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College on Methods and Experimental Techniques in Biophysics

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Electron Spin Resonance
Introduction
Applications

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These are preliminary lecture notes, intended only for distribution to participants.

INTRODUCTION

In this course we will loosely follow the book Theory of Magnetic Resonance, supplemented with discussions of how to interpret experimental data, followed by the presentation of results obtained from same biological systems.

On overall outline of the lectures is as follows.

1) Introduction
2) Instrumentation
3) Quantum Mechanics
4) Isotropic and Anisotropic Hamiltonian
5) Bloch equations
6) Line shape
7) Relaxation times
8) Powder
9) Irradiated Systems, Bones, Alanine, Mobility, Membranes
10) Conclusion

The systems that will be treated in the experimental discussions are biological, chemical, conduction electrons, free radicals, irradiated naturally occuring substances semiconductors and transition elements.

In spectroscopy photons are used to induce transitions between energy levels in atoms and molecules. For example, light incident on an atom can induce a transition if its energy hν equals the spacing between two energy levels

$$h\nu = E_1 - E_2$$

If the incident photon breaks up a molecule, such as

$$h\nu + CH_4 \rightarrow \cdot CH_3 + H\cdot \qquad (1)$$

it can produce two species with unpaired electrons so they can be studied in an electron open resonance (ESR) spectrometer. In this case the reaction product is the methyl radical $\cdot CH_3$ and a hydrogen atom $\cdot$ H, where the dot ($\cdot$) indicates an unpaired electron. Sometimes irradiation can produce a charged radical called a radical ion, such as a benzene anion or cation as follows

$$h\nu + C_6H_6 \rightarrow e + C_6H_6^+ \qquad (2)$$

$$e + C_6H_6 \rightarrow C_6H_6^- \qquad (3)$$

Transition ions are often paramagnetic because they have unfilled inner shells, such as

Mn^{2+} $3d^5$ $S = 5/2$

Fe^{2+} $3d^6$ $S = 2$

Fe^{3+} $3d^5$ $S = 5/2$ (4)

The absorption of a photon raises an electron to an excited state, and after a short time called the relaxation time the electron will return to the gound state. Relaxation times can range from nanoseconds in optical spectroscopy to many minutes in NMR.

2. Instrumentation

There are many branches of spectroscopy depending on the frequencies

We are interested in spectroscopy in the microwave region, with a wavelength of 3cm and frequencies of 10 GHz. This spectroscopy, called electron spin resonance is carried out in a magnetic filed of ~0.3T.

In carrying out an ESR experiment, microwave power from the klystron is incident on the sample located in the resonant cavity between the pole pieces of the magnet, and the magnetic field is scanned. At the proper combination of magnetic field and frequency corresponding to the resonant condition

$$h\nu = g\beta H \tag{5}$$

energy is absorbed, and the change in reflected power from the cavity is detected at the crystal detector, amplified, and displayed on a chart recorder.

3. Quantum Mechanics

During an ESR experiment the microwaves induce transitions between Zeeman levels of free radicals and transition metal ions, and the separations of these levels as well as the probability of inducing transitions are given by the laws of quantum mechanics.

When the spin Hamiltonian is known the energy levels can be found by a direct product matrix diagonalization method, and we will show how to set up the matrix for the spins that enter the Hamiltonian.

For each spin there is an S_x, S_y, and S_z matrix. For spin-1/2 those matrices are one half of the Pauli spin matrices, $S_i = 1/2\ \sigma_i$, as follows

$$S_x = \frac{1}{2}\begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix} \qquad S_y = \frac{1}{2}\begin{pmatrix} 0 & -i \\ i & 0 \end{pmatrix} \qquad S_z = \frac{1}{2}\begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} \qquad (6)$$

and for S = 1 they are

$$S_x = \frac{1}{\sqrt{2}}\begin{pmatrix} 0 & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix} \qquad S_y = \frac{1}{\sqrt{2}}\begin{pmatrix} 1 & -i & 0 \\ i & 0 & -i \\ 0 & i & 0 \end{pmatrix} \qquad S_z = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -1 \end{pmatrix} \qquad (7)$$

