Reconstruction of the probe angular distribution from a series of electron spin resonance spectra of tilted oriented samples

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ABSTRACT Model-independent methods for the reconstruction of the nitroxide spin probe angular distribution of labeled oriented biological assemblies from electron spin resonance (ESR) spectra were investigated. We found that accurate probe angular distribution information could be obtained from the simultaneous consideration of a series of ESR spectra originating from a sample at differing tilt angles relative to the Zeeman magnetic field. Using simulated tilt series data sets, we developed a consistent criteria for judging the reliability of the simulated fit to the data as a function of the free spectral parameters and thereby have increased the significance of the model-independent reconstruction of the probe angular distribution derived from the fit. We have also enhanced the angular resolution measurable with the model-independent methodology by increasing the rank of the order parameters that we can reliably deduce from a spectrum. This enhancement allows us to accurately deduce higher resolution features of the spin probe distribution. Finally we investigated the usefulness of fitting the tilt series data in multiple data sets such that tilt series data from many identical sample preparations are fitted simultaneously. This method proved to be useful in rapidly reducing a large amount of data by eliminating any redundant computations in the application of the enhanced model-independent analysis to identical sets of tilt series data. We applied the methodology developed here to ESR spectra from probe labeled muscle fibers to study the orientation of myosin cross-bridges in fibers. This application is described in the accompanying paper (Ajtai, K., A. R. French and T. P. Burghardt, 1989. *Biophys. J.* 56: 535–541)

INTRODUCTION

The electron spin resonance (ESR) spectrum from a nitroxide spin label specifically and rigidly attached to a static protein element of a biological assembly contains information concerning the angular distribution of the probe (Libertini et al., 1974; Hentschel et al., 1978; Friesner et al., 1979). The deduction of the probe angular distribution from the ESR spectrum is a problem that we worked on in the past (Burghardt and Thompson, 1985). Our efforts led to the development of a model-independent description of probe angular order that proved to be useful in sorting out the types of angular information that ESR could detect. This methodology was successfully applied to real data to reconstruct some of the features of the probe angular distribution.

The model-independent description of order in biological assemblies was developed for the analysis of ESR spectra and fluorescence polarization data (Burghardt, 1984; Burghardt and Ajtai, 1986). In these experimental techniques, extrinsic probes are specifically bound to immobile or slowly moving elements of the biological assembly. The probes are sensitive to the angular arrangement of the elements to which they are attached. The model-independent method treats the angular order of the biological assembly as a distribution of reference frames, with one frame fixed in each element. The angular distribution function is expanded in terms of complete, orthogonal angular functions, and the order parameters are the expansion coefficients. The order parameters are also related to the observed signal (the ESR spectrum); these two relations allow the inference of the order parameters from the data. The order parameters uniquely determine the angular distribution of the elements. The model-independent treatment of ESR shows that the ESR spectrum depends on an infinite set of independent order parameters and basis spectra. An infinite sum over the basis spectra, with order parameters as the weighting factors, describe any observable ESR spectrum. We numerically calculated a finite subset of basis spectra and demonstrated an effective method for measuring the order parameters from an observed ESR spectrum.

Although the ESR spectrum contains a large amount of information concerning the probe orientation there are several spectral parameters that must be correctly adjusted for the experimental conditions, to properly deduce the probe orientation distribution. The spectral parameters include, the g-tensor that couples the electron spin to the Zeeman field and the T-tensor that couples the electron spin to the nuclear spin of nitrogen in the nitroxide radical. Generally the spectral parameters are

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adjusted to minimize some scalar quantity, we refer to as Q, that reflects a quantitative comparison of the experimental data with theoretically generated data. The minimization of Q is a major computational problem because the brute force method of computing Q for all of the possible spectral parameter values can not be done in a finite amount of computing time. This practical constraint forces us to be able to compute Q only for a small subset of the possible spectral parameter values. Naturally, for a limited search through the spectral parameters, finding the correct global minimum of Q requires that Q have a deep minimum in the spectral parameter space at the correct choices for the spectral parameter values. We have considered this problem of choosing the best form of Q and describe here our findings. All of our comments are the result of our investigation of the system of a muscle fiber specifically labeled with a nitroxide spin label, although, the conclusions are probably generalizable to other oriented systems and other types of spin labels. The muscle fiber system and our findings based on the methods described herein have some biological significance and these aspects of the project are discussed in detail in the accompanying paper (Ajtai et al., 1989).

We obtained the best results from the consideration of a series of ESR spectra of the oriented sample that differ only in their orientation relative to the Zeeman magnetic field. We refer to this data set as a tilt series. The minimum number of spectra in a series is two and the maximum number is unlimited. One of the spectra in the tilt series always corresponds to the parallel alignment of the muscle fiber axis with the Zeeman field, this spectra is the zero tilt spectra. The angular order parameters of the distribution are computed from the zero tilt spectra by the numerical method described previously with some enhancements described later in this manuscript (Burghardt and Thompson, 1985). The zero tilt order parameters transform under the rotation of the sample by a well known formula for the transformation of spherical tensors under coordinate rotation (Davydov, 1963). These transformed-zero-tilt order parameter values are compared with order parameters measured directly from the tilted spectra.

The sum over all order parameters of the weighted difference between the transformed-zero-tilt order parameter and the corresponding order parameter computed from the tilted spectra is used as the scalar factor Q, to judge the quality of the choice of the spectral parameters. This method gave excellent results on simulated data from Gaussian angular distributions showing that Q had a deep global minimum at the correct choices for the spectral parameters. By minimizing Q, the correct choices for the spectral parameters could be found in a limited search through spectral parameter space even with poor initial guesses.

Real ESR data differs from simulated data by containing random and systematic error from many sources. We reduce the random error component in the real ESR spectra by applying the model-independent analysis of the tilt series of ESR spectra described above to multiple independent data sets. In this procedure multiple tilt series data sets (~10) from independent, identically prepared muscle fiber samples are simultaneously fitted and the sum of the Q factors are minimized. The minimization of Q is required for the spectral parameters that are identical for each data set consisting of a complete tilt series. This procedure reduces the total computing time of a complete data analysis by eliminating the repetition of identical computing tasks for each data set while allowing us to estimate the standard error of the order parameters using the variability of the values among the equivalent data sets. This technique was successfully applied to the muscle fiber data (Ajtai et al., 1989).

