roxamate complexes are present in the acetylhydroxamate complexes at pH 4.5 (A) and 3.0 (B), the monohydroxamate and the hexaaquo complexes coexist at the lower pH (B). By comparison, the resolution enhanced spectrum of alumichrome at pH 1.9 (C) strongly suggests equilibration between these three species plus a significant displacement toward the hexaaquo ion, shown as a reference, without digital filtering, in Figure 6D.

This study represents a first instance of the use of ^{27}Al NMR spectroscopy to derive direct information on (a) electric field gradients at metal coordination centers in biomolecules and (b) metal-binding affinity differences among homologous structures with common metal-binding sites. Since for a given ligand field configuration the line breadth increases with the molecular weight, the use of ^{27}Al NMR as a quadrupolar probe for metalloproteins should prove to be informative mainly in cases of high symmetry. For small peptides, as studied here, the technique provides a quantitative basis to ascertain the extent of entasis at the active site.

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The Effect of Spin Saturation on Nuclear Overhauser Effects

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Abstract: In homonuclear magnetic resonance, measurement of nuclear Overhauser effects (both transient and steady state) is affected by indirect saturation of the observed resonance if the chemical shift between the spin being irradiated and the spin observed is not very large compared to the rf power. This problem is discussed theoretically and practical formulas are derived to correct experimental results for the effect of partial saturation. Transient effects (Torrey oscillations) are also discussed.

Introduction

The nuclear Overhauser effect (NOE) has become a powerful and commonly used tool for the determination of molecular conformations and configurations and for the assignment of nuclear magnetic resonance (NMR) spectra. Formulas used for the interpretation of such measurements in both steady-state[1] and transient[2] experiments generally ignore the indirect saturation of the observed resonance due to the nearby off-resonance rf field, in effect assuming an infinite chemical shift between the irradiated and the observed spins. This convenient set of circumstances does not always hold true. In this paper, the theory of these indirect saturation effects is discussed for a two-spin system and conclusions are drawn which will be of help in multispin systems.

Theory

We start with the set of coupled Bloch equations for the spins:

$$\mathrm{d}U_i/\mathrm{d}t + \sum_j R_{ij}U_j = -\Delta_i V_i \qquad (1)$$

$$\mathrm{d}V_i/\mathrm{d}t + \sum_j R_{ij}V_j = \Delta_i U_i + \omega_1 M_{zi} \qquad (2)$$

$$\mathrm{d}M_{zi}/\mathrm{d}t + \sum_j \Gamma_{ij}(M_{zj} - M_{0j}) = -\omega_1 V_i \qquad (3)$$

where $\omega_1 = \gamma H_1$, the rf field power, and $\Delta_i = \omega_i - \omega_0$, the difference between the resonance frequency of spin i and the Larmor frequency, $\omega_0 = \gamma H_0$. Formulas for the spin-lattice

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relaxation matrix Γ with diagonal elements ρ_i and off-diagonal elements σ_{ij} and the transverse relaxation elements $R_{ii} \equiv R_{2i} = 1/T_{2i}$ have been given elsewhere.[1,2] In this paper, we will neglect the effect of the transverse cross relaxation, R_{ij} with $i \neq j$; this neglect is justifiable when resonances do not overlap but should be used with care when the rf field strength (ω_1) is comparable to the chemical shift between the two resonances. Since, in the end, we will assume total saturation of the irradiated spin, this neglect can be justified for the specific cases to be discussed.