The higher order S_x and S_y matrices are generated from the following pyramid which contains the magnitudes of the matrix elements at the position adjacent to the diagonal

$$\begin{array}{ll}
S = \frac{1}{2} & \sqrt{1} \\
S = 1 & \sqrt{2}\ \sqrt{2} \\
S = \frac{3}{2} & \sqrt{3}\ \sqrt{2\cdot 2}\ \sqrt{3} \\
S = 2 & \sqrt{4}\ \sqrt{3\cdot 2}\ \sqrt{2\cdot 3}\ \sqrt{4} \\
S = \frac{5}{2} & \sqrt{5}\ \sqrt{4\cdot 2}\ \sqrt{3\cdot 3}\ \sqrt{2\cdot 4}\ \sqrt{5} \\
S = 3 & \sqrt{6}\ \sqrt{5\cdot 2}\ \sqrt{4\cdot 3}\ \sqrt{3\cdot 4}\ \sqrt{2\cdot 5}\ \sqrt{6} \\
S = \frac{7}{2} & \sqrt{7}\ \sqrt{6\cdot 2}\ \sqrt{5\cdot 3}\ \sqrt{4\cdot 4}\ \sqrt{3\cdot 5}\ \sqrt{2\cdot 6}\ \sqrt{7}
\end{array} \qquad (8)$$

Where the S_x matrix is real and ±i is added to S_y, as given by Eqs. (6) and (7). The S_z matrices have the $M_s = S$ to $M_s = -S$.

These matrices are employed to expand a spin hamiltonian of the type

$$H = g_1\beta\,\vec{S}_i\cdot\vec{B} + g_2\beta\,\vec{B}\cdot\vec{S}_2 + A\vec{S}_1\cdot\vec{S}_2 \qquad (9)$$

by direct products, with unit matrices inverted for the magnetic field in the two Zeeman terms. In the case of $S_1 = 1/2$, $S_2 = 1/2$ the result is a sum of 4 × 4 matrices which are added to give the spin hamiltonian matrix. These matrix is diagonalize to find the energy levels.

4. Isotropic and Anisotropic Hamiltonian

The Hamiltonian of Eq. (9) is isotropic because the x, y and z directions are equivalent. When these directions are not equivalent we have in direct product notation, for the magnetic field oriented along the z direction.

$$H = g_1\beta\, S_{1z} x I + g_2\beta\, I x S_{2z} + A_x S_{1x} x S_{2x} + A_y S_{1y} x S_{zy} + A_z S_{1z} x S_{2z} \tag{10}$$

In a typical ESR case S_1 is an electron spin and S_2 is a nuclear spin, and the nuclear Zeeman term $g_2\beta I \text{ x } S_{2z}$ can be neglected because $g_2 << g_1$.

The Hamiltonian is often axially symmetric with

$$\begin{aligned} g_x &= g_y = g_\perp \\ g_z &= g_{\parallel} \end{aligned} \tag{11}$$

$$\begin{aligned} A_x &= A_y = A_\perp \\ A_z &= A_{\parallel} \end{aligned} \tag{12}$$

although the symmetry axis for the g-factor and hyperfine (A) tensor is not always the same. For the special case of an anisotropic g-factor and no hyperfine tensor we have the angular dependence

$$g^2 = \left(g_x^2 \cos^2\varphi + gy_y^2 \sin^2\varphi\right)\sin^2\theta + g_z^2\cos^2\theta \tag{13}$$

which, for axial symmetry (11) reduces to

$$g^2 = g_{\parallel}^2 \sin^2\theta + g_{\perp}^2 \cos^2\theta \qquad (14)$$

Anisotropies are often important, as for example with spin labels.

5 - Block Equations

The magnetism of the parmagnetic sample process under the action of the microwave power applied at the frequency ω and the components of the magnetization satisfy what are called the Bloch equations.

These equations take into account relaxation processes between the spins through the spin spin relocation time T_2, and the rate at which the precessing spins transfer energy to the lattice through the spin lattice relaxation time T_1.