The combination of the methods employing the data of a tilt series of ESR spectra and the simultaneous fitting of multiple tilt series data sets produced the reconstructed probe angular distribution of spin probes in the muscle fiber system. In this experimental work different physiological states of the fiber were induced by the presence or absence of nucleotides while the angular distribution of the probes was monitored by ESR as described in the accompanying paper (Ajtai et al., 1989). With this experimental application of the formalism presented herein we confirm the usefulness of our method and demonstrate its capabilities on an important biological system.

METHODS

Model-independent ESR

The probability density function, N, describes the angular distribution of spin probes relative to a laboratory fixed reference frame. The orientation of any probe is related to the laboratory frame by a rotation. We choose to describe this relationship using the Euler angles α, β, and γ that are denoted collectively by Ω (Davydov, 1963). We expand N in terms of Wigner functions $D^j_{m,n}$ that form a complete, orthogonal basis set of functions on the domain $0 \leq \alpha \leq 2\pi$, $0 \leq \beta \leq \pi$, $0 \leq \gamma \leq 2\pi$, denoted by Ω_0, such that,

$$N(\Omega) = \sum_{j=0}^{\infty} \sum_{m,n=-j}^{j} a^j_{m,n} \sqrt{\frac{2j+1}{8\pi^2}}\, D^j_{m,n}(\Omega). \tag{1}$$

The parameter $a^j_{m,n}$ is an order parameter of rank j. The order parameters are unknowns that we measure experimentally. The angular resolution of an experimental method is theoretically limited by the rank and total number of order parameters detected. The higher resolution features of the angular distribution are proportional to the higher rank order parameters (Burghardt and Ajtai, 1988).

The absorption probability of the microwave energy by a single nitroxide spin probe, $G(H, \Omega)$, is the spectral lineshape and is a function of the Zeeman field, H, and the Euler angles. G is also expanded in terms of the Wigner functions such that

$$G(\Omega, H) = \sum_{j=0}^{\infty} \sum_{m,n=-j}^{j} g^{j}_{m,n} \sqrt{\frac{2j+1}{8\pi^2}} D^{j}_{m,n}(\Omega). \tag{2}$$

The observed ESR spectrum for a macroscopic sample made up of many spins at different orientations, $F(H)$, is,

$$F(H) = \int_{\Omega_0} d\Omega G^*(H, \Omega) N(\Omega), \tag{3}$$

where * means complex conjugate. The orthogonality of the Wigner functions, such that,

$$\int_{\Omega_0} d\Omega D^{*j}_{m,n} D^{j'}_{m',n'} = \frac{8\pi^2}{2j+1} \delta_{j,j'} \delta_{m,m'} \delta_{n,n'}, \tag{4}$$

where $\delta_{j,j'}$ is the Kronecker delta, implies that Eq. 3 can be written as,

$$F(H) = \sum_{j=0}^{\infty} \sum_{m,n=-j}^{j} a^{j}_{m,n} g^{*j}_{m,n}(H) \tag{5}$$

and that the coefficients $g^{j}_{m,n}$ can be computed from the lineshape using the expression,

$$g^{j}_{m,n}(H) = \sqrt{\frac{2j+1}{8\pi^2}} \int_{\Omega_0} d\Omega D^{*j}_{m,n} G(H, \Omega) \tag{6}$$

The coefficients $g^{j}_{m,n}(H)$ are called the basis spectra and are calculated using Eq. 6. The $a^{j}_{m,n}$'s are the unknown order parameters calculated from the observed ESR spectrum, $F(H)$, using a direct numerical inversion method (referred to as method A) described below. When the order parameters are determined from the ESR spectrum, Eq. 1 is used to reconstruct the probe orientation distribution, N.

Measuring order parameters from the ESR spectrum (method A)

The $g^{j}_{m,n}$'s make up a set of basis spectra that span the space occupied by any ESR spectrum. The task is to measure the amount of each basis spectrum contained in an ESR spectrum, i.e., the value of the $a^{j}_{m,n}$'s (see Eq. 5). We do this by constructing projection operators $q^{j}_{m,n}(H)$ where,

$$a^{j}_{m,n} = \int_0^{\infty} dH\, q^{*j}_{m,n}(H) F(H), \tag{7}$$

which is equivalent to requiring,

$$\int_0^{\infty} dH\, q^{*j}_{m,n}(H)\, g^{j'}_{m',n'}(H) = \delta_{j',j}\, \delta_{m',m}\, \delta_{n',n}. \tag{8}$$

Eq. 8 is satisfied if,

$$q^{j}_{m,n}(H) = \sum_{j'=0}^{\infty} \sum_{m',n'=-j'}^{j'} A^{j,m,n}_{j',m',n'}\, g^{j'}_{m',n'}(H), \tag{9}$$

where

$$(A^{-1})^{j,m,n}_{j',m',n'} = \int_0^{\infty} dH\, g^{*j}_{m,n}(H)\, g^{j'}_{m',n'}(H). \tag{10}$$

There are an infinite number of basis spectra and matrix elements $(A^{-1})^{j,m,n}_{j',m',n'}$ that must be calculated to rigorously determine $q^{j}_{m,n}$ and consequently the order parameters. We compute $(A^{-1})^{j,m,n}_{j',m',n'}$ using Eq. 10 for a finite number of matrix elements such that $0 \leq j, j' \leq j_{\max}$ and determine the order parameters. We call order parameters and basis spectra with $j \leq j_{\max}$ the included order parameters and basis spectra. We estimate the contribution from basis spectra with $j > j_{\max}$, the ignored basis spectra, using a remainder basis spectrum, g_R, such that,

$$g_R(H) = F(H) - E(H), \tag{11}$$

where $E(H)$ is the best estimate for $F(H)$ when only order parameters and basis spectra corresponding to $j \leq j_{\max}$ are included. Eq. 11 is the correct choice for g_R because the infinite set of $g^{j}_{m,n}$'s is complete. The explicit expression for $E(H)$ is,

$$E(H) = \sum_{j=0}^{j_{\max}} \sum_{m,n=-j}^{j} a^{j}_{m,n} g^{*j}_{m,n}(H). \tag{12}$$