Equations 1-3 can be derived from the density matrix equations[3] under the following conditions: (a) cross correlation can be ignored (see ref 4 and discussion in ref 2); (b) all resonances of a given spin have the same frequency, i.e., no J coupling. Condition (b) is rarely if ever valid but it will be acceptable if for the irradiated spin ω_1 is greater than the spread of frequencies due to J coupling and for the observed spin the intensity is integrated over all lines of the resonance. The effect of small J couplings may perhaps be roughly accounted for by including them in R_2; i.e., $R_2 = \pi$ (frequency spread of the spin). The effect of J couplings on NOE measurements has been discussed elsewhere.[5]

If transverse cross relaxation is ignored, we can formally solve eq 1-3 by taking their Laplace transforms[6] with $M_z(0) = M_0$, the equilibrium value:

$$M_{zi}(s) = \frac{M_0(s + R_{1i})G_i(s)}{sQ_i(s)} - \frac{G_i(s)}{Q_i(s)} \sum_{j \neq i} \sigma_{ij} M_{zj}(s) \quad (4)$$

with the definitions

$$R_{1i} = \sum_j \Gamma_{ij} = \rho_i + \sum_{j \neq i} \sigma_{ij} \quad (5)$$

$$G_i(s) = [(s + R_{2i})^2 + \Delta_i^2] \quad (6)$$

$$Q_i(s) = (s + \rho_i)[(s + R_{2i})^2 + \Delta_i^2] + \omega_1^2(s + R_{2i}) \quad (7)$$

The cubic polynomial equation (7), as with all cubic polynomials, can be formally factored into a linear times a quadratic factor, $Q(s) = (b + s)[(a + s)^2 + \omega^2]$, which has roots $s = -b$, $-a + i\omega$, and $-a - i\omega$. In general, these roots must be found numerically. If eq 7 is expanded to give

$$Q(s) = s^3 + s^2(2R_2 + \rho) + s(\omega_e^2 + R_2^2 + 2R_2\rho) + \omega_e^2 R_e + R_2^2 \rho$$

defining

$$\omega_e^2 = \Delta^2 + \omega_1^2 \quad (8)$$

$$R_e = (\rho\Delta^2 + R_2\omega_1^2)/\omega_e^2 = \rho \cos^2\theta + R_2 \sin^2\theta \quad (9)$$

where θ is the angle of the effective field $= \tan^{-1}(\omega_1/\Delta)$, it can be seen that at large effective fields $\omega_e^2 \gg R_2^2$ or $R_2\rho$ or ρ^2, $-R_e$ is an approximate root of the polynomial. Then by synthetic division (dropping small terms) one can find the other roots $s = -R_a \pm i\omega_e$ with

$$R_a = R_2(1 + \cos^2\theta)/2 + \rho \sin^2\theta/2 \quad (10)$$

and

$$Q_i(s) \simeq (s + R_e)[(s + R_a)^2 + \omega_e^2] \quad (11)$$

Equations 8-11 clearly apply to any of the spins (i) so R_e, R_a, etc., can be defined for each spin with the appropriate parameters. Numerical solutions of eq 7 with typical parameters and $R_2 = 2\rho$, $\omega_e \sim 10R_2$ show these approximate roots accurate to about 0.1% or better. When $\omega_e = 3R_2$, accuracy is generally 1% or better. When R_2 becomes much greater than ρ, the errors increase somewhat (see Appendix).

This theory presumes (from eq 1 onward) that we are in the "nonviscous" liquid limit, $\omega_e^2\tau_c^2 \ll 1$.

Results

We now consider the case of two spins where one of the spins is being strongly irradiated on resonance. Previous work[2] has shown that, for the high-power limit, $\omega_1 \gg |\sigma|$, one can assume essentially instantaneous saturation of the irradiated spin, setting M_z (satd spin) = 0 in the equations for the observed spins. This means that eq 4 for the observed spin contains only the first term on the right-hand side with $R_1 = \sigma + \rho$. In the case that $\Delta \rightarrow \infty$, eq 4 gives the expected result:

$$(M_z - M_0)/M_0 = (\sigma/\rho)[1 - \exp(-\rho t)]$$

Using $M_z(t \rightarrow \infty) = \lim_{s\rightarrow 0} sM_z(s)$,[6] one obtains an important result for the steady state:

$$M_z(t \rightarrow \infty)/M_0 = \left[1 + \frac{\sigma}{\rho}\right]\left[\frac{R_2^2 + \Delta^2}{R_2^2 + \Delta^2 + \omega_1^2 R_2/\rho}\right] \quad (12)$$

Hence, the NOE in the presence of partial saturation is equal to the usual ($\Delta = \infty$) NOE multiplied by a saturation factor. The only important limitation to eq 12 is that the rf power is sufficient to totally saturate the irradiated spin, i.e., $\omega_1^2/R_{2i}\rho_i \gg 1$ for the irradiated spin. (The parameters in eq 12 refer, of course, to the observed spin; subscripts have been omitted for simplicity.) Equation 12 is exact only in the high-power limit (see Appendix).