The solution to these equations in a coordinate system x', y', z' rotating with the applied radio frequency field H_1 are given by.

$$M_{x'} = \frac{\chi_0 \omega_0 T_2 H_1 \left[(\omega - \omega_0) T_2\right]}{1 + (\omega - \omega_0)^2 T_2^2 + \gamma^2 H_1^2 T_1 T_2} \qquad (15a)$$

$$M_{y'} = \frac{\chi_0 \omega_0 T_2 H_1}{1 + (\omega - \omega_0)^2 T_2^2 + \gamma^2 H_1^2 T_1 T_2} \qquad (15b)$$

In the laboratory coordinate system x, y, z the x component of the magnetization is

$$M_x = M_{x'} \cos\omega t + M_{y'} \sin\omega t \qquad (16)$$

and if we define the susceptibility

$$\chi(\omega) = \chi'(\omega) - i\chi''(\omega) \qquad (17)$$

and substitute Eqs. (15a) and (15b) into eq. (16) we obtain

$$M_x = H_1\left[\chi(\omega)e^{+i\omega t} + \chi*(\omega)e^{-i\omega t}\right] \tag{18}$$

$$= 2H_1\left[\chi'(\omega)\cos\omega t - \chi''(\omega)\sin\omega t\right] \tag{19}$$

which gives for the susceptibility terms

$$\chi' = \frac{1}{2}\chi_0\omega_0T_2\frac{(\omega-\omega_0)T_2}{1+(\omega-\omega_0)^2T_2^2+\gamma^2H_1^2T_1T_2} \tag{20a}$$

$$\chi'' = \frac{1}{2}\chi_0\omega_0T_2\frac{1}{1+(\omega-\omega_0)^2T_2^2+\gamma^2H_1^2T_1T_2} \tag{20b}$$

which are measured in an ESR experiment.

The real part $\chi'(\omega)$ gives a dispersion signal which involves a shift in frequency and the imaginary part $\chi''(\omega)$ gives absorption which is detected as a change in quality factor Q.

The absorption signal Y(H) can be written as

$$Y(H) = \frac{sH_1y_m^0}{1+s(H-H_0)^2\gamma^2T_2^2} \tag{21}$$

and its first derivative Y′(H) is often detected

$$Y'(H) = \frac{16s^2(H-H_0)\gamma T_2H_1y_{m'}^0}{3^{3/2}\left[1+s(H-H_0)^2\gamma^2T_2^2\right]^2} \tag{22}$$

where s is the saturation factor defined by

$$s = \left(1+H_1^2\gamma^2T_1T_2\right)^{-1} \tag{23}$$

and we changed the variable from frequency to magnetic field because the field is generally scanned in ESR. In NMR it is customary to scan frequency. The radio frequency field H_1 is proportional to the square root of the microwave power

$$H_1 = k\sqrt{P} \tag{24}$$

and Y′(H) has the maximum value

$$y'_m = s^{3/2} H_1 y^{0'}_m = \frac{k\sqrt{P} y^{0'}_m}{\left(1 + kP\gamma^2 T_1 T_2\right)^{3/2}} \tag{25}$$

which is calculated from the condition

$$\frac{dy'_m}{dH_1} = 0 \tag{26}$$

corresponding to

$$s = \frac{2}{3} \tag{27}$$

The power dependence is shown on the figure. The spin spin (T_2) and spin lattice (T_1) relaxation times are given by

$$T_2 = \frac{2}{\gamma \Delta H^0_{1/2}} = \frac{2}{\sqrt{3}\gamma \Delta H^0_{pp}} \tag{28}$$

$$T_1 = \frac{\sqrt{3}\gamma\Delta H^0_{pp}}{\gamma(2\Delta H_1)^2} \text{ NMR case} \tag{29}$$

$$T_1 = \frac{1.97\times10^{-7}\Delta H^0_{pp}}{g(2H_1)^2} \text{ ESR case} \tag{30}$$