In practice the computer program calculates the next highest contributing basis spectrum with $j > j_{\max}$ and equates it to g_R in the first trial fit. Next an iterative loop begins where g_R is recalculated using Eqs. 11 and 12. g_R is always used in Eqs. 9 and 10 insuring the infinite set of basis spectra are used in the calculation of each revised value of the included order parameters. The iterative procedure stops with g_R is unchanged in two successive passes through the loop. This procedure utilizing the remainder basis spectrum to compute the included order parameters is an enhancement over the previously used method (Burghardt and Thompson, 1985). We show below how this enhancement improves the overall reliability of the determination of the order parameters.

Application to nitroxide ESR

We checked the effectiveness of the enhanced model-independent procedure for determining order parameters outlined above, by applying the formalism to simulated ESR spectra from a Dirac delta function angular distri-

TABLE 1 Order parameters from an ESR spectrum with a Dirac delta function probe angular distribution

$a^j_{m,n}$	Theory	Enhanced	% difference	Standard	% difference
$a^0_{0,0}$	0.113	0.113	0.	0.113	0.
$a^2_{0,0}$	0.000	0.000	0.	0.000	0.
$a^4_{0,0}$	−0.131	−0.145	11.	−0.135	0.
$a^6_{0,0}$	0.090	0.086	4.	0.095	6.
$a^8_{0,0}$	0.072	0.058	19.	0.076	6.
$a^{10}_{0,0}$	−0.138	−0.147	7.	−0.119	14.
$a^{12}_{0,0}$	0.020	0.000	100.	0.042	110.
$a^{14}_{0,0}$	0.124	0.121	2.	0.176	42.
$a^{16}_{0,0}$	−0.103	−0.145	41.	−0.050	49.
$a^{18}_{0,0}$	−0.056	−0.067	20.	0.048	186.
$a^{20}_{0,0}$	0.140	0.094	33.	0.255	80.
$a^{22}_{0,0}$	−0.038	−0.036	5.	0.143	476.
$a^{24}_{0,0}$	−0.115	−0.162	41.	0.062	154.
$a^{26}_{0,0}$	0.114	0.159	39.	0.366	221.

Shown are measured and theoretical order parameters for the Dirac delta function probe angular distribution $N = N_0\,\delta(\cos\beta - \cos[54.7°])$. The measured values are from a model-independent analysis with the standard, or the enhanced method using a remainder basis spectra as outlined in Eqs. 11 and 12. The simulated spectra were calculated with $\epsilon = 2.5$ G, $g_x = g_y = 2.0070$, $g_z = 2.0020$, $T_x = T_y = 7.0$ G, and $T_z = 30.0$ G.

bution of spins. ESR spectra generated as such depend on all ranks of order parameters and basis spectra. This is an appropriate test of the method because we would like to show that we can accurately measure order parameters with rank j, such that $0 \leq j \leq j_{\max}$, from a spectrum that depends on order parameters and basis spectra of rank $0 \leq j \leq j_{\max}$ and $j > j_{\max}$.

We calculated the ESR spectra using the model angular distribution,

$$N(\Omega) = \frac{1}{4\pi^2}\,\delta(\cos\beta - \cos\beta_0), \tag{13}$$

where $\delta(\cos\beta - \cos\beta_0)$ is a Dirac delta function. Eq. 13 describes a spin angular distribution that has a single polar angle given by β_0 and is randomly distributed in α and γ. The order parameters for this distribution are calculated exactly using Eqs. 1 and 4 and are,

$$a^j_{m,n} = \sqrt{\frac{2j+1}{8\pi^2}}\,P_j(\beta_0)\,\delta_{m,0}\delta_{n,0}, \tag{14}$$

where P_j is a Legendre polynomial (Arfken, 1970).

The lineshape $G(H, \Omega)$, used in all of our calculations is the first derivative of a Lorantzian. This choice is appropriate for nitroxide spin labels (Hudson and Luckhurst, 1968; Berliner, 1976) and is,

$$G(H,\Omega) = \sum_{m_1=-1}^{1} \frac{\mathrm{d}}{\mathrm{d}H}\,\frac{\epsilon}{\epsilon^2 + [H - H_0(\Omega, m_1)]^2}, \tag{15}$$

where ϵ is the linewidth and,

$$H_0(\Omega, m_1) = \frac{c - m_1|T \cdot \mathbf{K}|}{|g \cdot \mathbf{K}|}, \tag{16}$$

m_1 is the nuclear spin projection quantum number, T is the tensor coupling nuclear spin with the electron spin, $\mathbf{K}$ is a unit vector in the direction of the Zeeman field, g is the tensor coupling the electron spin to the Zeeman field, and $c = 2\pi\nu/g_e$, where ν is the microwave frequency exciting the spin transitions and g_e is the electron gyromagnetic ratio (McCalley et al., 1972).

Using Eqs. 3, and 13–16 we computed $F(H)$ for several delta function angular distributions and fitted this simulated data with and without the enhancing procedure. The order parameters calculated using these methods for a typical case are compared in Table 1. Comparison of the exact and measured order parameters demonstrate the effectiveness of the enhancing procedure.

When the numerical inversion method described above is applied to real data the spectral parameters are not precisely known, and we perform a limited search through spectral parameter space to find the most appropriate parameter values. The adjustable spectral parameters are in the expression for the lineshape G and are the values of the g and T-tensors (corresponding to six independent parameters) that depend on the structure of the nitroxide probe and the probe's local environment, and the constant c (the linewidth, ϵ, in Eq. 15 is related to the normalization of the absorption curve, $\epsilon = 2.5$ G in all of the calculations). These seven parameter values are varied to minimize the scalar quantity, Q, representing a quantitative comparison of experimental with theoretically generated data. Factor Q could be chosen to be the square of the difference between the ESR spectrum and the spectral curve generated from model-dependent choices for the probe angular distibution , i.e., the χ^2 criteria. We found this criteria to be too ambiguous or incorrect for the number of independent spectral parameters in a nitroxide spectrum, when used with the model-independent method for reconstructing probe angular distributions. The form of Q that worked consistently with model data, such that a limited search through the spectral parameters always converged on the correct values, is described in the next section.

