JOURNAL OF MAGNETIC RESONANCE 61, 220–232 (1985)

A Statistical Theory for Powder EPR in Distributed Systems

W. R. HAGEN, D. O. HEARSHEN, R. H. SANDS, AND W. R. DUNHAM

Biophysics Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor, Michigan 48109

Received February 24, 1984

A statistical interpretation is presented for "g strain," the dominant broadening in the EPR spectra of metallo-proteins. The direct cause of g strain is described by a three-dimensional tensor $\mathbf{p}$, whose principal elements are random variables. The p and g tensors are not necessarily colinear. The observed EPR linewidth results from a distribution in the effective g value as a function of (a) the joint distribution function of the elements of the p tensor and (b) the spatial relationship between the two principal axis systems involved. The theory is reformulated in terms of matrices that facilitate a direct comparison with earlier work. Two previous theories of g strain represent different subsets of the general theory, namely, the case of zero rotation between axis systems and the case with nonzero rotation and full correlation between elements of the p tensor. © 1985 Academic Press, Inc.

INTRODUCTION

The term "g strain" is a label for inhomogeneous line broadening of EPR in metalloproteins. Empirically, g strain is loosely defined as a lineshape that approximates a normal distribution (*1*) with a linewidth that is roughly proportional to the applied magnetic field (*2, 3*). For many years "g strain" has been used as a working term in spite of the apparent lack of any theoretical explanation.

In biological applications of magnetic resonance, a common technique is the purely spectro*metric* use of powder EPR to characterize centers by comparing them in terms of atom number and valency, to determine relative concentrations, and to monitor signal intensity over a set of samples to deduce apparent thermodynamic and kinetic parameters. At the root of this application is the hypothesis that a spectroscopic splitting factor is an unequivocal manifestation of a center and that anisotropic inhomogeneous broadening (i.e., g strain) is a noninformative practical nuisance, which may be described by simple algorithms to allow for the generation of computer fits. Although this phenomenological approach is essentially indifferent to any link with a physical model, it has undeniably resulted in a major contribution to our present-day knowledge of metallo-enzymes. However, in recent years it has become increasingly clear that this idea falls short of accurately accounting for the shape of observed spectra and that questions concerning the multiplicity and stoichiometry of paramagnetic prosthetic groups are troubled by the limited resolution and the lack of rigor that is associated with the assumptions (*4, 5*).

In this paper, to substitute for this simplistic model, we present a statistical theory to describe the origin of the spectroscopic splitting factor as well as its distribution.

0022-2364/85 $3.00
Copyright © 1985 by Academic Press, Inc.
All rights of reproduction in any form reserved.

The theory incorporates two previously proposed models of g strain (*4–6*). In an accompanying paper (*7*) we present some fast numerical techniques that make the application of our theory practical.

THE STATISTICAL THEORY OF g STRAIN

Magnetic-resonance data can be conveniently parameterized as line position, amplitude, and width. Each of these three quantities has been related to intrinsic properties of the spin system via spin Hamiltonians and equations of motion. For example, the linewidth can be explained in terms of spin relaxation times (properties of the ensemble of spins) by solving a system of simultaneous equations which model the resonating spin ensemble as a damped harmonic system (*8*). Under nonsaturating conditions the linewidth is parameterized by T_2, the transverse relaxation time. In the case of many metal centers in proteins, however, the linewidth is much larger than can be attributed to T_2. Moreover, the linewidth is approximately proportional to the applied magnetic field, which implies that it results from an electron Zeeman interaction. Zeeman interactions are often described by a g tensor, hence the name "g strain."

Some of the linewidth is attributable to magnetic hyperfine interactions and spin–spin interactions; however, these terms cannot account for the observed field dependence. Apparently, the bulk of the broadening results from a *distribution* in the Zeeman parameters. In consequence, the usual concepts and mathematics which describe resonance position, amplitude and width must be rederived for they are functions of a set of distributed variables. Therefore, the usual equations from spin-Hamiltonian formalisms do not necessarily apply.

In our treatment, we maintain the greatest generality compatible with the derivation of a set of equations whose parameter set is small enough to allow convergence of spectral simulations in realistic times. For example, we hypothesize that the distributed variables are arguments of a joint distribution function, and that they are not necessarily components of the usual g tensor (i.e., the g tensor of a nondistributed system). However, for simplicity, we shall assume the distribution function to be of dimensionality not higher than three.

These assumptions signal the reader that what follows is not the standard derivation of magnetic-resonance lineshapes. The usual concept of a resonance position as g_{eff} must be substituted with $\langle g \rangle$, an expectation value. The linewidth is given by σ_g^2, the second central moment of the distribution. Intensity is also a function of the moments of the distribution.

THE EXPECTATION VALUE AND VARIANCE OF g

When considering the Zeeman energy for a *single* spin system in a magnetic field B, the quantity $\mathbf{g}$ is a second-rank Cartesian tensor in the effective spin Hamiltonian (*9*).

$$\mathscr{H} = \beta \mathbf{B} \cdot \mathbf{g} \cdot \mathbf{S}. \qquad [1]$$

With reference to its principal axis system, the g tensor mathematically describes the interaction between the magnetic field and the spin vector as a function of field orientation. For a *single* $S = \frac{1}{2}$ spin system this leads, in the absence of hyperfine interactions, to the resonance condition

$$h\nu = g\beta B \quad [2]$$

with

$$g = |\mathbf{n} \cdot \mathbf{g}| \quad [3]$$

in which **n** is a unit vector along **B**. This gives the resonance position for a single spin system as a function of magnetic-field orientation. To simulate an EPR spectrum of the randomly oriented sample (powder or frozen amorphous solution), we sum the individual line positions given by Eq. [3] for an isotropic distribution of magnetic-field orientations. Here we assume that the powder consists of a randomly oriented ensemble of the *same* spin system. We shall maintain the assumption of random orientation in what follows; however, the reader must be aware that the orientation distribution is completely separate from the distribution discussed below.