also of interest is the expresion

$$\frac{1}{T_m} = \frac{1}{T_2} + \frac{1}{T_2^*} \tag{31}$$

and the half width of the absorption line is

$$\Delta H_{1/2} = \frac{2}{\gamma T_m} \tag{32}$$

A typical operating condition is

$$\frac{2\pi}{\omega_0} < t_w < T_m \tag{33}$$

For pulse work we define a 90° pulse of length

$$\gamma H_1 t_w = \frac{1}{2}\pi \tag{34}$$

and a 180° pulse

$$\gamma H_1 t_w = \pi \tag{35}$$

When the z component of the magnetization is reversed in direction it returns to its equilibrium value M_0 with the time constant T_1

$$M_z(t) = M_0\left(1 - 2e^{-t/T_1}\right) \tag{36}$$

Typical operating conditions are

$$\frac{2\pi}{\omega_0} < t_w < T_m < T_2^* < \tau << T_2 \qquad (37)$$

for saturation recovery experiments.

6. Line Shapes

Magnetic resonance spectra, ESR and NMR alike, generally consist of resonant lines which have Lorentzian (Y^L) or Gaussian (Y^G) shapes, as follows

$$Y^G(H) = y_m \exp\left[-0.693\left(\frac{H-H_0}{\frac{1}{2}\Delta H_{1/2}}\right)^2\right] \tag{38}$$

$$Y^L(H) = \frac{y_m}{1+\left[(H-H_0)/\frac{1}{2}\Delta H_{1/2}\right]^2} \tag{39}$$

where $\Delta H_{1/2}$ is the full line width at half height, i. e. between points where $Y^G(H) = \frac{1}{2}y_m$. The corresponding first derivative line shapes are

$$Y'^G(H) = 1.649y'_m\left(\frac{H-H_0}{\frac{1}{2}\Delta H_{pp}}\right)\exp\left[-\frac{1}{2}\left(\frac{H-H_0}{\frac{1}{2}\Delta H_{pp}}\right)^2\right] \tag{40}$$

$$Y'^L(H) = \frac{16y'_m\left[(H-H_0)/\frac{1}{2\Delta H_{pp}}\right]}{\left[3+\left\{(H-H_0)/\frac{1}{2}\right\}\Delta H_{pp}{}^2\right]^2} \tag{41}$$

where ΔH_{pp} is the line width between the peaks on the first derivative spectrum, The relationship between the half amplitude and peak to peak widths is

$$\begin{aligned} \Delta H_{1/2} &= (2\,\ell n\,2)^{1/2} && \text{(Gaussian)} \\ \Delta H_{1/2} &= \sqrt{3}\Delta H_{pp} && \text{(Lorentzian)} \end{aligned} \qquad (42)$$

We have been discussing what are called homogenous lines. Inhomogeneous lines are superpositions of many homogeneous ones and a common case is a gaussian distribution of lorentizian spin packets with $\Delta H^{L} << \Delta H^{G}$. This is called a gaussian convolution of lorentzian lines, and has interesting saturation properties because a low power level will saturate a Lorentzian spin packet, but much more power is needed to saturation the convolution.

ΔH^{L}

7. Relaxation Times

We already encountered the relaxation times T_1 and T_2 in the discussion of the Bloch equations. We know from Eq. (28) that T_2 is inversely proportional to the line width below saturation, with the proportionality constant $2/\gamma$ for the Lorentzian line shape that results from the solution of Bloch's equation at low power $\left(\gamma^2 H_1{}^2 T_1 T_2 << 1\right)$. In this section we will give the physical significance of T_1.

Consider photons hν incident on a two level system (S=1/2) with N_+ spins in the upper level with M=1/2 and N_- spins in the lower level with M= -1/2. The photons induce transitions between the lower to the upper levels, and if P↓ and P↑ are the probabilities of inducing downward and upward transitions respectively then the rate of change of the spin in the two states are given by

$$-\frac{dN_+}{dt} = \frac{dN}{dt} = N_+ P\downarrow - N_- P\uparrow \tag{43}$$

where

$$\begin{aligned} n &= N_+ - N_- \\ N &= N_+ + N_- \end{aligned} \tag{44}$$

For equal upward and downward transition probabilities.

$$P = P\downarrow = P\downarrow \tag{45}$$

we have

$$\frac{dn}{dt} = 2nP \tag{46}$$

which has the solution

$$n = n(o)e^{-2Pt} \tag{47}$$

where n(o) is the population difference when radiation starts. We see that when t → ∞ then n → 0, and this equalization of the populations is called saturation.

