Theoretical nuclear magnetic resonance–lineshape study of a two-site chemical exchange model of sodium ions ($I = {}^3/_2$) in an intracellular environment

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ABSTRACT We present a theoretical calculation of the lineshape function based on the solution of the semiclassical Liouville equation, of a two-site chemical exchange model of biological relevance. The bound site is allowed to be in the slow region regime that is the inverse quadrupole interaction of one bound site is in the same range as the reorientational correlation time. We compare different chemical exchange models, and several different physical situations are investigated. The variation of the width at half height (WHH) and the relative intensity (I/I_0) is shown to be important, experimentally accessible quantities that are useful in order to discriminate between different model systems.

1. INTRODUCTION

Counterion NMR is a well-established and powerful technique for studying ion binding properties on a molecular level in complex solution systems, ranging from surfactant micelles and lyotropic liquid crystals to solutions of macrobiomolecules and living cells. A common situation in these experiments is that there are more free ions (i.e., ions with the undisturbed first hydration shell and thus with a fluctuating cubic symmetry) than ions residing in different possible binding sites. Ions in heterogeneous regions of the solution, i.e., bound ions, may not be possible to observe directly, because they are a small fraction of the total population or because their linewidth is very broad. However, these ions may considerably affect the observed lineshape due to chemical exchange. The problem is thus to extract information about the bound sites indirectly from the observed lineshape. This paper is concerned with a discrete two-site exchange model (DEM) for quadrupole nuclei with quantum number $I = 3/2$ and with the problem of characterizing the bound site from experimentally accessible quantities. We have theoretically studied the influence of a slow motion site (P_A) on the observed lineshapes. The analysis comprises a study of the calculated lineshape by (*a*) naked eye, (*b*) measurement of the variation of the linewidth at half height, and (*c*) measurement of the relative intensity (I/I_0) as a function of chemical exchange rate ($1/\tau_{ex}$).

In a paper by Westlund and Wennerström (1989) the two-site DEM lineshape of quandrupole nuclei with quantum number $I > 1$ was given for two isotropic sites. In another paper (Westlund and Wennerström [1989]) we extended the analysis to a two-site DEM model with a bound anisotropic site exchanging with an isotropic site. In the publication by Berggren and Westlund (1989), we studied a three-site model with two bound sites, one isotropic and the other one anisotropic, involved in chemical exchange with a free site. The possibilities of characterizing the bound site solely from the variation of the lineshape with chemical exchange were small, because the spectral data from these papers are dominated by a narrow, central Lorenzian-like lineshape. The variation of WHH (width at half height) with chemical exchange rate may exhibit one maximum, two maxima, or none over the range of slow to fast chemical exchange. The addition of the observable I/I_0 may be helpful in order to differentiate between possible two- or three-site models. However, it is always possible that the resulting physical picture may not be unique.

In this paper the discrete chemical exchange model (DEM) is applied to the study of quadrupolar spins exchanging between two sites: one site characterized by slow isotropic tumbling, i.e., the reorientational correlation time τ_R of the site is comparable to the inverse of the quadrupole interaction strength, that is $\sqrt{\langle (H_Q(t)^2\rangle}\ \tau_R \geq 1$, whereas the other site is described by relatively rapid isotropic rotational motion. For brevity of discussion, these sites will be referred to as the bound and the free states, respectively. Furthermore, the model allows for fast chemical exchange conditions, i.e. τ_{ex} comparable or smaller than τ_R. In the conventional DEM lineshape theory (Westlund and Wennerström [1982, 1989], Berggren and Westlund [1989]) it is assumed that the chemical exchange rate is slow compared with the molecular motions responsible for the spin relaxation of the spin residing in the site. The chemical exchange process was introduced through a master equation coupling the Redfield equation of motion of the