In the following analysis we hypothesize that the concept of the *single* spin system be replaced by the concept of an *ensemble* of spin systems. The ensemble has the property that the interaction between applied field and magnetic moment will be described by Eq. [3] for any of its members. This hypothesis has the interpretation that each magnetic-field orientation has an associated ensemble comprising spin systems with slightly different physical properties and therefore g tensors that are potentially slightly different. Given the Hamiltonian of Eq. [1], resulting in Eq. [3], the hypothesis implies that g is a random variable. The statistical properties associated with the random variable g depend upon a particular mathematical form for **g**, which we represent formally by the equation $g = g(x_1, \ldots, x_n)$ where $x_1, \ldots, x_n$ are random variables.

We wish to determine the expectation value and variance of g either by formal integration (this necessitates a specific form for the joint distribution of the x_i's; cf. p. 206 in Ref. (*10*)), or, if we expand the function, g, in a Taylor series, it can be shown that if g is smooth in the neighborhood of the center of the expansion, and if the probability density is sufficiently "concentrated" in this region (i.e., the probability that any of the n random variables takes on a value outside this region is small), then the expectation value and variance of g can be approximated by (cf. p. 216 in Ref. (*10*))

$$\langle g \rangle = \hat{g} + \frac{1}{2}\left(\sum_{i=1}^{n} \frac{\partial^2 g}{\partial x_i^2}\sigma_i^2 + 2\sum_{j>i}^{n} \frac{\partial^2 g}{\partial x_i \partial x_j} r_{ij}\sigma_i\sigma_j\right) \quad [4]$$

$$\sigma_g^2 = \sum_{i=1}^{n}\left(\frac{\partial g}{\partial x_i}\right)^2 \sigma_i^2 + 2\sum_{j>i}^{3}\left(\frac{\partial g}{\partial x_i}\right)\left(\frac{\partial g}{\partial x_j}\right) r_{ij}\sigma_i\sigma_j \quad [5]$$

where σ_i^2 is the variance of x_i, r_{ij} is the correlation coefficient between x_i and x_j, $\hat{g} \equiv g(\bar{x}_i, \ldots, \bar{x}_n)$, and all partial derivatives are evaluated at $x_k = \bar{x}_k$.

A SPECIAL CASE: THE PRINCIPAL g VALUES AS RANDOM VARIABLES

In an earlier paper (*6*) it was proposed that the random variables, x_i, that define the g tensor were actually the principal g values themselves. We can then write in the principal axis system

$$g = (\sum_{i=1}^{3} l_i^2 g_i^2)^{1/2} \qquad [6]$$

where the direction cosines, l_i, are the elements of **n**. Evaluating the partial derivatives, $\partial^2 g/\partial g_i^2$ and $\partial^2 g/\partial g_i \partial g_j$, Eqs. [4] and [5] give

$$\langle g \rangle = \hat{g} + \frac{1}{2\hat{g}} \left(\sum_{i=1}^{3} l_i^2 \left(1 - \frac{l_i^2 \bar{g}_i^2}{\hat{g}^2} \right) \sigma_i^2 + 2 \sum_{j>i}^{3} l_i^2 l_j^2 \frac{\bar{g}_i \bar{g}_j}{\hat{g}^2} r_{ij} \sigma_i \sigma_j \right) \qquad [7]$$

$$\sigma_g^2 = \frac{1}{2\hat{g}^2} (\sum_{i=1}^{3} l_i^4 \bar{g}_i^2 \sigma_i^2 + 2 \sum_{j>i}^{3} l_i^2 l_j^2 \bar{g}_i \bar{g}_j r_{ij} \sigma_i \sigma_j). \qquad [8]$$

Note that the second term in Eq. [7] is a second-order correction in $\sigma_i^2/\hat{g}$. In practice, σ_i is usually orders of magnitude smaller than $\hat{g}$. For example, in a system where $\sigma_i/\hat{g}$ is relatively large, low spin ferric cytochrome *c*, we can approximate the second term in Eq. [7] using the mean of the three apparent principal linewidths, σ_{av}, equal to 0.09 in *g*-value units and the mean of the three principal *g* values, $\hat{g}_{av}$, equal to 2.18 (*4*) by

$$\frac{\langle g \rangle - \hat{g}_{av}}{\hat{g}_{av}} \simeq \frac{\sigma_{av}^2}{2\hat{g}_{av}^2} = 8 \times 10^{-4}. \qquad [9]$$

This gives a shift of about 0.1 mT for $\langle g \rangle$ in an X-band spectrum where σ_i ranges from ~6 to ~39 mT (*4*).

We have written computer programs based on the assumption of a distribution in the principal axis *g* values, and while we were successful in fitting some experimental spectra, there were also other spectra for which this type of program was inadequate (*6*). We extend this model while preserving two important features, namely, the number (three) of random variables and their linear relationship with the *g* tensor.