In writing there equations we did not take into account the possibility of relaxation. Consider a spin system which has a population difference n which differs from the equilibrium population difference n_0. Since there is no irradiated power we only take into account the probabilities of spontaneous transitions down (W↓) and up (W↑), and we obtain

$$\frac{dN}{dt} = N_+W\downarrow - N_-W\uparrow \tag{48}$$

which gives

$$\frac{dN}{dt} = (n - n_o)(W\uparrow + W\downarrow) \tag{49}$$

If we define

$$\frac{1}{T_1} = W\uparrow + W\downarrow \tag{50}$$

this expression becomes

$$\frac{dn}{dt} = \frac{n - n_o}{T_1} \tag{51}$$

which has the solution

$$n - n_o = \frac{n - n_o}{T_1} \tag{52}$$

This only took into account relaxation. Now we will take into account both incident radiation and relaxation.

$$\frac{dn}{dt} = \left.\frac{dn}{dt}\right)_{\text{lattice}} + \left.\frac{dn}{dt}\right)_{\text{radiation}} \tag{53}$$

$$\frac{dn}{dt} = \frac{n - n_o}{T_1} + 2np \tag{54}$$

At equilibrium

$$\frac{dn}{dt} = 0$$

and we have

$$n = n_o \frac{1}{1 + 2PT_1} \tag{55}$$

Notice that for low incident power the population remains at equilibrium

$$PT_1 << 1 \qquad n \approx n_o$$

and for high power levels

$$PT_1 >> 1 \qquad n \approx 0$$

saturation occurs.

The equilibrium population difference n_0 is determined by the Boltzmann factor. To show this we write for the energy difference

$$\Delta E = E_+ - E_- = h_\upsilon = g\beta H \tag{56}$$

We know from statistical mechanics that the populations are

$$\begin{aligned} N_+ &= N \exp\left(-E_+ / kT\right)/Z \\ N_- &= N \exp\left(-E_- / kT\right)/Z \end{aligned} \tag{57}$$

where the partition function Z is

$$Z = \exp(-E_+ / kT) + \exp(-E_- / kT) \tag{58}$$

This given for the equilibrium population difference

$$n_0 = [N / Z]\left(\exp(-E_+ / kT) - \exp(-E_- / kT)\right) \tag{59}$$

For room temperature we have

$$\begin{aligned} T &= 300k \\ \frac{g\beta H}{k} &\approx 0.5k \end{aligned}$$

so if we expand the exponential in a power series we obtain

$$\begin{aligned} n_0 &= Ng\beta H/kT \\ &= Nhv/kT \end{aligned} \tag{60}$$

The observed signal is proportional to the population difference n_0, and combining this with Eq (55) we obtain

$$\text{signal } \alpha \ \frac{Nh\nu/kT}{1+2PT_1} \approx \frac{Nh\nu}{kT} \tag{61}$$

for low power, $2PT_1 << 1$.

We can clarify the difference between T_1 and T_2 by the analogy with a gas. Consider a gas at a temperature T in a container where walls are at the room temperature T_R. If some of the gas is heated the rapid diffusion of the gas molecules will come the cause the gas to relax to a uniform temperature in a relative short time T_s, and it will take a longer time T_w for the gas to reach the same temperature of the container.

With this analogy in mind we can write

T_2 time for internal equilibrium

T_1 time for external equilibrium

Sometimes it is useful to distinguish between the two relaxation times T_2 and $T_{2'}$

$$\frac{1}{T_2} = \frac{1}{T_2'} + \frac{1}{2T_1} \tag{62}$$

where T'_2 is the spin-spin relaxation time.

