ± 0.09
tim	−18.2	0.312	1.0	50% N, Cα (234)	-18.4 ± 0.4	0.27 ± 0.03
calpain	−12.0	0.199	1.0	50% N, Cα (167)	-11.6 ± 0.3	0.18 ± 0.04
calmodulin	−15.1	0.503	1.0	50% N, Cα (143)	-15.1 ± 0.4	0.43 ± 0.04
jun	−20.3	0.195	1.0	50% N, Cα (42)	-20.6 ± 1.4	0.18 ± 0.08
tim	−18.2	0.312	0.5	All (960)	-18.1 ± 0.1	0.30 ± 0.01
calpain	−11.8	0.194	0.5	All (678)	-11.6 ± 0.1	0.19 ± 0.01
calmodulin	−15.1	0.503	0.5	All (580)	-14.8 ± 0.2	0.54 ± 0.02
jun	−20.2	0.201	0.5	All (170)	-20.0 ± 0.3	0.18 ± 0.02

Note. For each of the four known structures shown, triose phosphate isomerase (*18*), calpain (*20*), calmodulin (*21*), and jun (*19*), the same procedure was employed. First, if necessary, protons were added to the structures in CNS (*7*). Second, couplings were predicted using the algorithm of Zweckstetter and Bax (*17*), assuming the proteins were dissolved in 25 mg/mL phage. Predicted values of D_a^{PQ} and R are listed in columns two and three. The indicated subset of coupling data was used in the maximum likelihood method by first normalizing all couplings to the N–H bond length and gyromagnetic ratios, adding the error indicated, and grid searching in D_a^{PQ} and R from −2.5 to −25 and 0 to 0.7, respectively. The standard deviations given are those predicted from 100 isotropic data sets of the appropriate size, D_a^{PQ}, R, and coupling error. In column 4, "50% N, Cα" indicates that half of all possible N–H and Cα–H couplings, randomly chosen, were used and "All" refers to all one-bond backbone couplings, i.e., N–H, N–Cα, Cα–H, Cα–C′, and C′ (i)–N(i + 1).