THE PRINCIPAL *g* VALUES AS FUNCTIONS OF RANDOM VARIABLES

The random variables are not constrained to be the principal *g* values. The most general linear function of three random variables is

$$\mathbf{g} = \mathbf{g}^0 + R(\alpha, \beta, \gamma)\mathbf{p}R^{\dagger}(\alpha, \beta, \gamma) \qquad [10]$$

where **p** is a tensor whose elements are random variables, $\mathbf{g}^0$ is a tensor whose elements do not fluctuate, and $R(\alpha, \beta, \gamma)$ is the three-dimensional rotation which transforms the **p** principal axis system to the $\mathbf{g}^0$ principal axis system. This gives *g* in the $\mathbf{g}^0$ principal axis system as

$$g = (\sum_{i=1}^{3} l_i^2 (g_i^0 + p_{ii})^2 + \sum_{j \neq i}^{3} l_i^2 p_{ij}^2 + 2 \sum_{j>i}^{3} l_i l_j (g_i^0 + p_{ii}) p_{ij} + 2 \sum_{k \neq j \neq i}^{3} l_j l_k p_{ij} p_{ik})^{1/2} \qquad [11]$$

or alternatively in the **p** principal axis system

$$g = (\sum_{i=1}^{3} l_i^2 (p_i + g_{ii}^0)^2 + \sum_{j \neq i}^{3} l_i^2 g_{ij}^{02} + 2 \sum_{j>i}^{3} l_i l_j (p_i + g_{ii}^0) g_{ij}^0 + 2 \sum_{k \neq j \neq i}^{3} l_j l_k g_{ij}^0 g_{ik}^0)^{1/2}. \qquad [12]$$

The specific goal of a calculation dictates the choice of Eq. [11] or Eq. [12]. For instance, in the determination of the second-order term in $\langle g \rangle$, Eq. [12] is easier to use because the partial derivatives are less complicated:

$$\frac{\partial^2 g}{\partial p_n^2} = \frac{1}{\hat{g}}\left(1 - \frac{l_n^2(l_n q_n + \sum_{j\neq i}^{3} l_j g_{nj}^0)^2}{\hat{g}^2}\right) \qquad [13]$$

$$\frac{\partial^2 g}{\partial p_n \partial p_m} = -\frac{1}{\hat{g}} \cdot \frac{(l_n^2 q_n + \sum_{j\neq n}^{3} l_n l_j g_{nj}^0)(l_m^2 q_m + \sum_{j\neq m}^{3} l_m l_j g_{mj}^0)}{\hat{g}^2} \qquad [14]$$

where $q_n = \bar{p}_n + g_{nn}^0$. Both expressions in brackets are less than unity (in fact significantly less) since the numerators are smaller than $\hat{g}^2$, regardless of the relative sizes of $\bar{p}_n$ and g_{nn}^0. The numerators are in fact part of the sum comprising $\hat{g}$. Thus, the second term in the expansion of $\langle g \rangle$ remains second order.

Since in both the first and second partial derivatives, $\bar{p}_n$ always occurs in conjunction with g_{nn}^0, we can redefine $\mathbf{g}^0$, without loss of generality, to include the first moment of $\mathbf{p}$, i.e., let $p_i' = p_i - \bar{p}_i$. The new p_i' have now been defined to have zero first moments and in the $\mathbf{p}$ principal axis frame, $g_{ii}^{0\prime} = g_{ii}^0 + \bar{p}_i$. Therefore, whenever this mathematical treatment applies, the first conclusion is that it cannot be used to determine *a posteriori* the relative sizes of g_{ii}^0 and $\bar{p}_i$. For simplicity of notation, in what follows we have dropped all of the primes; however, the reader is to understand that $\mathbf{p}$, $\mathbf{g}^0$, $\mathbf{g}$, and the angles α, β, γ, which appear below have been redefined and refer to the primed variables.

The variance of g can be calculated using Eq. [11], which is preferred for this purpose because $\langle g \rangle$ has a simple form in the $\mathbf{g}^0$ principal axis system. This facilitates the calculation of the probability of the resonance, which is a function of $\langle g \rangle$. To conform with the use of $\bar{g}$ in Eq. [8], let $\bar{g}_i \equiv g_i^0 + \bar{p}_{ii}$. Using the definitions of $\mathbf{g}^0$ and $\mathbf{p}$, $\partial g/\partial p_n$ is

$$\frac{\partial g}{\partial p_n} = \frac{1}{\hat{g}^2}\left(\sum_{i=1}^{3} l_i^2 \bar{g}_i R_{in}^2 + \sum_{j>i}^{3} l_i l_j \bar{g}_i R_{in} R_{jn}\right) \qquad [15]$$

where in this coordinate system $\partial p_{ij}/\partial p_n = R_{in}R_{jn}$ and $\hat{g} = (\sum_{i=1}^{3} l_i^2 \bar{g}_i^2)^{1/2}$. With the added complexity of the rotation introduced, Eq. [5] yields a fourth-order polynomial whose terms are all products of direction cosines, $l_1^a l_2^b l_3^c$, where a, b, and c are integers greater than, or equal to zero, and $a + b + c = 4$. The result is

$$\hat{g}^2 \sigma_g^2 = \sum_{i=1}^{3} A_i l_i^4 + 2\sum_{j\neq i}^{3} B_{ij} l_i^3 l_j + 2\sum_{j>i}^{3} D_{ij} l_i^2 l_j^2 + 2\sum_{k\neq j\neq i}^{3} E_k l_i l_j l_k^2. \qquad [16]$$