Improved fitting convergence using a series of ESR spectra

The tilt series of ESR spectra from an oriented spin labeled sample is generated by measuring spectra as a function of the orientation of the sample relative to the Zeeman magnetic field. Under these conditions the spectral parameters of the nitroxide probe are constant while the order parameters are transformed by the relation,

$$a'^{j}_{m,n}(\Omega_1) = \sum_{k=-j}^{j} a^{j}_{k,n} D^{j}_{k,m}(\Omega_1), \tag{17}$$

where $a'^{j}_{m,n}(\Omega_1)$ is the order parameter of the rotated sample, $a^{j}_{m,n}$ are the order parameters of the unrotated sample, and Ω_1 denotes the Euler angles of the sample rotation. The generalized ESR signal from a tilted sample is, from Eqs. 5 and 17,

$$F(H, \Omega_1) = \sum_{j=0}^{\infty} \sum_{m,n=-j}^{j} \left[\sum_{k=-j}^{j} a^{j}_{k,n} D^{j}_{k,m}(\Omega_1) \right] g^{*j}_{m,n}(H). \tag{18}$$

The Wigner functions $D^{j}_{k,m}(\Omega_1)$ obey the orthogonality condition of Eq. 4 implying that,

$$\frac{8\pi^2}{2j+1} \sum_{k=-j}^{j} a^{j}_{m,k}\, g^{j}_{n,k}(H) = \int_{\Omega_0} \mathrm{d}\Omega_1 F(H, \Omega_1) D^{j}_{m,n}(\Omega_1). \tag{19}$$

Eq. 19 shows that simple linear combinations of the order parameters of the same rank, j (on the left hand side of Eq. 19) are related to an observable quantity (right hand side). The direct analytical method for measuring order parameters presented by Eq. 19, is described in detail for the related technique of fluorescence polarization (Burghardt, 1984). The measurement of the ESR spectrum from the many tilt angles required to make the calculation of Eq. 19 accurate is impractical due to the time required to collect the data. Fortunately, the alternative method of measuring all of the order parameters from a single ESR spectrum, method A, can be modified to incorporate the constraint of Eq. 17.

In this modified approach only a few (two) tilted spectra are required. The zero tilt order parameters are computed directly from the zero tilt ESR spectrum using the numerical inversion method A. Then, using Eq. 17, the order parameters of the rotated sample are computed from the zero tilt order parameters. The rotated order parameters are also computed directly from the tilted ESR spectra using method A. The weighted difference between these independent computations of $a'^{j}_{m,n}(\Omega_1)$, denoted by Q and defined by the relation,

$$Q = \sum_{j=0}^{j_{\max}} \sum_{m,n=-j}^{j} w_{j,m,n} \left| \left[a'^{j}_{m,n}(\Omega_1) - \sum_{k=-j}^{j} a^{j}_{k,n} D^{j}_{k,m}(\Omega_1) \right] \right|, \tag{20}$$

where $w_{j,m,n}$ is a weighing factor, is minimized to find the correct choice for the spectral parameters. Model calculations were performed to verify that Q was an appropriate parameter to be minimized in the analysis of tilt series spectra for the reconstruction of the probe angular distribution. These model calculations and the verification of the usefulness of Q are described in the next section on model calculations.

The relaxation of excited spin states is governed by the interactions the electron spin has with its local environment that can cause the transfer of spin angular momentum. Usually two dominant relaxation modes are recognized and referred to as spin-lattice and spin-spin relaxation modes. The Lorentzian lineshape is appropriate when the spin-spin relaxation mode is dominant but in a real spin system other modes contribute to broaden the resonance lineshape (Goldman et al., 1972). Our choice of the Lorentzian lineshape to compute the basis spectra, $g^{j}_{m,n}$, clearly introduces model-dependence into our spectral analysis.

The constraint on the order parameters summarized by Eq. 17, and incorporated in our analysis programs via Eq. 20, compensates for the practical necessity of choosing an imperfect lineshape. Eq. 17 is a requirement based on the rotational transformation rules for order parameters. This requirement is rigorously correct and strictly model-independent. The fact that the minimization of Q is a superior constraint to the minimization of χ^2 is demonstrated by the convergence to the correct spectral parameters in the model calculation described below.

Model calculations

We tested the effectiveness of minimizing Q, defined in Eq. 20, for identifying the correct spectral parameters in a tilt series of ESR spectra by making model calculations of ESR spectra and applying the fitting procedure to the simulated data. We choose as a model the Gaussian angular distribution of the form,

$$N = N_0 \exp\left[-\left(\frac{\cos\beta - \cos\beta_0}{w_1} \right)^2 - \left(\frac{\cos\gamma - \cos\gamma_0}{w_2} \right)^2 \right], \tag{21}$$

where N_0 is a normalization determined by requiring that the sum of N over all possible orientations is unity, w_1 is the width of the angular distribution in the β dimension, and w_2 is the width of the distribution in the γ dimension. This model distribution is appropriate for probe labeled myosin cross-bridges in muscle fibers with zero tilt where β corresponds to a polar angular degree of freedom measured relative to the fiber axis and γ to a torsional degree of freedom of the probe (Mendelson and Wilson, 1982). This expression for N is substituted in Eq. 3 and the simulated ESR spectrum is calculated for the zero tilt spectrum. Theoretical order parameters are computed directly from the model distribution with the relation,

$$a^{j}_{m,n} = \sqrt{\frac{2j+1}{8\pi^2}} \int_{\Omega_0} \mathrm{d}\Omega\, D^{j*}_{m,n} N(\Omega). \tag{22}$$

The tilted spectra are calculated for the Gaussian angular distribution using the constructions shown in Fig.