Using the high-power approximation and always neglecting relaxation terms when added to ω_1 terms we get finally

$$M_z(t) = M_z(t \rightarrow \infty) + M_0 A \cos^2\theta[1 - R_1/R_e]\exp(-R_e t) + M_0 \sin^2\theta \exp(-R_a t)[B \cos \omega_e t + C \sin \omega_e t] \quad (13)$$

with $C \sim (R_a - R_e + R_2 + \sigma)/\omega_e$ and $A \sim B \sim 1$. These values for A, B, and C are valid when ω_e is large. More exact formulas are given in the Appendix. The oscillating terms in eq 13 are called Torrey[7] oscillations.

Since transient NOEs are most useful and necessary when the correlation time is long, subsequent discussion will focus on this case. For this case $\sigma \simeq -\rho$, the steady-state $M_z(\infty)$ is quite small, and the saturation effect (eq 10) is relatively unimportant. Also, when the correlation time is long $R_2 > \rho$ and the effect of replacing ρ with R_e as the decay constant for the nonoscillating term is to speed up the rate at which the magnetization approaches its steady-state value. Another important effect of the finite chemical shift is the presence of the Torrey oscillations.[7] Fortunately, a number of effects conspire to make this term decay rapidly. These are (a) $R_a > R_e$ when $R_2 > \rho$ and $\Delta > \omega_1$; (b) magnetic field inhomogeneities; (c) inhomogeneity in the rf field; (d) the presence of unresolved J couplings. The latter three effects cause rapid decay because of cancellation of the various cosine waves.

A numerical example is instructive. For the case of two protons at 360 MHz separated by 2 Å with correlation time $\tau_c = 1 \times 10^{-8}$ s and dipolar coupling only (see Table II, ref 2), we have $\sigma = -8.87$, $\rho = 8.98$, $R_2 = 22.34$. A really marginal case is when $\omega_1 = 2\pi(10\text{ Hz})$, barely enough to saturate, and $\Delta = 2\pi(30\text{ Hz})$, only slightly more than four times the line width. Derived parameters are $\omega_e = 199\text{ s}^{-1}$, $R_a = 21.7\text{ s}^{-1}$, and $R_e = 10.3\text{ s}^{-1}$.

$$M_z(t)/M_0 = 0.01 + 0.89e^{-10.3t} + e^{-21.7t}(0.10 \cos 199t + 0.01 \sin 199t)$$

It was previously[2] suggested that the half-life was a convenient measure of the rate of decay. At 57 ms $M_z/M_0 = 0.50 \pm 0.03$, the "uncertainty" being due to the oscillating term. A more appropriate "half-life" may be when M_z/M_0 falls to about $\cos^2\theta/2$ (= 0.45 in this case). At $t = \ln 2/R_e = 67$ ms, we have $M_z = 0.455 \pm 0.023$. This decay constant is more closely related to R_e and hence ρ.

Conclusion

The very important multispin case cannot be readily solved without resort to numerical methods. However, previous work[2] has shown that the decay in such cases is multiexponential, too complicated for any but qualitative conclusions. In that spirit, the present work makes several suggestions for the interpretation of NOE experiments when the effect of indirect saturation is likely to be important. For steady-state experiments, values should be corrected for saturation using eq 12. Rather than comparing half-lives in transient experiments, it would be better to determine "effective half-lives", the time when the intensity falls to $\cos^2\theta/2$. Even then, one can expect the half-lives of nearby resonances to be shorter than expected from geometry because of a mixing of "T_2 type" processes, as in eq 9. Also, one should watch for, and if necessary correct for, terms oscillating at ω_e.