The coefficients A–E have the following definitions:

$$A_i = \bar{g}_i^2\left(\sum_{l=1}^{3} R_{il}^4 \sigma_l^2 + 2\sum_{n>m}^{3} R_{im}^2 R_{in}^2 r_{mn}\sigma_m\sigma_n\right)$$

$$2B_{ij} = \bar{g}_i(\bar{g}_i + \bar{g}_j)\left(\sum_{l=1}^{3} 2R_{il}^3 R_{jl}\sigma_l^2 + \sum_{l\neq m\neq n}^{3} R_{im}R_{in}T_{kl}r_{mn}\sigma_m\sigma_n\right)$$

$$2D_{ij} = (\sum_{l=1}^{3} R_{il}^2 R_{jl}^2 \sigma_l^2 + 2 \sum_{n>m}^{3} R_{im}R_{in}R_{jm}R_{jn}r_{mn}\sigma_m\sigma_n)$$
$$\times ((\bar{g}_i + \bar{g}_j)^2 + 2\bar{g}_i\bar{g}_j) + \bar{g}_i\bar{g}_j(\sum_{l\neq m\neq n}^{3} R_{kl}^2 r_{mn}\sigma_m\sigma_n)$$

$$2E_k = (\bar{g}_k(\bar{g}_i + \bar{g}_j) + (\bar{g}_k + \bar{g}_i)(\bar{g}_k + \bar{g}_j))(\sum_{l=1}^{3} R_{il}R_{jl}R_{kl}^2\sigma_l^2 + \sum_{l\neq m\neq n}^{3} R_{km}R_{kn}T_{kl}r_{mn}\sigma_m\sigma_n)$$
$$- \bar{g}_k(\bar{g}_i + \bar{g}_j) \sum_{l\neq m\neq n}^{3} R_{il}R_{jl}r_{mn}\sigma_m\sigma_n \quad [17]$$

where $i \neq j \neq k$, $r_{mn} = r_{nm}$, and the matrix elements of T have the definition: $T_{ij} = R_{ij} + R_{i+1,j-1}R_{i-1,j+1}$ (the indices are cyclically permuted). As a check, for a rotation of zero degrees, i.e., $R_{ij} = \delta_{ij}$, the coefficients reduce to: $A_i = \bar{g}_i^2\sigma_i^2$, $B_{ij} = E_k = 0$ for all i, j, k, and $D_{ij} = \bar{g}_i\bar{g}_j r_{ij}\sigma_i\sigma_j$. Then Eq. [16] reduces to Eq. [8], the previous result.

We now proceed to rederive Eq. [16] using matrix algebra. This is done for two reasons: (1) to define a more compact notation for the purposes of discussion and comparison with other work, and (2) the significance of the rotation R in Eq. [16] will be made clearer. The preceding analysis was necessary to provide a rigorous statistical basis for what follows and to estimate the size of the second-order correction to the first moment, $\langle g \rangle$.

A MATRIX FORMULATION OF g STRAIN

The standard deviations and correlation coefficients defined in Eqs. [4] and [5] can be arranged in a real, symmetric ($n \times n$) covariance matrix (*12*). For $n = 3$:

$$ {}_1\mathbf{S} = \begin{bmatrix} \sigma_1^2 & r_{12}\sigma_1\sigma_2 & r_{13}\sigma_1\sigma_3 \\ r_{12}\sigma_1\sigma_2 & \sigma_2^2 & r_{23}\sigma_2\sigma_3 \\ r_{13}\sigma_1\sigma_3 & r_{23}\sigma_2\sigma_3 & \sigma_3^2 \end{bmatrix}. \quad [18]$$

Defining a weighted row vector

$$ {}_1^2\Lambda^\dagger = (\lambda_1^2, \lambda_2^2, \lambda_3^2) \equiv (l_1^2 g_1, l_2^2 g_2, l_3^2 g_3) \quad [19]$$

Equation [8] can be rewritten in matrix form as

$$g^2\sigma_g^2 = {}_1^2\Lambda^\dagger \cdot {}_1\mathbf{S} \cdot {}_1^2\Lambda. \quad [20]$$

The significance of the subscript index, 1, and the superscript, 2, will become clear below. We identify the matrix ${}_1\mathbf{S}$ with the linewidth tensor that describes the apparent width of an EPR spectrum. σ_g is then defined as the standard deviation of the distribution as a function of orientation, and it can be related to the width at half height by means of a proportionality constant (*4*).

To facilitate comparison with our previously used algorithms (see below), the g weighting may be decoupled from the vector ${}_1^2\Lambda$, thus reducing it to the vector ${}_1^2\mathbf{L} = (l_1^2, l_2^2, l_3^2)$, by a proper redefinition of the elements of the linewidth tensor. If

$$ {}_1W_{ij} = r_{ij}\bar{g}_i\sigma_i\bar{g}_j\sigma_j \qquad (r_{ii} = 1) \tag{21}$$

then Eq. [20] is equivalent to

$$ \sigma_g^2 = \hat{g}^{-2}({}_1^2\mathbf{L}^\dagger \cdot {}_1\mathbf{W} \cdot {}_1^2\mathbf{L}) \tag{22}$$

Note that ${}_1^2\mathbf{L}$ (and hence ${}_1^2\Lambda$) does not span the group of real, orthogonal matrices, since its elements are all positive.

THE CASE OF NONCOLINEAR p AND g IN MATRIX NOTATION

Analogous to the extension of Eq. [8] to Eq. [16], with the introduction of **p** the generalization of Eq. [20] will have the form

$$ \hat{g}^2\sigma_g^2 = \Lambda^\dagger \cdot \mathbf{DPD}^\dagger \cdot \Lambda \tag{23}$$

where **P** is an appropriate matrix representation of **p**. However, the transformation matrix **D** is *not* a 3×3 matrix in $\mathcal{D}^{(1)}$, an irreducible representation of the pure rotation group (cf. Ref. (*12*)). The covariances $r_{ij}\sigma_i\sigma_j$, associated with the g tensor, can be seen to contain terms in $(\sigma_m^3\sigma_n)^{1/2}$, where σ_m^2 is the variance of the mth principal value of the p tensor. These terms cannot result from a similarity transformation on ${}_1\mathbf{p}$. The basis set, $\{x, y, z\}$, is too small to span the space of the required operation. From this perspective, Eq. [16], from the derivation in the previous section, is Eq. [8] redefined on a new basis set. We are led to define both Eq. [8] and Eq. [16] in terms of a proper irreducible representation. The transformation between Eq. [8] and Eq. [16] is then well characterized. Since Eq. [16] contains no cubic, quadratic, linear, or constant terms, it can be written as a linear combination of products of four direction cosines: $(l_il_jl_kl_m)$. Because two of the indices i, j, k, m, have to be equal, and given the definitions of the direction cosines, in particular $\sum_{i=1}^{3} l_i^2 = 1$, it is readily apparent that the irreducible representation of the basis which spans the set of products of four direction cosines is the set of five second-order spherical harmonics. In terms of Cartesian coordinates, and arranged as the elements of a unit length vector,