1. Coordinates (x_L, y_L, z_L) refer to the laboratory fixed frame. The molecular frame, denoted by (x_M, y_M, z_M), is fixed in the probe molecule with an orientation such that the g and T-tensors are diagonal (the principal magnetic frame). Tilted samples are obtained by rotation through angle ψ about the y_L axis. Euler angles $(\alpha', \beta', \gamma')$ describe the rotation of the (x, y, z) frame to the probe molecule fixed frame. The rotation from the laboratory frame to the probe fixed frame can take two paths, one is the upper pathway in Fig. 1 where a series of four rotations through angles $(\psi, \alpha', \beta', \gamma')$ occur. Another path is the lower one where a series of three Euler rotations through angles (α, β, γ) occur. To compute the tilted spectra we use Eq. 3 with the expression for N given below. It is a straightforward but tedious calculation, using the coordinate transformations of Fig. 1, to derive the relationship between the angles $(\alpha, \beta\ \gamma)$ and $(\alpha', \beta', \gamma')$ and to show that for any ψ,

$$N = N_0 \exp\left[-\left(\frac{\cos\beta' - \cos\beta_0}{w_1}\right)^2 - \left(\frac{\cos\gamma' - \cos\gamma_0}{w_2}\right)^2\right], \quad (23)$$

where

$$\cos\beta' = \sin\beta \cos\alpha \sin\psi + \cos\beta \cos\psi \quad (24)$$

$$\sin\beta' = \cos\alpha' (\sin\beta \cos\alpha \cos\psi - \cos\beta \sin\psi) + \sin\alpha' \sin\beta \sin\alpha \quad (25)$$

and

$$\begin{aligned}\cos\gamma' = {} & \cos\beta' \cos\alpha' (\cos\beta \cos\gamma \cos\alpha \cos\psi - \sin\gamma \sin\alpha \cos\psi \\ & + \sin\beta \cos\gamma \sin\psi) \\ & + \cos\beta' \sin\alpha' (\cos\beta \cos\gamma \sin\alpha + \sin\gamma \cos\alpha) \\ & + \sin\beta' (\sin\gamma \sin\alpha \sin\psi + \sin\beta \cos\gamma \cos\psi \\ & - \cos\beta \cos\gamma \cos\alpha \sin\psi). \end{aligned} \quad (26)$$

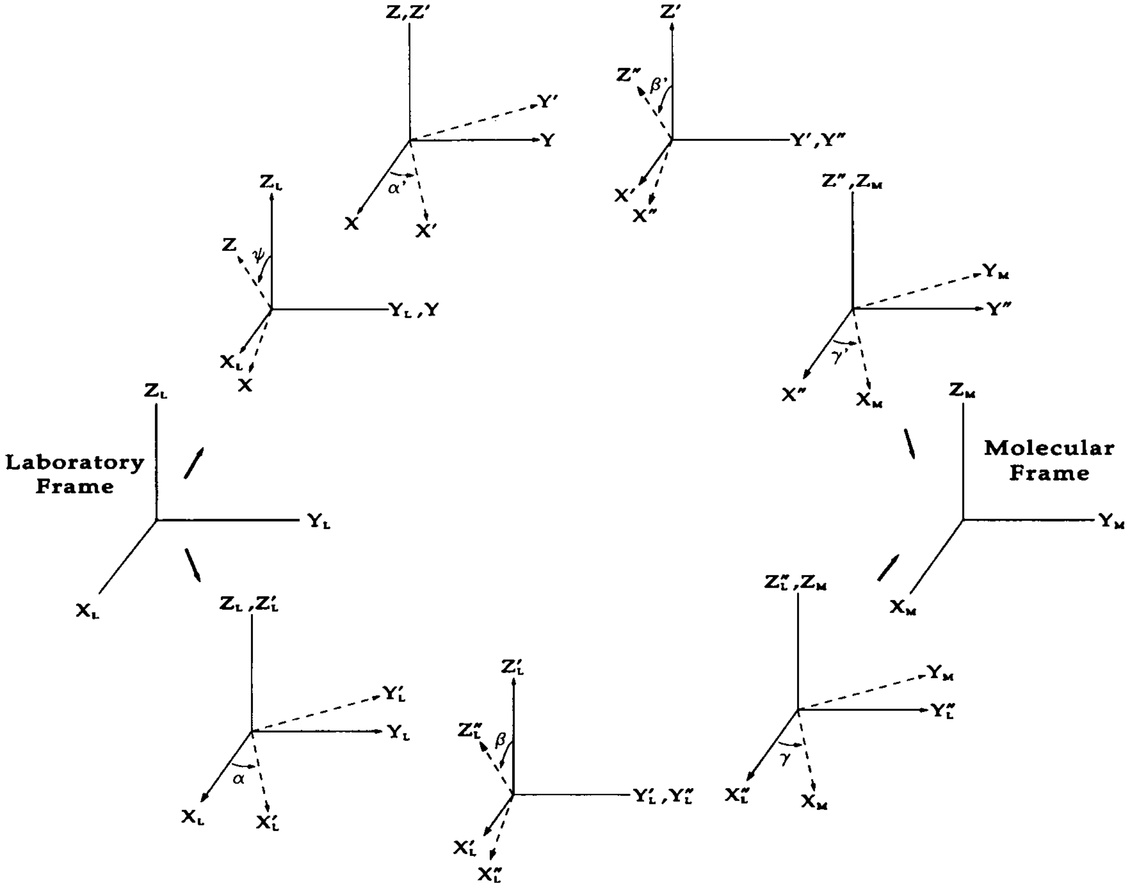

FIGURE 1 Rotational transformations used to compute the tilted angular distributions. Subscripts L signifies the laboratory fixed frame and M signifies the principal magnetic frame fixed in the spin probe. Rotation angles (α, β, γ) describe the relationship between the lab frame and the molecular frame. Rotation angles $(\psi, \alpha', \beta'\ \gamma')$ applied in the sequence shown in the upper pathway describe the same relationship. In the (x', y', z') coordinate frame (*upper pathway*) the model probe angular distribution is given by Eq. 23. The purpose of these constructions is to illustrate the relationship between the angles (α, β, γ) used in the lower pathway and the angles $(\psi, \alpha', \beta', \gamma')$ used in the upper pathway so that Eqs. 3 and 23 can be used to compute the model ESR tilt series spectra.