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Appendix

While the approximate roots of the cubic equation (7) given earlier are remarkably accurate, more accurate formulas may be needed, especially when $R_e > \rho$. The next order of approximation is given below. Defining $r = (R_2 - \rho)/2$ and $E = 8r^3(\cos^2\theta)^2\sin^2\theta/\omega_e^2$, the more accurate roots are $-R_e'$, $-R_a' \pm i\omega_e'$ with

$$R_e' = R_e - E,\ R_a' = R_a + E/2$$

and

$$\omega_e' = \omega_e[1 - x^2\sin^2\theta(1 + 3\cos^2\theta)]^{1/2}$$

$x = r/\omega_e$ is the parameter of smallness. In all cases tested, the residual error is an order of magnitude less than the correction.

The constants A, B, and C in eq 13 are given to accuracy $\sim 16x^4$ by

$$\begin{aligned}
A &= 1 + 12x^2\cos^2\theta\sin^2\theta \\
B &= (1 - Z)B_0 - WC_0 \\
C &= (1 - Z)C_0 + WB_0 \\
B_0 &= 1 - 12x^2\cos^4\theta \\
C_0 &= x(3\cos^2\theta + 1) - 30(x\cos^2\theta)^3 \\
Z &= R_aR_{\rm l}/(R_a^2 + \omega_e^2) \\
W &= R_1\omega_e/(R_a^2 + \omega_e^2) \\
R_{\rm l} &= \sigma + \rho
\end{aligned}$$

Since ω_1 must be large to saturate the irradiated spin and since we have already assumed that Δ of the observed spin is large enough to prevent significant overlap of these resonances, it is unlikely that these corrections will be needed in any case of practical utility. However, the cautious investigator will use them to ensure that the corrections are indeed small.

Equation 12 of this paper is not exact because the high-power limit neglects the "feedback" of magnetization from the observed spin to the irradiated spin. The exact result is obtained if the power factor (the third term of the denominator of eq 12) is divided by

$$1 - (\sigma^2R_2/(\sigma\omega_1^2 + R_2\rho^2))$$

a factor which will be 1 when $\omega_1 \gg \sigma$.

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Aqueous Lanthanide Shift Reagents. 8. Chiral Interactions and Stereochemical Assignments of Chemically and Isotopically Chiral Ligands[1]

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Abstract: The phenomenon of NMR spectral resolution of enantiomeric nuclei of α-hydroxycarboxylates by paramagnetic lanthanide ions in aqueous solution is described in detail and its modes of application are demonstrated. Phenomenological equations are presented describing the spectral resolution of enantiotopic groups, the "self"-resolution of nonracemic mixtures of enantiomers by the mere addition of lanthanide ions, and the resolution of racemic mixtures in the presence of another chiral ligand. Spectral resolution occurs in mixed-ligand complexes of 3:1 ligand/metal stoichiometry but is absent in the 2:1 complexes, indicating that steric crowding around the central lanthanide ion forcing the ligands into stereochemically persistent positions is one of the important structural features of the tris chelates in aqueous solution. Approaches to the configurational assignment of chemically and of isotopically chiral ligands are described. Using these approaches the configurations of (+)-citramalate and of (−)-citramalate are assigned as L and D, respectively, and that of glycolate-*d* made by the action of lactate dehydrogenase on glyoxylate-*d* as L. These assignments are in agreement with determinations by other methods.

Introduction

Enantiomeric nuclei, i.e., nuclei that are interchangeable via reflection in a plane of symmetry, are isochronous in NMR spectra. When enantiomeric molecules, or molecules containing enantiotopic groups, are placed in chiral environments, the enantiomeric nuclei become diastereotopic and in principle anisochronous. The phenomena whereby the enantiotopic carboxyls of citric acid or the methylene hydrogens of ethanol