$$ {}_2^2\mathbf{L}^\dagger = \left(\frac{3l_3^2 - 1}{2}, \frac{\sqrt{3}}{2}(l_1^2 - l_2^2), \sqrt{3}l_1l_2, \sqrt{3}l_1l_3, \sqrt{3}l_2l_3\right). \tag{24}$$

The weighted vector ${}_2^2\Lambda$ is easily obtained from ${}_2^2\mathbf{L}$ by realizing that the g weighting amounts to a redefinition of the form $\lambda_i = l_i\sqrt{g_i}$. The characterization of the transformation matrix **D** follows immediately from the determination of the basis. Since the basis is the set of second order spherical harmonics, **D** must be an element of $\mathcal{D}^{(2)}$. Thus, in our notation Eq. [23] can be rewritten as

$$ \hat{g}^2\sigma_g^2 = {}_2^2\Lambda^\dagger \cdot {}_2\mathbf{D}\ {}_2\mathbf{P}\ {}_2\mathbf{D}^\dagger \cdot {}_2^2\Lambda. \tag{25}$$

Next, we determine the representation of the covariance matrix ${}_2\mathbf{S}$ in the new basis by expanding Eq. [8] in terms of second-order spherical harmonics (see Table 1).

TABLE 1

Expansion of the Variance of g into Second-Order Spherical Harmonics in Cartesian Coordinates

	λ_1^4	λ_2^4	λ_3^4	$\lambda_1^2\lambda_2^2$	$\lambda_1^2\lambda_3^2$	$\lambda_2^2\lambda_3^2$
$[(3\lambda_3^2 - r^2)/2]^2$	$\frac{S_{11}}{4}$	$\frac{S_{11}}{4}$	S_{11}	$\frac{S_{11}}{2}$	$-S_{11}$	$-S_{11}$
$2[(3\lambda_3^2 - r^2)/2][\sqrt{3}(\lambda_1^2 - \lambda_2^2)/2]$	$-\frac{\sqrt{3}S_{12}}{2}$	$\frac{\sqrt{3}S_{12}}{2}$	0	0	$\sqrt{3}S_{12}$	$-\sqrt{3}S_{12}$
$[\sqrt{3}(\lambda_1^2 - \lambda_2^2)/2]^2$	$\frac{3S_{22}}{4}$	$\frac{3S_{22}}{4}$	0	$-\frac{3S_{22}}{2}$	0	0
$[\sqrt{3}\lambda_1\lambda_2]^2$	0	0	0	$3S_{33}$	0	0
$[\sqrt{3}\lambda_1\lambda_3]^2$	0	0	0	0	$3S_{44}$	0
$[\sqrt{3}\lambda_2\lambda_3]^2$	0	0	0	0	0	$3S_{55}$

Note. Where $r^2 = \sum_{i=1}^{3} \lambda_i^2$.

From this table we obtain six independent equations with six unknowns:

$$\begin{aligned} 4\sigma_1^2 &= S_{11} - 2\sqrt{3}S_{12} + 3S_{22} \\ 4\sigma_2^2 &= S_{11} + 2\sqrt{3}S_{12} + 3S_{22} \\ \sigma_3^2 &= S_{11} \\ 4r_{12}\sigma_1\sigma_2 &= S_{11} - 3S_{22} + 6S_{33} \\ 2r_{13}\sigma_1\sigma_3 &= -S_{11} + \sqrt{3}S_{12} + 3S_{44} \\ 2r_{23}\sigma_2\sigma_3 &= -S_{11} - \sqrt{3}S_{12} + 3S_{55}. \end{aligned} \qquad [26]$$

Solving this set we find the elements S_{ij} of the ${}_2\mathbf{S}$ matrix:

$$ {}_2\mathbf{S} = \begin{bmatrix} \sigma_1^2 & \frac{\sigma_2^2 - \sigma_1^2}{\sqrt{3}} & 0 & 0 & 0 \\ \frac{\sigma_2^2 - \sigma_1^2}{\sqrt{3}} & \frac{2\sigma_1^2 + 2\sigma_2^2 - \sigma_3^2}{3} & 0 & 0 & 0 \\ 0 & 0 & \frac{\sigma_1^2 + \rho_{12} + \sigma_2^2 - \sigma_3^2}{3} & 0 & 0 \\ 0 & 0 & 0 & \frac{\sigma_1^2 + \rho_{13} + \sigma_3^2 - \sigma_2^2}{3} & 0 \\ 0 & 0 & 0 & 0 & \frac{\sigma_2^2 + \rho_{23} + \sigma_3^2 - \sigma_1^2}{3} \end{bmatrix} \qquad [27]$$

where $\rho_{ij} = 2r_{ij}\sigma_i\sigma_j$.