Eqs. 24–26 are nonlinear equations determining $(\alpha', \beta', \gamma')$ in terms of (α, β, γ). For particular values of (α, β, γ) we use Eq. 24 to compute β' directly and then use this value in Eq. 25 to solve for α' with Newton's rule for finding the roots of an equation (Arfken, 1970). We use β' and α' in Eq. 26 to solve for γ'.

We computed the tilt series ESR spectra corresponding to $\psi = 0, 30°, 60°$, and $90°$ shown in Fig. 2. We applied the model-independent analysis to the simulated data using only the $\psi = 0$ and $\psi = 90°$ spectra as a model for the real data collected from muscle fibers described in the accompanying paper (Ajtai et al., 1989). The minimization of Q, as defined in Eq. 20, always caused the search through spectral parameter space to converge to the correct spectral parameter values. When the minimization of χ^2 guided the search through parameter space, the best fit did not occur for the correct values of the spectral parameters. This result is summarized in Table 2 where we also show a comparison between the theoretical order parameters computed using Eq. 22 and those measured from the model-independent analysis of the tilt series of simulated ESR data. We find good agreement between the theoretical and measured order parameters.

Determining the significance of the order parameters

The ability to determine the order parameter $a^j_{m,n}$ from an ESR spectrum depends on the extent to which the spectrum is influenced by the basis spectrum $g^j_{m,n}$. This feature is easiest to understand using limiting examples.

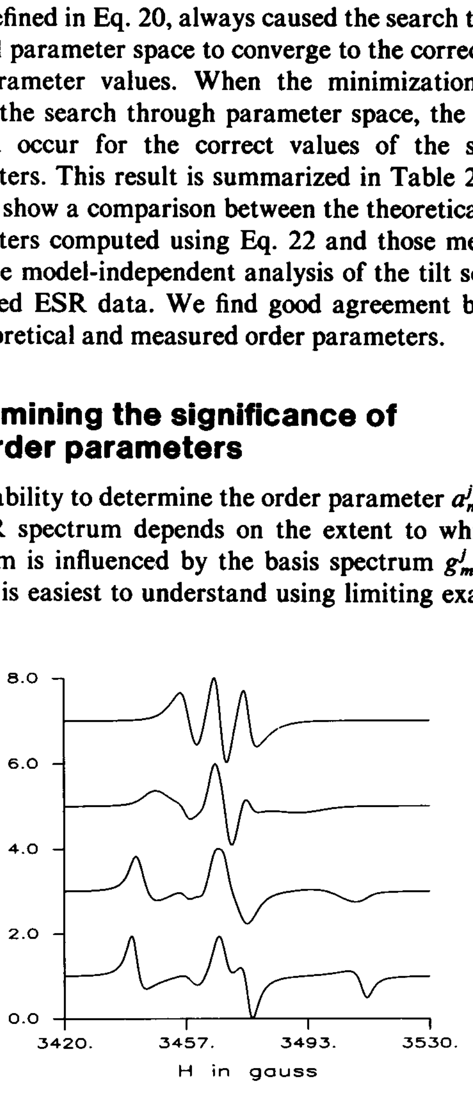

FIGURE 2 Model ESR tilt series spectra from the Gaussian probe angular distribution of Eq. 23 and model-independent fit for $\psi = 0$ (*top*), $\psi = 30°$ (*upper middle*), $\psi = 60°$ (*lower middle*), and $\psi = 90°$ (*bottom*). The model spectra and their fits are too similar to be resolved in these plots. Order parameters measured from these data are listed in Table 2. The spectral parameters and the model parameters of the Gaussian angular distribution are listed in the caption of Table 2.

TABLE 2 Selected order parameters from the simulated data of Fig. 1

	$\psi = 0$		$\psi = 90°$		
$a^j_{m,n}$	Measurement	Theory	Measurement	Theory	$w_{j,m,n}$
$a^0_{0,0}$	0.1125	0.1125	0.1125	0.1125	0.0
$a^2_{0,0}$	−0.1127	−0.1129	0.0565	0.0563	0.1743
$a^2_{0,2} + a^2_{0,-2}$	0.0521	0.0532	−0.0267	−0.0266	0.0060
$a^4_{0,0}$	0.0881	0.0855	0.0332	0.0332	0.2212
$a^4_{0,2} + a^4_{0,-2}$	−0.0363	−0.0358	−0.0135	−0.0134	0.0002
$a^4_{0,4} + a^4_{0,-4}$	−0.1449	−0.1481	−0.0574	−0.0555	0.0
$a^6_{0,0}$	−0.0599	−0.0602	0.0188	0.0188	0.1238
$a^6_{0,2} + a^6_{0,-2}$	0.0236	0.0236	−0.0073	−0.0074	0.0003
$a^6_{0,4} + a^6_{0,-4}$	0.1041	0.0803	−0.0264	−0.0251	0.0
$a^8_{0,0}$	0.0357	0.0360	0.0098	0.0098	0.1168
$a^8_{0,2} + a^8_{0,-2}$	−0.0142	−0.0140	−0.0036	−0.0038	0.0021
$a^8_{0,4} + a^8_{0,-4}$	−0.0252	−0.0450	−0.0131	−0.0123	0.0
$a^{10}_{0,0}$	−0.0186	−0.0190	0.0047	0.0047	0.0772
$a^{10}_{0,2} + a^{10}_{0,-2}$	0.0075	0.0073	−0.0018	−0.0018	0.0045
$a^{10}_{0,4} + a^{10}_{0,-4}$	0.0441	0.0230	−0.0052	−0.0057	0.0
$a^{12}_{0,0}$	0.0090	0.0090	0.0020	0.0020	0.0803
$a^{12}_{0,2} + a^{12}_{0,-2}$	−0.0028	−0.0035	−0.0007	−0.0008	0.0073
$a^{12}_{0,4} + a^{12}_{0,-4}$	0.0052	−0.0106	−0.0010	−0.0024	0.0
$a^{14}_{0,0}$	−0.0033	−0.0038	0.0008	0.0008	0.0875
$a^{14}_{0,2} + a^{14}_{0,-2}$	0.0022	0.0015	−0.0002	−0.0003	0.0091
$a^{14}_{0,4} + a^{14}_{0,-4}$	0.0122	0.0045	−0.0002	−0.0009	0.0
$a^{16}_{0,0}$	0.0015	0.0015	0.0002	0.0003	0.0893