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exchanging sites. Thus, the master equation includes well-defined relaxation rates of each site. However, for the case of one site in the slow-motion region, the reorientational correlation time may be longer than the mean life time of the spin. Thus, both modulation due to molecular reorientation and chemical exchange must be introduced in the exchange model. In a recent paper by Linse and Halle (1989), the electrical field gradient (EFG) of Na^+ ions has been calculated in a heterogeneous system. The hydration shell of the ion is disturbed in the perturbed region and when the ion leaves the heterogeneous part of the solution the direction of the principal frame changes, but also the magnitude of the electric field gradient changes, introducing a modulation of the quadrupolar interaction. Introducing the chemical exchange process on the level of the stochastic Liouville equation of motion the restriction on the exchange process being slow is lifted. When the chemical exchange process is introduced in the stochastic Liouville equation one can distinguish between two different models. First following the model first analyzed by Eliav et al. (1988) the direction of the principal frame of the EFG of the ion is not changed upon changing site, but because the quadrupolar interaction is different in the two sites there is an exchange modulated contribution to the linewidth. The other model, suggested by Marshall (1970) and later also by Vasavada and Kaplan (1985), the direction of the principle frame of the EFG is randomized when the spin changes site. The effective modulation of the quadrupolar interaction of the sites is due to the reorientation and the chemical exchange.

We use the DEM theory in this study, although the CDM lineshape theory developed by Halle and Westlund (1988) might be a more realistic description of the system than the DEM. However, we believe that the DEM theory is simpler to use in the slowmotion regime and sufficiently accurate for the purposes of this paper, though the discrepancy between the models appears when there is a shift between the two sites, i.e., $\Delta\omega \neq 0$, and this is not the case in this study.

The outline of this paper is as follows. In section 2, the stochastic Liouville formalism is reviewed. The lineshape function is obtained by solving the stochastic Liouville equation in the frequency domain. Then the lineshape is obtained by an inversion of the full Liouville matrix. In section 3 the chemical exchange is included into the master equation according to the two different models discussed above. The important experimentally accessible quantities are the variation of the linewidth at half height (WHH) and the relative intensity I/I_0. The variation of these two quantities are shown and discussed in section 4, and in section 5 we sum up the conclusions of this work.

2. THEORY

This section will review the lineshape theory based on solving the stochastic Liouville equation of motion in the frequency domain Freed et al. (1971), Muus and Atkins (1972), Benetis et al. (1983), and Lynden-Bell (1971). We start with the density matric $|\rho)$, which is a function both of spin operators and of the reorientation of the spin system, which in our case is the quadrupole ion–macromolecule complex. The equation of motion then reads

$$\frac{d}{dt}|\rho) = -iL|\rho),$$

where the Liouville operator is generated by the Hamiltonians defining the system. The Zeeman interaction is given by H_{Zeeman}

$$H_{Zeeman} = -\gamma B_0 \hat{I}_z,$$

where γ is the gyromagnetic ratio and B_0 the static magnetic field. The nuclear spin system is not considered as a pseudo-isolated system but as strongly coupled to the reorientational degrees of freedom through the quadrupole interaction given by the Hamiltonian H_Q^α of site α.

$$H_Q^\alpha[\Omega_{LM}(t)] = \beta \sum_n (-1)^n A_n^2 V_0^{(P)\alpha} \{D_{0,-n}^2[\Omega_{LM}(t)$$
$$+ \eta \cos 2\alpha \, d_{\pm 2,-n}^2(\Omega_{LM}(t))]\}$$

with $\beta = eQ/\{I(2I-1)h\}$ and $\Omega_{LM} = (\alpha, \beta, \gamma)$, the Euler angle relating the principal coordinate system (M) of the field gradient tensor to the fixed laboratory frame (L). A_n^2 is the nth component of the irreducible spherical spin tensor operator and $V_0^{(P)}$ is the zero tensor component in the principal coordinate system of the field gradient tensor.