With the covariance matrix defined in this basis, Eq. [20] may be rewritten as

$$g^2\sigma_g^2 = {}_2^2\Lambda^\dagger \cdot {}_2\mathbf{S} \cdot {}_2^2\Lambda. \qquad [28]$$

Since a rotation in $\mathcal{D}^{(2)}$ on ${}_2^2\Lambda$ stays within the set of second-order spherical harmonics, and since the rotation can equally well be applied to ${}_2\mathbf{S}$, we can identify

${}_2\mathbf{S}$ with ${}_2\mathbf{P}$ in Eq. [25]. An explicit expression for the rotation matrix ${}_2\mathbf{D}$ in Eq. [25] is derived in the Appendix.

FULL CORRELATION—COMPARISON WITH EARLIER WORK

Based on the assumption of a permanent, strain-induced g shift in frozen solutions of metallo-proteins one of us previously proposed (*4, 5*) the algorithm

$$W = |{}^{\dagger}\mathbf{L} \cdot \Delta\mathbf{g} \cdot {}^{\dagger}\mathbf{L}| \qquad [29]$$

to describe the anisotropic width of some inhomogeneously broadened EPR spectra. Whereas there was no rigorous theoretical explanation of this previous model, we can now understand, via our present parameterization, why Eq. [29] was effective in nearly reproducing highly asymmetric EPR powder shapes of certain iron proteins.

It will now be shown, by a substitution of variables, that Eq. [29] closely approximates, under certain conditions, a subset of the solutions covered by the general expressions Eqs. [16] or [25], namely, those cases in which there is full correlation between variables.

First compare the diagonal form of Eq. [29], i.e., $\Delta g_{ij} = 0$, with

$$\sigma^2 = {}^{2}_{\dagger}\mathbf{L} \cdot {}_1\mathbf{S} \cdot {}^{2}_{\dagger}\mathbf{L}. \qquad [30]$$

Equation [30] and the diagonal form of Eq. [29] are identical within the substitutions

$$\begin{aligned} \sigma^2 &\equiv W^2 \\ \sigma_i &\equiv |\Delta g_{ii}| \\ r_{ij} &\equiv \frac{\Delta g_{ii} \cdot \Delta g_{jj}}{|\Delta g_{ii} \cdot \Delta g_{jj}|}. \end{aligned} \qquad [31]$$

Now consider what happens when we generalize the diagonal form of Eq. [29] by implementing a rotation in $\mathcal{D}^{(1)}$. Applied to the coordinate system, the elements of the vector $\mathbf{L}$ will become linear combinations of the original direction cosines. Since, to obtain W^2, the right-hand side is squared after completion of the inner product, the result is a *full* fourth order polynomial in l_1, l_2, l_3, containing the same terms as Eq. [16] or Eq. [25] which include the rotation in $\mathcal{D}^{(2)}$, except that the correlation coefficients are fixed at ± 1, and the partial derivatives of g with respect to p_i are missing. The partial derivatives could be included by an appropriate redefinition of the variances, σ_i^2, if they did not include a factor of $\hat{g}^{-1}$ which is not a constant, but a function of (θ, ϕ).

If $\mathbf{g}$ is not highly anisotropic, i.e., if $\hat{g} \simeq 2$, Eq. [29] approximates Eq. [25], for $r_{ij} = \pm 1$, when we define

$${}_1\mathbf{P} \equiv \begin{bmatrix} r_{23}\sigma_1 & 0 & 0 \\ 0 & r_{13}\sigma_2 & 0 \\ 0 & 0 & r_{12}\sigma_3 \end{bmatrix} \qquad [32]$$

and make the substitution

$$\Delta\mathbf{g} \equiv {}_1\mathbf{D}\ {}_1\mathbf{P}\ {}_1\mathbf{D}^{\dagger} \qquad [33]$$

Consequently, in the case of full correlation between the random variables p_1, p_2, and p_3 (and therefore the functions of random variables g_1, g_2, and g_3), the generalized linewidth algorithm, Eq. [25], reduces to a form that contains only (3×3) matrices, namely

$$\hat{g}^2\sigma_g^2 = ({}_1^1\Lambda^{\dagger} \cdot {}_1\mathbf{D}\ {}_1\mathbf{P}\ {}_1\mathbf{D}^{\dagger} \cdot {}_1^1\Lambda)^2. \qquad [34]$$

The superscripts and subscripts attached to Λ and $\mathbf{L}$ can now be explained. They are used to distinguish the vectors according to these definitions: (1) the subscript refers to the subspace to which each vector belongs and (2) the superscript refers to the power to which the elements (direction cosines) of the vector are raised. The second definition is necessary to distinguish ${}_1^2\mathbf{L}$ from ${}_1^1\mathbf{L}$ and ${}_2^2\mathbf{L}$. The latter two are basis vectors for $\mathcal{D}^{(1)}$ and $\mathcal{D}^{(2)}$, respectively, while ${}_1^2\mathbf{L}$ has a peculiar definition useful only in defining Eqs. [20] and [22]. This gives an insight into the differences between Eqs. [20] and [34]. These two equations, derived from the two previous theories (*4–6*), represent different subsets of the general solution Eq. [25]. Equation [20] is the subset with a rotation of zero degrees and arbitrary correlation coefficients. Equation [34] is a subset with fixed correlation coefficients (± 1) and an arbitrary rotation.

PROPERTIES OF THE DISTRIBUTION

The statistical model presents a mathematical description of the line*width* of the projection of the g tensor onto the external magnetic field (referred to as the effective g value). This linewidth is calculated using some rather general assumptions, without knowledge of the exact form of the joint distribution function of the p variables or consequently the distribution function of the effective g value. Knowledge of the identity of the p variables (and hence a model for the g tensor) does not necessarily lead to knowledge of the correct distribution function for the p variables. Therefore, we assume that the p variables follow a joint normal distribution. Some justification for this has been made elsewhere (*13*).