Shown are measured and theoretical order parameters for the probe angular distribution of Eq. 23 with $\beta_0 = 90°$, $w_1 = 15°$, $\gamma_0 = 45°$, and $w_2 = 15°$. Listed are the order parameters $a^j_{m,n}$ for $j = 0, 2, 4, \ldots, 16$ $m = 0$, and $n = 0, \pm 2, \pm 4$ for spectra with $\psi = 0$ and $\psi = 90°$. Also listed are the weighting factors used in the calculation of Q from Eq. 20. The most heavily weighted order parameters are the most reliable. The weighting factor $w_{0,0,0}$ is set to zero because the value of $a^0_{0,0}$ is fixed by the normalization of N. The measured order parameters are derived from the spectra in Fig. 2 using a model-independent analysis of tilt series data, where Q is minimized. Minimizing Q by adjusting the spectral parameters led to the correct choices for these parameters. The steepest descent to the minimum Q, for the correct spectral parameters, was achieved for a Gaussian weighting of the order parameters with a width $w_0 = 1.8$ (see Eqs. 20 and 28). The spectral parameters used to generate the spectra used here and shown in Fig. 2 were $\epsilon = 2.5$ G, $c = 3{,}470$ G, $g_x = 2.0080$, $g_y = 2.0060$, $g_z = 2.0020$, $T_x = 8.8$ G, $T_y = 7.2$ G, $T_z = 34.8$ G. When χ^2 was minimized to find the spectral parameters the fit converged to the same parameters with the exception that $c = 3{,}469.8$ G. This fault indicated that χ^2 was not a useful criteria for minimization.

Consider a spin probe with axially symmetrical g and T-tensors such that $g_x = g_y$ and $T_x = T_y$. Eqs. 15 and 16 show that the lineshape of this kind of probe is independent of angles α and γ implying from Eq. 6 that $g^j_{m,n} = g^j_{0,0}\,\delta_{m,0}\,\delta_{n,0}$. From Eq. 5 this restriction on $g^j_{m,n}$ implies that the ESR spectrum depends only on the order parameters $a^j_{m,n} = a^j_{0,0}\,\delta_{m,0}\,\delta_{n,0}$. These order parameters describe a distribution independent of α and γ implying that a change in the probe distribution involving the α and γ degrees of freedom leaves the ESR spectrum unchanged. Similarly, if the probe is symmetrical such that all of the elements of the g and T-tensors are identical, then all orientations of the probe are equivalent and any change in the probe distribution leaves the ESR spectrum unchanged. For the case of the nitroxide spin label the g and T-tensors are anisotropic but we know from experience that the probe is more sensitive to distribution changes in the β than in the γ rotational degree of freedom.

These examples show that some order parameter values are more significant than others. We found an objective method to judge this significance and thereby assign a weighting factor, $w_{j,m,n}$, to each order parameter. This weighting factor is used in the determination of Q (see Eq. 20).

The overlap of the basis spectra, $g^j_{m,n}\,(H)$, with each other is given by the matrix elements $(A^{-1})^{j,m,n}_{j',m',n'}$, defined in Eq. 10. Matrix A is used to determine the values of the order parameters through the projection operators of Eq. 9. When the $g^j_{m,n}(H)$' s are not independent we cannot compute A because if $g^j_{m,n}$ and $g^{j'}_{m',n'}$ are equal to within a multiplicative constant (i.e., their shapes overlap), where at least one of the three indices are different, then A^{-1} is singular and its inverse does not exist. Even when the basis spectra are only approximately equal, matrix inversion may cause singularities. This problem leads to the difficulty of distinguishing the order parameters that correspond to the overlapping basis spectra. We found that basis spectral overlap is a direct measure of the certainty of the corresponding order parameter.

With this observation we constructed the weighting factors, $w_{j,m,n}$, using the elements of A^{-1} such that if

$$s_{j,m,n} = -1 + \left| [(A^{-1})^{j,m,n}_{j,m,n}]^{-1} \left| \sum_{j'=0}^{j_{\max}} \sum_{m',n'=-j'}^{j'} \left| (A^{-1})^{j,m,n}_{j',m',n'} \right| \right. \right. \tag{27}$$

then

$$w_{j,m,n} = \frac{\exp\,[-(s_{j,m,n}/w_0)^2]}{\sum\limits_{j,m,n} \exp\,[-(s_{j,m,n}/w_0)^2]}, \tag{28}$$

where w_0 is an adjustable width that is set to give Q, in Eq. 20, its deepest minimum at the correct spectral parameters values when used on appropriate model data. We chose model data that were similar to that of a nitroxide probe on a muscle fiber to set the valve of w_0 (see Table 2).

Minimization of multiple tilt series data sets

The tilt series data sets from identical muscle fiber preparations differ due to the random error and in the spectral parameter c. We minimize Q for multiple tilt series data sets by adjusting c for each data set while holding all of the other six spectral parameters constant (i.e., the g and T-tensor values). New values for the g and T-tensors are tried and in each trial we minimize Q by adjusting c. The best choices for the g and T-tensor values were given by the global minimum of Q determined in this manner. We determined the average value and standard deviation of the order parameters by utilizing the order parameter values measured for the individual data sets.

Selection Rules for order parameters determined by ESR

The basis spectra of ESR of nitroxide spin labels, $g^j_{m,n}(H)$, are computed using Eqs. 6, 15, and 16. These relations are used to show that certain selection rules apply to the order parameters that are detectable with ESR. The lineshape, $G(\Omega, H)$, does not depend on the Euler angle α so that from Eq. 6 we find,

$$g^j_{m,n} = 2\pi\sqrt{\frac{2j+1}{8\pi^2}} \cdot \int_0^{\pi} \sin\beta\,\mathrm{d}\beta \int_0^{2\pi} \mathrm{d}\gamma G(\Omega, H) D^{*j}_{m,n}(\Omega)\delta_{m,0} \tag{29}$$

implying from Eq. 5 that only $a^j_{m,n}$ with $m = 0$ contributes to the detectable ESR spectrum.