8. Powder

We mentioned earlier that sometimes the spin Hamiltonian parameters depend on the direction, and this shifts the positions of the spectral lines of single crystals as a function of the orientation of the applied magnetic filed relative to the direction of the spin Hamiltonian principal axes.

In a powder sample the observed spectrum is the superposition of the randomly oriented microcrystallites. For an axially symmetric g-factor with the components $g_{||}$ and $g_{\perp}$, without any hyperfine term, we have

$$\begin{aligned} \mathcal{H} &= \beta \vec{H} \cdot \vec{g} \cdot \vec{S} \\ E &= \pm \frac{1}{2} \beta g H \\ \Delta E &= \beta g H \\ g^2 &= g_{\perp}^2 \sin^2\theta + g_{||}^2 \cos^2\theta \end{aligned} \tag{63}$$

For any orientation we have

$$H = \frac{\text{constant}}{g} \tag{64}$$

and in particular

$$\begin{aligned} H_{||} &= \frac{\text{constant}}{g_{||}} \qquad && \theta = 0 \\ H_{\perp} &= \frac{\text{constant}}{g_{\perp}} \qquad && \theta = \pi / 2 \end{aligned}$$

combining Eq. (63) and (64) gives

$$H = C/\left[g_\perp^2\left(1-\cos^2\theta\right)+g_\parallel^2\cos^2\theta\right]^{1/2}$$
$$= C/\left[g_\perp^2+\cos^2\theta\left(g_\parallel^2-g_\perp^2\right)\right]^{1/2} \qquad (65)$$

where C is a constant, and if we write

$$\delta^2 = g_\parallel^2 - g_\perp^2 \qquad (66)$$

this becomes

$$C = gH$$
$$C=\left(g_\perp^2 + \delta^2\cos^2\theta\right)^{1/2}H \qquad (67)$$

which gives for the cosine of the angle θ

$$\cos\theta=\frac{\left[(C/H)^2-g_\perp^2\right]}{\delta} \qquad (68)$$

We can show that the number of microcrystathiles dN oriented in the range of angles from θ to θ+dθ is given by

$$dN = N_0\sin\theta d\theta \qquad (69)$$

From Eq (68) we obtain

$$\left(\frac{(C/H)^2-g_\perp^2}{\delta^2}\right)^{-1/2}(C/H)\left(C/H^2\right)dH = \sin\theta d\theta \qquad (70)$$

9. Irradiated Systems, Bone, Alanine, Mobility, Membranes

One of the principal ways to produce free radicals in a material is to irradiate it. In a typical case the incoming particle will strike a molecule and ionize it

$$\begin{matrix}\text{Incoming}\\\text{Particle}\end{matrix} \quad + \quad \begin{matrix}\text{Molecule}\\\text{or}\\\text{Sensitizer}\end{matrix} \quad \rightarrow \quad [\text{Molecule}]^{+} + e^{-}$$

and the resulting electron will produce the damage in its surroundings.

$$e^{-} \quad + \quad \begin{matrix}\text{Another}\\\text{Molecule}\end{matrix} \quad \rightarrow \text{Radiation Damage}$$

The various particles that are used to create the damage may be classifies as follows

$$\begin{bmatrix}\text{Incoming}\\\text{Particle}\end{bmatrix} = \begin{cases}\text{Photon (hv)} & \begin{cases}\text{Visible}\\\text{U.V.}\\\text{x ray}\\\gamma\text{ ray}\end{cases}\\[2ex] \begin{matrix}\text{electrons}\\\text{protons}\\\alpha\text{ particles}\end{matrix}\end{cases}$$

The fact that the electron is primary reaction product is the reason why all radiations give almost same damage. There are generally intermediate steps in the process of producing the final radiation damage, as follows

$$\_\_\_\_\_ + P \quad \Rightarrow \quad P^{+} + e^{-} \qquad \text{Primary}$$

$h\nu + P$

greek nu as in here

which gives

$$\left(\frac{\delta^2}{(C/H)^2 - g_\perp^2}\right)^{1/2} \frac{C^2}{H^3} dH = \sin\theta d\theta \tag{71}$$

$$= dN$$

or

$$\frac{dN}{dH} = \frac{1}{H^2}\left(\frac{1}{H_\perp^2 - H^2}\right)^{1/2} \tag{72}$$