In the semiclassical approximation the probability $P(\Omega, t)$ that an ion-macromolecule complex has undergone a total change in orientation of Ω_{LM} in a time t is independent of the original orientation and obeys a Markov diffusion operator $\Gamma = D\nabla_\Omega^2$, representing isotropic reorientation.

$$\frac{\partial}{\partial t} P(\Omega_{LM}, t) = \Gamma_\Omega P(\Omega, t).$$

The Liouville system operator is then given by

$$L = L_{Zeeman}^0 + L_Q + i\Gamma_\Omega.$$

Because the lineshape experiments are carried out in the frequency domain it is convenient to consider the Laplace transform

$$|\rho(\omega, \Omega)) = \int_0^\infty \exp\,[i\omega t]\,|\rho)\,\mathrm{d}t,$$

which gives

$$|\rho(\omega, \Omega)) = [iL - i\omega \mathrm{E}]^{-1}|\rho_{\mathrm{eq}}(0)),$$

assuming the initial condition at $t = 0$ to be a system at equilibrium. With the notation of the two last equations, a matrix element in the Liouville space is written $(A\,|L|\,B) = \mathrm{tr}\,\{A^{\dagger}[HB - BH]\}$, where $(A\,| = A^{\dagger}$.

To solve the Laplace transformed equation we introduce a complete set of basis operators which span the Liouville space. For the nuclear subsystem the basis vectors $|\Sigma, \sigma)$ are chosen so that they transform as irreducible spherical tensor operators

$$|\Sigma, \sigma) = \sum_m (-1)^{I-m-\sigma}(2\Sigma + 1)^{1/2} \cdot \begin{pmatrix} I & I & \Sigma \\ m+\sigma & -m & -\sigma \end{pmatrix} |I, m + \sigma\rangle\langle I, m|,$$

where $|I, m\rangle$ is a Hilbert space eigenvector of I^2 and I_Z and $\left(\begin{smallmatrix} I & I & \Sigma \\ m+\sigma & -m & -\sigma \end{smallmatrix}\right)$ is a $3 - j$ symbol as defined by Brink and Slatcher (1961).

For the molecular reorientational space a convenient orthonormal basis is formed by the functions $\theta^L_{K,M} = |L, K, M)$, which are closely related to the symmetric top eigenfunctions

$$|L, K, M\rangle = [(2L + 1)/8\pi^2]^{1/2} D^L_{K,M}(\Omega).$$

The basis vectors are now formulated as the direct product of the bases $\{|\Sigma, \sigma) \times |L, K, M)\}$. In this representation the lineshape of one site is given by the frequency dependence of one element in the superoperator matrix $\mathbf{M}^{-1}$ of the Laplace transformed equation of the system, where the matrix $\mathbf{M}$ is defined by

$$\mathbf{M} = i[L_{\mathrm{Zeeman}} - \omega \mathbf{E} + L_{\mathrm{Q}} + i\Gamma_{\Omega}],$$

with $\mathbf{E}$ representing a unit matrix. The matrix elements of the superoperator matrix $\mathbf{M}$ are given in greater detail in the Appendix.

3. CHEMICAL EXCHANGE

Chemical exchange between two discrete sites may be introduced into the lineshape description with a generalized Bloch equation following Kaplan (1958*a* and *b*). This approach was introduced for a Redfield equation of motion and consequently the chemical exchange process did not appear in the intrinsic Redfield relaxation expressions of the sites. The validity of this approach is determined by the condition $\tau_{\mathrm{ex}} \gg \tau_{\mathrm{R}}$. If the chemical exchange process is introduced on the level of the stochastic Liouville equation describing two sites the master equation then reads:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} |\rho^{\mathrm{A}}(\mathrm{t}, \Omega)) \\ |\rho^{\mathrm{B}}(\mathrm{t}, \Omega)) \end{pmatrix} = \begin{pmatrix} -iL^{\mathrm{A}} - \tau_{\mathrm{A}}^{-1}\mathbf{E} & \tau_{\mathrm{B}}^{-1}\mathbf{E} \\ \tau_{\mathrm{A}}^{-1}\mathbf{E} & -iL^{\mathrm{B}} - \tau_{\mathrm{B}}^{-1}\mathbf{E} \end{pmatrix}\begin{pmatrix} |\rho^{\mathrm{A}}(\mathrm{t}, \Omega)) \\ |\rho^{\mathrm{B}}(\mathrm{t}, \Omega)) \end{pmatrix}.$$