Assuming a joint normal distribution for the p variables does not immediately lead to the distribution function for the effective g value. This distribution function must be derived from the joint distribution function for the p variables using an appropriate method (cf., Ref. (*11*)). For the case where the p tensor is colinear with the g tensor, the distribution function for the effective g value has been shown to be closely approximated by a normal distribution (*13*). We assume this to hold for the noncolinear case. Furthermore, the lineshape of a *powder* EPR spectrum in this case will prove to be dominated not so much by the details of the lineshape, but by contributions to the powder from lines with widely varying linewidths (*7*).

CONCLUDING REMARKS

The previous mathematical treatment forms the theoretical basis for a computer program which fits g-strained EPR data. A unique fit to precise data results in a set of values for the expected g values, for the variances of the principal elements of

the p tensor, for the correlation coefficients associated with the variances, and for the angles of rotation that relate the principal axis systems of **g** and **p**. Numerical problems that are associated with the design of such a program are addressed in an accompanying paper (*7*).

In the derivation of Eq. [16] the *first* moments of the p values and the *second-central* moments of the g values ($\mathbf{g}^0$) were, for convenience, set equal to zero. The situation in which these quantities are not equal to zero is mathematically equivalent to these assumptions. Therefore, one cannot discriminate between the two possibilities on basis of fits to the EPR spectra.

If the principal axis systems of **g** and **p** are colinear, then it is impossible to separate **g** and **p**. In fact, it is also unnecessary since one can simply interpret g strain in terms of distributions of the usual terms which give rise to g values, namely, crystal-field splittings, spin-orbit interactions, etc.

If the coordinate systems are not colinear, then distributions in the principal-axis values of the g tensor cannot be the cause of g strain. In this case it is useful to examine the properties of the covariance matrix (cf. Eq. [18]). The matrix is positive definite or positive semidefinite. Thus, in its diagonal form, no element can be negative, and the number of degrees of freedom in the "distribution" space is equal to the number of nonzero elements. In Eq. [18] the matrix is (3×3). If the rank of the matrix is 2 or 1, after diagonalization, then the variables σ_n and r_{nm} can be redefined to give a covariance matrix with no zero eigenvalues and rank less than 3. Regardless of whether the rank of the positive definite form of the covariance matrix is 1, 2, or 3, its inverse has elements which define the argument of the joint distribution function (*11*). The covariance matrix contains information that should allow one to determine the origin of g strain. For instance, if its rank is less than three, the p variables are not independent, and can be redefined in terms of a smaller set of variables. This redefinition has physical implications, as do the sizes of the second central moments.

In the accompanying paper we analyze a g-strain broadened spectrum of a metallo-protein to illustrate the use of fast numerical techniques that make application of the foregoing practical. In this example, we find the absolute values of the three correlation coefficients to essentially equal unity. Furthermore, we are in the course of an ambitious research effort to analyze the g-strained EPR, measured at many microwave frequencies, of representative examples from different classes of metallo-proteins. So far, we have found no indication of correlation coefficients that significantly deviate from the value ± 1. Full positive or full negative correlation means that the rank of the covariance matrix is unity. Consequently, all p variables are fully dependent and can be redefined in terms of a single variable. Thus, it appears that the cause of g strain in the EPR of metallo-proteins is a single phenomenon describable by a scalar quantity as, for example, a hydrostatic pressure.

APPENDIX: ROTATION OF COORDINATE SYSTEM IN $\mathcal{D}^{(2)}$

A rotation in three dimensions is specified by its Eulerian angles $\{\alpha, \beta, \gamma\}$. We adopt Whitakers convention (see, for example, Appendix 1 in Ref. (*14*)) according to which an axis transformation from X'', Y'', Z'' into X, Y, Z starts with a rotation

about the Z'' axis by an angle α, followed by a rotation about the new Y' axis by an angle β, and completed by a rotation about the Z axis by an angle γ.

The general expression for the elements ${}'D_{\mu'\mu}$ of the rotation matrix ${}'_j\mathbf{D}$ in the irreducible representation $\mathcal{D}^{(j)}$ of the rotation group with respect to a basis of spherical harmonics of order j is defined by Wigner (Eq. [15.27] in Ref. (*12*)) as

$$ {}'_j\mathbf{D}_{\mu',\mu} = \sum_k C_k e^{i\mu'\alpha} \cos^{2j+\mu-\mu'-2k}\left(\frac{\beta}{2}\right) \sin^{2k+\mu'-\mu}\left(\frac{\beta}{2}\right) e^{i\mu\gamma} \qquad \text{[A-1]} $$

with

$$ C_k = (-1)^k \frac{((j+\mu)!(j-\mu)!(j+\mu')!(j-\mu')!)^{1/2}}{(j-\mu'-k)!(j+\mu-k)!k!(k+\mu'-\mu)!} \qquad \text{[A-2]} $$

where [maximum $(0, \mu - \mu') \leqslant k \leqslant$ minimum $(j - \mu', j + \mu)$].