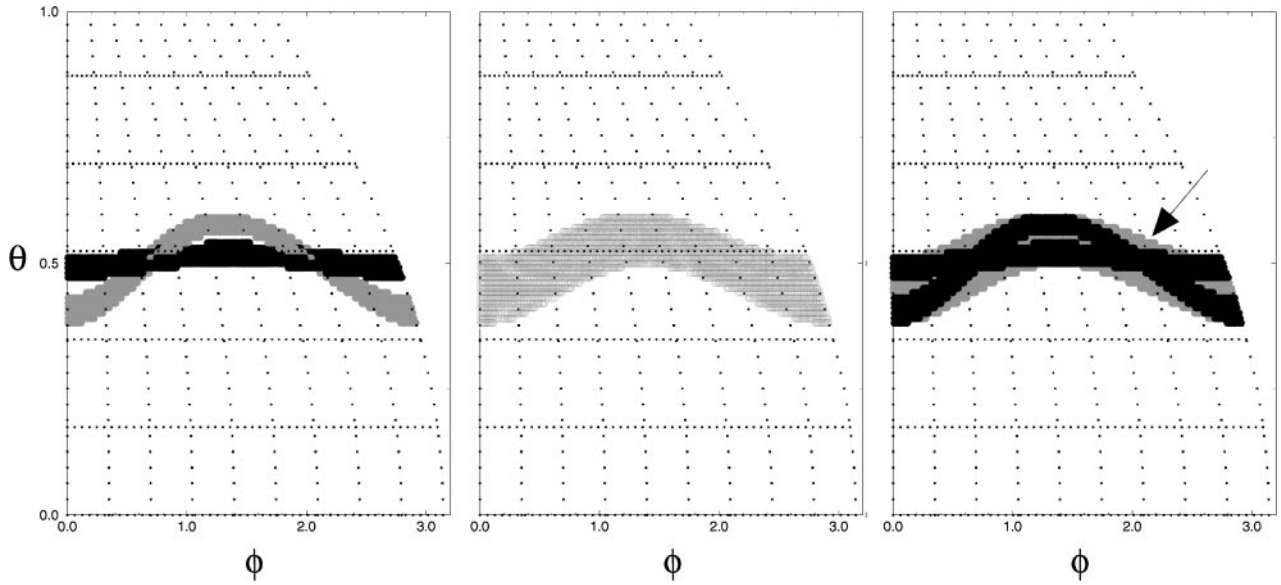

FIG. 1. A mapping procedure for transforming errors in D_a^{PQ} and R into errors in coupling values is illustrated for a coupling of 10 ± 1 Hz, given a D_a^{PQ} of 34.5 ± 3 Hz and an R of 0.11 ± 0.077. Panel A shows a subset (the values shown are mirrored on the other sides of both the θ and ϕ axes) of the allowed values of θ and ϕ at the lower (black) and upper (gray) extreme values of R (given the errors associated with D_a^{PQ} and the coupling value). The couplings which would be generated by this set of angles at the mean values of D_a^{PQ} and R were then determined by substitution into Eq. [1]. The center of this distribution of couplings was taken as a new coupling value with error equal to the distance to the two extrema. In this case the adjusted value of the coupling is 10.449 ± 5.017 Hz. The possible polar angles which would generate couplings within these bounds, given an errorless D_a^{PQ} of 34.5 and R of 0.11, are shown in B. In C the distributions in A (Black) and B (shaded) are overlaid. Note that the allowed angles in A are a subset of those in B. The arrow indicates angles allowed in B which are not allowed in A. All plots shown are equal area Sauson–Flamsteed map projections (*22*), after (*23*).

and R parameters, assuming that the distribution of internuclear vector orientations was isotropic. The errors in Table 2 are the standard deviations of the two parameters extracted from these data sets. These estimates are likely to be slightly low, since this process does not account for the effects of anisotropy in the orientation of the ensemble of internuclear vectors. Nonetheless, in all cases save the extended calmodulin structure (*21*), the target values lie within one estimated standard deviation from the best-fit estimates. This is true, surprisingly, even in the case of the set of N–H couplings determined for Jun, a coiled-coil homodimer (*19*). In Jun the N–H vectors are predominantly parallel to the long axis of the coiled-coil and thus are extremely anisotropic. It appears, however, that the small number of N–H vectors in Jun that do not follow this trend are sufficient to prevent the failure of the maximum likelihood method. The seven α-helices of calmodulin lie roughly in a plane perpendicular to the short axis of the molecule, and the positive extreme (in N–H couplings) is unrepresented. Nonetheless, the method gives a fairly close estimate in this case as well. As expected, just as with isotropic random data, inclusion of more coupling types and larger number of couplings improves accuracy.

Clearly accurate values of D_a^{PQ} and R can be extracted for sets of dipolar coupling data using a maximum likelihood approach, even when the number of couplings available is small. It also appears that we can assign error estimates to the values of these parameters reasonably accurately. Unfortunately, the module that CNS uses for structure refinement with dipolar data (*2*, *7–9*) does not make use of information about the errors associated with D_a^{PQ} and R; only errors associated with the coupling values themselves can be entered. A simple mapping procedure has been devised to circumvent this difficulty, which is illustrated in Fig. 1. In essence one adjusts each coupling and error to allow the corresponding internuclear vector to sample all values of θ and ϕ which are consistent with D_a^{PQ}, R, the original coupling value, and all their associated errors. In our experience with refinement of nucleic acid solution structures using dipolar coupling data, structures converge quite well with errors in D_a^{PQ} and R of the sizes seen here and errors in coupling data of several percent (data not shown).

DISCUSSION

We have presented a method that allows the rapid and unambiguous estimation of the parameters D_a^{PQ} and R for a set of dipolar couplings of arbitrary size. The method appears to be robust, in that it works when there are significant errors associated with the coupling data and when the data are derived from real protein structures where there is significant anisotropy. This method should yield estimates for D_a^{PQ} and R that are more accurate than those calculated by other methods, e.g. that of Clore *et al.* (*8*), for two reasons. First, the intrinsically low frequency of occurrence of internuclear vectors with θ near 0 (due to the

weighting of the population by sin θ) is implicitly corrected for by our methodology, as is the difficulty of estimating the mode of sparse data sets. Second, the maximum likelihood method uses all data available rather than the subset which define the mode and extrema, which should make it more efficient at extracting information about the values of D_a^{PQ} and R. In the limit of large, isotropic coupling sets both advantages should disappear.

Additionally we have presented a method for estimating the error associated with our most likely values for D_a^{PQ} and R and a mapping procedure for translating these errors into errors associated with couplings. This procedure allows incorporation of errors in D_a^{PQ} and R into refinement in CNS by simply inputting the adjusted coupling and error as "sani" restraints, and using a harmonic potential to refine one's structures. Algorithms for performing these various functions are available on our website at http://proton.chem.yale.edu.

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