Here $\mathbf{E}$ represents a diagonal matrix and L^{α} represents the Liouville supermatrix of site α. τ_{i} is the mean life time of site i, i = A or B, i.e., $(\tau_{\mathrm{i}})^{-1} = (P_{\mathrm{i}}\,\tau_{\mathrm{ex}})^{-1}$, where τ_{ex} is the time of chemical exchange. In this equation the chemical exchange is assumed to be instantaneous so each orientational-spin state of site A exchange with corresponding state of site B. However, as the spin has changed site the spin and reorientational motion are differently governed by the Liouville superoperator of that site. This approach was recently investigated by Eliav et al. (1988) for a two-site DEM model comprising a binding site in the slowmotion region and one site representing a Redfield site. The slow motion site is characterized by a long reorientational correlation time $\tau_{\mathrm{R}} \simeq 10^{-6}$ s which may be comparable with the inverse of the expected quadrupole interaction strength. The reorientational motion of the slow motion site is then comparable or longer than the mean life time of the site when the interaction strength is different in the two sites we obtain an exchange modulating in the expression of the linewidth. The linewidth is determined by an averaged interaction and an averaged reorientational dynamics. We here refer to this chemical exchange model as model I.

In the papers by Marshall (1970) and Vasavada and Kaplan (1985), they suggest a master equation where the reorientational motion is totally randomized when a spin moves between the sites. The Liouville equation of motion describing each site separately is unchanged but the exchange process is modified according to this equation.

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} |\rho^{\mathrm{A}}(\mathrm{t}, \Omega)) \\ |\rho^{\mathrm{B}}(\mathrm{t}, \Omega)) \end{pmatrix} = \begin{pmatrix} -iL^{\mathrm{A}} - \tau_{\mathrm{A}}^{-1}\mathbf{E} & \tau_{\mathrm{A}}^{-1}\mathbf{F} \\ \tau_{\mathrm{B}}^{-1}\mathbf{F} & -iL^{\mathrm{B}} - \tau_{\mathrm{B}}^{-1}\mathbf{E} \end{pmatrix}\begin{pmatrix} |\rho^{\mathrm{A}}(\mathrm{t}, \Omega)) \\ |\rho^{\mathrm{B}}(\mathrm{t}, \Omega)) \end{pmatrix}.$$

The diagonal matrix $\mathbf{F}$ only has one nonzero element. In the operator representation of this paper $(|LKM)\Sigma\sigma))$ only $|000)11)_{\mathrm{A}}$ is exchanging with $|000)11)_{\mathrm{B}}$. The condition of fast chemical exchange is taken into account as for the other model but the intrinsic effective correlation time is in the Redfield limit given by

$$\frac{1}{\tau_{\mathrm{i}}^{\mathrm{eff}}} = \frac{1}{\tau_{\mathrm{R}}^{\mathrm{i}}} + \frac{1}{\tau_{\mathrm{i}}} \text{ where i refers to the site i.}$$

This chemical exchange model we here refer to as model II. Thus, the effect of the chemical exchange is quite different for the two models and the use of the models must be determined by the system under study.

We have investigated both exchange approaches presented as models I and II. We thus construct the extended Liouville matrix and calculate the inverse of the two elements needed using a modified Lanczos algorithm developed from the paper by Schneider and Freed (1989). In the direct product operator representation the DEM lineshape is given by the frequency dependence of two operators, namely $|000)11)_A$ and $|000)11)_B$.

4. RESULTS AND DISCUSSION

Lineshapes of two-site chemical exchange, $P_A \leftrightarrow P_B$

The equations of models I and II define two different two-site chemical exchange models. The lineshape depends on a set of parameters which are the resonance frequencies of the two sites ω_0^A and ω_0^B, the reorientational correlation times τ_R^A and τ_R^B, the lifetimes of the sites $P_A\,\tau_{ex}$ and $P_B\,\tau_{ex}$, and the quadrupolar interactions $\chi^A = \beta V_0^{(P)A}$ and $\chi^B = \beta V_0^{(P)B}$.

The bound site, which we also denote the 'slow motion' site, is characterized by the condition $\tau_R^B\chi^B > 1$. Within the relative large parameter space, we focus in the present context on effects due to the exchange rate $1/\tau_{ex}$. The bound site is in the slow motion region, and the other site is characterized as a free hydrated site. We consider both a low-field and a high-field case determined by $\omega_0^B\tau_R^B < 1$ and $\omega_0^B\tau_R^B > 1$, respectively. For simplicity we have neglected the shift difference, i.e., we set $\omega_0^A = \omega_0^B$.