By a suitable similarity transformation ${}'_j\mathbf{D}$ can be converted to a real rotation matrix, ${}_j\mathbf{D}$ with respect to a basis set in terms of Cartesian coordinates. The result for ${}_1\mathbf{D}$ can be found in several textbooks (e.g., Refs. (*12, 14*)). We will now derive the expression for the matrix ${}_2\mathbf{D}$ which occurs in Eq. [25]. For $\beta = \gamma = 0$ we have, with respect to a basis of spherical harmonics, $\{Y_{2-2}, Y_{2-1}, Y_{20}, Y_{21}, Y_{22}\}$:

$$ {}'_2\mathbf{D}(\alpha, 0, 0) = \begin{bmatrix} e^{-2i\alpha} & 0 & 0 & 0 & 0 \\ 0 & e^{-i\alpha} & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & e^{i\alpha} & 0 \\ 0 & 0 & 0 & 0 & e^{2i\alpha} \end{bmatrix} . \qquad \text{[A-3]} $$

The transformation to a basis in Cartesian coordinates, $\{(3z^2 - r^2)/2, \sqrt{3}(x^2 - y^2)/2, \sqrt{3}xy, \sqrt{3}xz, \sqrt{3}yz\}$ is described by the matrix (cf. Appendix 2.1 in Ref. (*12*))

$$ \mathbf{X}_c = {}_2\mathbf{M}\mathbf{X}_s $$

$$ {}_2\mathbf{M} = \frac{1}{\sqrt{2}} \begin{bmatrix} 0 & 0 & \sqrt{2} & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 \\ i & 0 & 0 & 0 & -i \\ 0 & 1 & 0 & -1 & 0 \\ 0 & i & 0 & i & 0 \end{bmatrix} . \qquad \text{[A-4]} $$

We can compute the real rotation matrix ${}_2\mathbf{D}(\alpha, 0, 0) = {}_2\mathbf{M}\ {}'_2\mathbf{D}(\alpha, 0, 0)\ {}_2\mathbf{M}^\dagger$ (${}_2\mathbf{M}^\dagger$ is the adjoint of ${}_2\mathbf{M}$) using the double-angle formulas for sine and cosine and their definitions in terms of exponentials:

$$ {}_2\mathbf{D}(\alpha, 0, 0) = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 - 2\sin^2\alpha & -2\sin\alpha\cos\alpha & 0 & 0 \\ 0 & 2\sin\alpha\cos\alpha & 1 - 2\sin^2\alpha & 0 & 0 \\ 0 & 0 & 0 & \cos\alpha & -\sin\alpha \\ 0 & 0 & 0 & \sin\alpha & \cos\alpha \end{bmatrix} . \qquad \text{[A-5]} $$

An equivalent expression is obtained for ${}_2\mathbf{D}(0, 0, \gamma)$ by replacing α with γ in Eq. [A-5]. We can compute ${}_2\mathbf{D}(0, \beta, 0)$ in an analogous manner using Eqs. [A-1], [A-2], and [A-4] with $\alpha = \gamma = 0$. For this case, additional nontrivial trigonometric substitutions are necessary to obtain ${}_2\mathbf{D}$ purely in terms of the whole angle β.

$$
{}_2\mathbf{D}(0, \beta, 0) = \begin{bmatrix}
1 - \frac{3}{2}\sin^2\beta & \frac{\sqrt{3}}{2}\sin^2\beta & 0 & -\sqrt{3}\cos\beta\sin\beta & 0 \\
\frac{\sqrt{3}}{2}\sin^2\beta & 1 - \frac{1}{2}\sin^2\beta & 0 & \cos\beta\sin\beta & 0 \\
0 & 0 & \cos\beta & 0 & \sin\beta \\
\sqrt{3}\cos\beta\sin\beta & -\cos\beta\sin\beta & 0 & 1 - 2\sin^2\beta & 0 \\
0 & 0 & -\sin\beta & 0 & \cos\beta
\end{bmatrix}. \qquad \text{[A-6]}
$$

The general rotation ${}_2\mathbf{D}(\alpha, \beta, \gamma)$ can be constructed from these matrices as

$$
{}_2\mathbf{D}(\alpha, \beta, \gamma) = {}_2\mathbf{D}(\gamma, 0, 0)\, {}_2\mathbf{D}(0, \beta, 0)\, {}_2\mathbf{D}(0, 0, \alpha). \qquad \text{[A-7]}
$$

ACKNOWLEDGMENTS

Financial support is acknowledged from the European Molecular Biology Organization, ALTF-146-82 (W.R.H.) and from the U.S. Public Health Service, GM12176 (R.H.S.)

REFERENCES

1. J. FRITZ, R. E. ANDERSON, J. A. FEE, G. PALMER, R. H. SANDS, J. C. M. TSIBRIS, I. C. GUNSALUS, W. H. ORME-JOHNSON, AND H. BEINERT, *Biochim. Biophys. Acta* **253,** 110 (1971).
2. L. H. STRONG, Ph.D. thesis, University of Michigan, 1976.
3. R. H. SANDS AND W. R. DUNHAM, *Quart. Rev. Biophys.* **7,** 443 (1975).
4. W. R. HAGEN, *J. Magn. Reson.* **44,** 447 (1981).
5. W. R. HAGEN AND S. P. J. ALBRACHT, *Biochim. Biophys. Acta* **702,** 61 (1982).
6. D. O. HEARSHEN, W. R. DUNHAM, R. H. SANDS, AND H. J. GRANDE, *in* "Electron Transport and Oxygen Utilization" (Ho, C., Ed.), p. 395, Elsevier, New York, 1982.
7. W. R. HAGEN, D. O. HEARSHEN, L. J. HARDING, AND W. R. DUNHAM, *J. Magn. Reson.*, submitted.
8. F. BLOCH, *Phys. Rev.* **70,** 460 (1946).
9. R. J. ELLIOT AND K. W. H. STEVENS, *Proc. Roy. Soc. London Ser. A* **218,** 553 (1953).
10. A. PAPOULIS, "Probability, Random Variables, and Stochastic Processes," McGraw-Hill, New York, 1965.
11. R. VON MISES, "Mathematical Theory of Probability and Statistics," Academic Press, New York, 1964.
12. E. P. WIGNER, "Group Theory," Academic Press, New York, 1959.
13. D. O. HEARSHEN, Ph.D. thesis, University of Michigan, 1983.
14. H. WATANABE, "Operator Methods in Ligand Field Theory," Prentice-Hall, Englewood Cliffs, N.J., 1966.