Furthermore, using the relation,

$$D^j_{0,n}(\alpha, \beta, \gamma) = \sqrt{\frac{4\pi}{2j+1}}\, Y_{j,n}(\beta, \gamma), \tag{30}$$

where $Y_{j,n}(\beta, \gamma)$ is a spherical harmonic with the symmetry rules that $Y_{j,n}(\pi - \beta, \pi + \gamma) = (-1)^j Y_{j,n}(\beta, \gamma)$ (Arfken, 1970) it can be shown from Eqs. 15, 16, and 29 that $g^j_{0,n} = 0$ unless $j = 0, 2, 4, \ldots$. Finally, with the relation $Y_{j,n}\,(\pi - \beta, \gamma) = (-1)^{j-n} Y_{j,n}(\beta, \gamma)$ it can be shown from Eqs. 15, 16, and 29 that $g^j_{0,n} = 0$ unless $n = 0, \pm 2, \pm 4, \ldots \pm j$, and that when $n \neq 0$ $g^j_{0,n} = g^j_{0,-n}$. Taken together these selection rules imply from Eq. 5 that the ESR spectrum is independent of $a^j_{m,n}$ unless $j = 0, 2, 4, \ldots, m = 0$, and $n = 0, \pm 2, \pm 4, \ldots \pm j$. They also imply that the linear combination of order parameters $a^j_{0,n} + a^j_{0,-n}$ are detected (rather than the individual order parameters) when $n \neq 0$.

The selection rules do not imply that any of the order parameters are zero they simply point out what order parameters are detectable with ESR on a nitroxide probe. From these rules it is clear that certain changes in the probe angular distribution would go undetected with nitroxide ESR. An example of an undetectable probe orientation change is when $\beta \longrightarrow \pi - \beta$, implying that all probe distributions derived from nitroxide ESR studies are even on the interval $0 \leq \beta \leq \pi$. This ambiguity in the spectrum causes the angular resolution in β, near $\beta = 90°$, to decrease since the ESR spectrum detects this distribution as a sum of two identical distributions centered at β and $\pi - \beta$. It is a simple calculation to show that this (even) sum of distributions can peak at $\beta = 90°$ even though the individual distribution peaks at $\beta < 90°$ due to the overlap of the curves near $\beta = 90°$. The MSL probe is oriented on the fiber such that the observed polar orientation distribution peak is at $\beta = 90°$. The consequences of this property of the MSL probe are discussed in the accompanying paper.

DISCUSSION

The introduction of a model-independent analysis of ESR data, from slowly moving spin probes labeling components of a biological assembly, established the theoretical limitations of the technique for detecting probe order (Burghardt and Thompson, 1985). A disturbing and difficult problem with this, and any other analysis method for ESR, was the high number of free spectral parameters that needed to be correctly measured before a determination of the probe angular distribution could be attempted. The simplest solution to this difficulty is to identify the correct spectral parameters from the ESR spectrum of a model system that contains no angular order where just the spectral parameters contribute to the shape of the spectrum. However, there is no guarantee that the model system will accurately reflect the conditions of the probe when it is inserted in the oriented sample of interest.

Our experience with the model-independent analysis method proved to us that the standard method of determing the spectral parameters, i.e., using a best fit approach of judging the quality of the spectral parameter choices, was ambiguous. This is demonstrated by Table 2 where we show that even for perfect data, the χ^2 test proved to be incorrect in judging the correct spectral parameters. With the tilt series ESR data set and the minimization of the factor Q for judging spectral parameter choices we have found a useful and superior method. When this method is applied to real data, as described in the accompnying paper (Ajtai et al., 1989), the convergence to a minimum Q is rapid and not dependent on the initial choices of the spectral parameters.

We also introduced here an independent enhancement over the original analysis method. This enhancement, summarized by Eqs. 11 and 12, significantly increases the resolution of the angular distribution computed from the ESR spectra. We investigated the utility of this enhancement on simulated data from model angular distributions where the width of the distribution is infinitesimal, i.e., a Dirac delta function distribution. The enhancement works by feeding back the difference between the observed ESR spectrum and its fit calculated from a subset of the basis spectra into the model-independent procedure for determining the order parameters. In this way, all of the members of the infinite set of basis spectra that can contribute to an ESR spectrum, contribute to the determination of the finite number of order parameters that correspond to the included basis spectra. Simulated data from a Dirac delta function tested the utility of this enhancement because the ESR spectrum generated from such a narrow distribution contains significant contributions from all orders of basis spectra. We obtained excellent results on this simulated data demonstrating that we can accurately calculate the order parameters for this distribution to any reasonable order.

We experimented with the usefulness of fitting the tilt series data in multiple data sets such that tilt series data sets from identically prepared samples are simultaneously fitted by minimizing the sum of the Q factors. This method significantly reduced the computing time required to get convergence to a best choice for the spectral parameters by eliminating some of the computing steps that would be done repetitively if the data sets were treated separately. We found this to be very helpful in reducing a large amount of data from the application described in the accompanying paper (Ajtai et al., 1989). This procedure has some similarity to the so called global analysis methods used with the so called global analysis methods used with time-resolved fluorescence decay data (Beechem et al., 1983; Knutson et al., 1983).

The analytical methods described herein including, the minimization of Q for a tilt series of ESR spectra, the enhancement of the detected angular resolution using a remainder basis spectrum fed back into the model-independent analysis to measure higher resolution order parameters, and minimization of factor Q for multiple tilt series data sets, were applied to spin labeled muscle fibers. With these improvements we were able to detect a subtle alteration in the ESR spectrum when a nucleotide bound to the myosin cross-bridge and interpret this change as a rotation of the probe due to a cross-bridge rotation.

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