We start with a presentation of the observable lineshapes and the question we address is the following: Is it

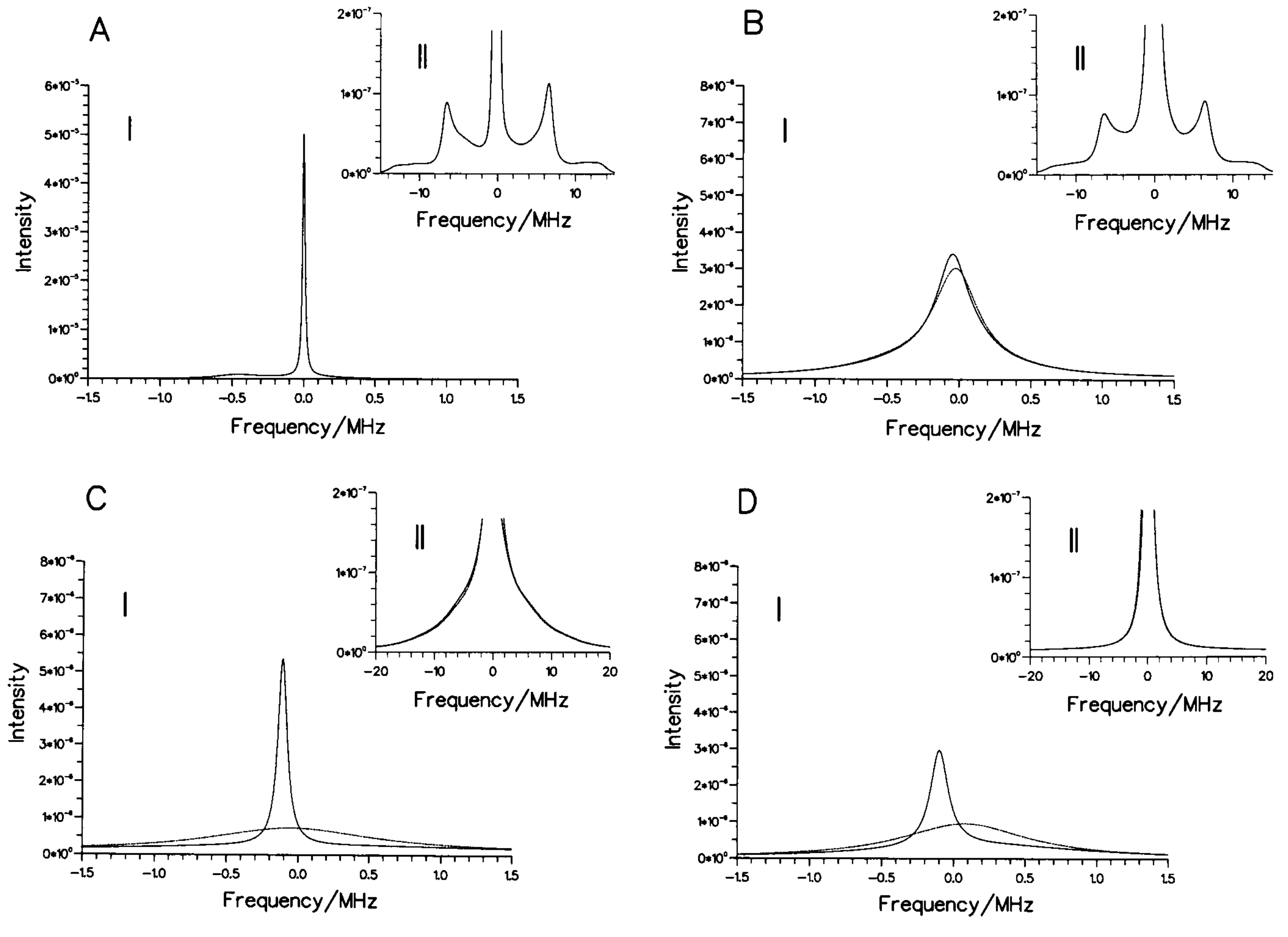

FIGURE 1 Illustrations of lineshapes for model I (*solid line*), model II (*dashed line*). Chemical exchange times (τ_{ex}) are (*A*) $2 \cdot 10^{-4}$ s; (*B*) $8 \cdot 10^{-6}$ s; (*C*) $8 \cdot 10^{-7}$ s; and (*D*) $8 \cdot 10^{-8}$ s. Parameters for *A*–*D* are given in Table 2. There are two frequency resolutions for each time of exhange, giving the central signal in detail (I) and the full power spectrum (II), including the high- and low-frequency splittings of the lineshape. In *A* the two lineshapes are identical as accepted, though $\tau_R^A \leq \tau_{ex}$. All lineshapes are calculated for the populations $P_A = P_B = 0.5$.

possible by mere inspection of the lineshape to conclude whether the bound site is a slow motion site? Fig. 1 presents a number of lineshapes ranging from slow to fast exchange condition where model I is represented by a solid line and model II by a dashed line. The lineshapes denoted I refer to the observable central signals and cover a narrow frequency range compared to the lineshapes denoted II. The latter lineshapes reveal a fine structure, which is, in most cases, impossible to observe under normal experimental condition because it is hidden in the baseline noise.