Notice that when $\theta \to 0$ $H \to H_\perp$ $\left(H_\perp^2 - H^2\right) \to 0$

$$\frac{dN}{dH} \to \infty$$

when $\theta \to \pi/2$ $H \to H_{\parallel}$ $(H_\perp - H) \to \neq 0$

$$\frac{dN}{dH} \to \text{finite}$$

and we conclude that the powder pattern line shape has an asymmetric form.

We see from eq 72 that the infinity in the lineshape arises from the presence of a constantly increasing density of resonant lines as the applied filed changes in the direction $H_{\parallel} \to H_\perp$.

10. Spin Labels

One of the principal ways to study active sites in biological macromolecules is to attach probes to the sites and measure the changes that occur in the probes during the course of biochemical conformational or other changes. We are all familiar with the use of radioactive probes. There is another widely used class of probe molecules called spin labels which are paramagnetic and hence are detected by an electron spin resonance spectrometer.

The most commonly used spin labels are nitroxides radicals, and examples of two are Tempo and Doxyl.

The g factor and hyperfine coupling constant of the nitrogen nuclear are completely anisotopic. In a low viscosity liquid such as water the triplet of narrow lines . is observed, due to the nitrogen nuclear spin I = 1 and the averaging out of the anisotropies. In the liquid state the a complex powder pattern is obtained. The signal at high viscosity is a pattern in between these two arising form the partial averaging of the anisotropic tensors, and the precise form of the spectrum depends critically on the extent of the motion of the spin label. When the label is attached to an active site where its motion is restricted, and if the extent of the hindrance of rotational, vibrational and conformational changes depends on the reorientation that are taking place in the local environment of the spin label.

called the slow.

$$1/???? = \frac{2 \cdot 10^8}{\left(\sqrt{\frac{h_{??}}{h_{(-1)}}} - 1\,\Delta H\right)} \text{ sec}^{-1}$$

$1/\tau_c(+1)$

h_0

$$\frac{1}{\tau_c(+1)} = \frac{2 \cdot 10^8}{\sqrt{\frac{h_0}{h(-1)} - \Delta H}} \text{ sec}^{-1}$$

$$\cancel{\mu_{(-1)}}\quad 1/\tau_{c(\underset{\ominus}{+}1)} = \frac{3.6\cdot 10^{9}}{\cancel{\{}\sqrt{\frac{h_0}{h_{(-1)}}} - \cancel{1}\,\Delta H_0\cancel{\}}}\ \mathrm{sec}^{-1}$$

$$\frac{1}{\tau_c(\pm 1)}\quad \cancel{\mu_{(+1)}} = \frac{1.2\cdot 10^{10}}{\cancel{\{}\sqrt{\frac{h_{(+1)}}{h_{(-1)}}} - \cancel{1}\,\Delta H_{(+1)}\cancel{\}}}\ \mathrm{sec}^{-1}$$

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - 0.5\left(A_{xx} + A_{yy}\right)}\cdot\frac{a_0'}{a_0}$$

where

$$a_0 = (A_{\parallel} + 2A_{\perp})/3$$

and

$$a_0' = \left(A_{xx} + A_{yy} + A_{zz}\right)/3$$

The result is

$$A_{\perp} = A_{\perp}' + 1.4\left\{1 - (A_{\parallel} - A_{\perp}')/\left[A_{zz} - 0.5\left(A_{xx} + A_{yy}\right)\right]\right\}$$

for the condition $S > 0.45$, and

$$A_{\perp} = A_{\perp}' + 0.8$$

for the case $S < 0.45$

The rotational correlation time is

$$\frac{1}{\tau_R^{calc}} = \frac{1}{\tau_0} + \frac{f(r)}{\tau_s} + \frac{1 - f(r)}{\tau_1}.$$