Now, consider the observable model II, type I, lineshapes of Fig. 1, *A*–*D*, which may be interpreted in terms of two different sites, with one broad and one narrow signal, respectively, involved in chemical exchange. Consequently, we observe a broadening and a coalescence. Such a model yields one maximum in the plot of WHH as a function of chemical exchange. But in Eliav et al. (1988) the authors reported two observed maxima. It is, however, possible to obtain the reported variation of WHH with a three-site model, which was presented by Berggren and Westlund (1989), where all the sites are within the Redfield condition. In Fig. 2 the results of such a three-site model, which are given in Berggren and Westlund (1989), are illustrated. The free site has a population fraction $P_F = 0.918$, and two bound isotropic sites are designed. The lineshape of the first bound site, $P_{B1} = 0.08$, is shifted and broad, relative the F site, while the lineshape of the second one, $P_{B2} = 0.002$, is not shifted but broader than the B1 site. The variation of WHH with chemical exchange shows two maxima. The first maximum in the WHH plot (*a*) is the broadening of the lineshape due to the contribution from the B1 site. The quadrupole interaction, χ^{B1}, is in the same range as the chemical exchange rate at the first maximum. The second maximum appears at a chemical exchange rate similar to the quadrupole interaction of B2 site. The change of the relative intensity over the range of chemical exchange is also presented in Fig. 2. The relative intensity makes it possible to separate the three-site model from the two-site model with the bound site in the slow motion regime.

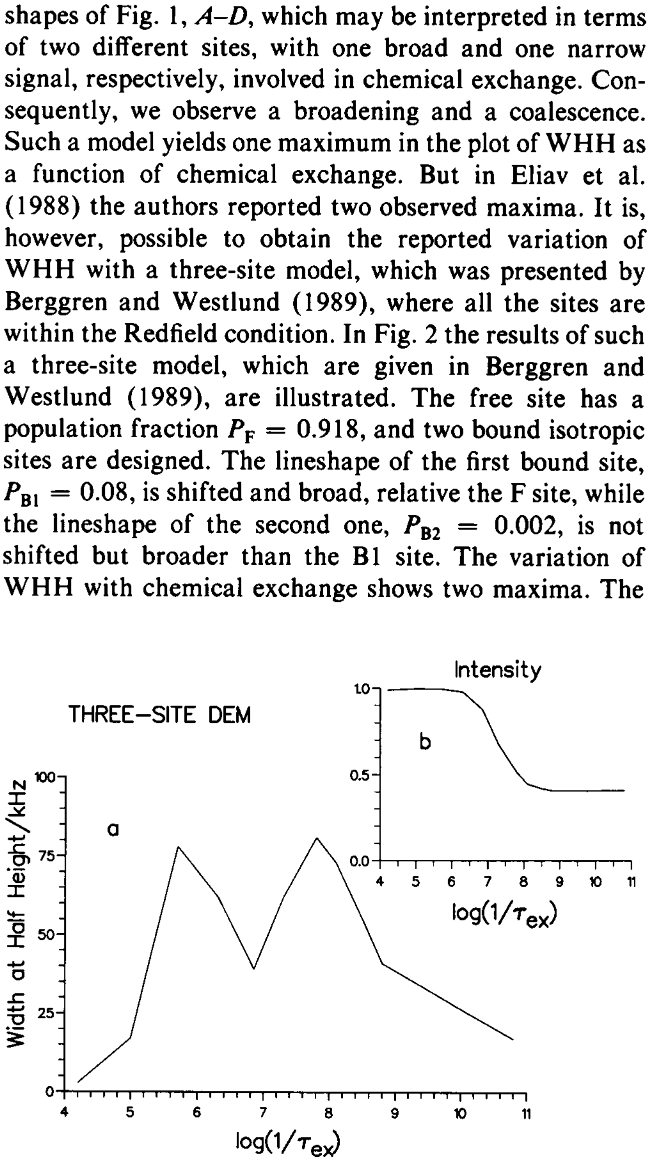

FIGURE 2 Plots of the width at half height (*a*) and the relative intensity (*b*) as functions of log $(1/\tau_{ex})$ for the three-site situation, where all sites are in the Redfield region. The parameters of these calculations are presented in Table 1.

In Table 1 we summarize the parameters of the three-site model. We may conclude that the observation of two maxima in the WHH dependence as a function of chemical exchange rate is not a sufficient condition for concluding the presence of a slow-motion site.

Dependence of the linewidth at half height, WHH, and relative intensity (I/I_o) with chemical exchange rate

The variation of WHH with the chemical exchange rate (temperature) is an accessible and important experimental quantity. A variety of effects may be expected according to theoretical studies of two-site models. It was shown by Westlund and Wennerström (1982) that the dependence on temperature could range from almost no variation to the appearance of one maximum over the range of fast to slow chemical exchange rates.

Eliav et al. (1988) reported in their lineshape study of $I = 3/2$ nuclei that the WHH plot exhibited two maxima. These two maxima are due to the averaging of slow-motion splittings, when the exchange rate becomes comparable with the slow motion splitting of the $|3/2, 1/2\rangle \leftrightarrow |3/2, -1/2\rangle$ transition and the $|3/2, \pm 3/2\rangle \leftrightarrow |3/2, \pm 1/2\rangle$ transitions. In Fig. 3 the results of Eliav et al. (1988) are presented, here refered to as model I. The parameters used in Fig. 3 are given in Table 2. It is also possible to observe that in the extreme narrowing regime only the chemical exchange rate dominates the appearance of the lineshape.

TABLE 1 **Parameters of sites B1, B2, and F**

Site B1 (Bound site 1)	Site B2 (Bound site 2)	Site F (Free site)
$\chi^{B1} = 10^6$ rad/s	$\chi^{B2} = 8\ 10^8$ rad/s	$\chi^F = 0.3\ 10^6$ rad/s
$P_{B1} = 0.08$	$P_{B2} = 0.002$	$P_F = 0.918$
$\tau_o^B = 1.5\ 10^{-13}$ s	$\tau^{B2} = \tau_o^{B1}$	$\tau_o^F = \tau_o^{B1}/100$
$\omega_{B1} = \omega_0 - 0.4\ 10^6$ Hz	$\omega_{B2} = \omega_0$	$\omega_0 = 7.1\ 10^7$ Hz

$$\tau^\alpha = \tau_o^\alpha \times \exp(3000/T)$$
$$\lambda = 2.08 \times T \times 1.6\ 10^{12} \times \exp(-6000/T)$$

The three-site DEM is constructed as by Berggren and Westlund (1989).

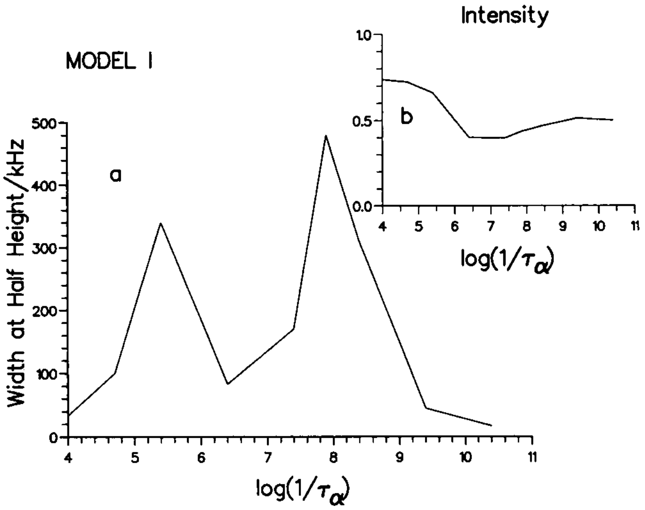

FIGURE 3 The width at half height (*a*) and the relative intensity (*b*) of model I as functions of log $(1/\tau_\alpha)$, $\alpha = A$ or B. Some of these lineshapes were outlined in Fig. 1 (*solid line*), and the parameters used are given in Table 2. The populations are $P_A = P_B = 0.5$. The relative intensities are calculated for the same frequency interval in all cases (−1.4e6 Hz − 1.4e6 Hz).

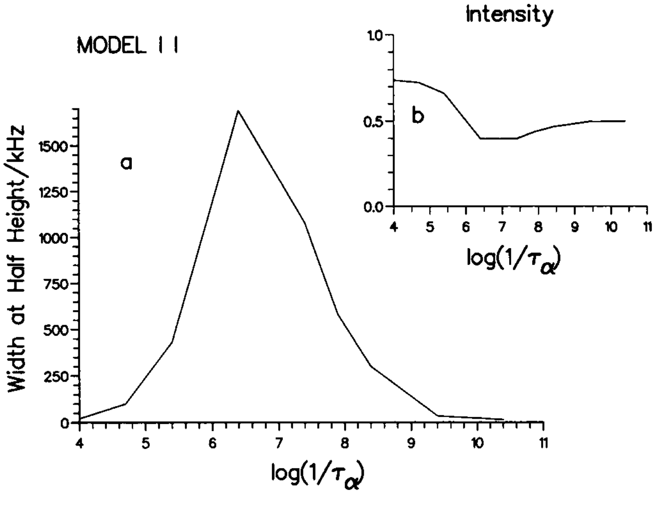

FIGURE 4 Plots of the width at half height (*a*) and the relative intensity (*b*) of model II as a function of log $(1/\tau_\alpha)$. Some of these lineshapes were outlined in Fig. 1 (*dashed line*). The parameters are the same as in Table 2. The populations are $P_A = P_B = 0.5$, and intensity is integrated over the $-1.4 \cdot 10^6 - 1.4\ 10^6$ Hz interval.

In Fig. 3 *b* the variation of I/I_0 with chemical exchange rate is shown. The I/I_0 value in the slow exchange limit is sensitive to P_A and P_B, because I/I_0 has a low limit of $P_B + 0.4\ P_A$. This value is due to the superposition of the free site ($P_B = 0.5$) and the central signal of the bound site ($P_A = 0.5 \times 0.4 = 0.2$). The fast exchange limit value is ≥ 0.4, which is due to an averaged anisotropic signal, where the central signal contributes with $I/I_0 = 0.4$.

Using the same parameters as in Fig. 3, we calculated WHH and relative intensity also for model II with one site in the slow motion regime, which is presented in Fig. 4. Only one maximum is observed in the plot of WHH with rising exchange rate for the plot in (*a*) and is obviously an effect of the totally randomized orientations between exchanging sites.

Comparing with Fig. 3 *a* we loose the characteristics of two peaks. In Fig. 4 *b* the I/I_0 for the same case as in Fig. 4 *a* is presented, and the same limits of the relative intensity as observed in Fig. 3 *b* is valid also here.

The low-field and high-field data are expected to show different WHH variation, as is indeed observed and presented in Fig. 5 for model II. For the case of high-field this is due to the nonextreme narrowing condition,

TABLE 2 Parameters of sites A and B

Site A	Site B
$\chi^A = 6.57\ 10^6$ rad s^{-1}	$\chi^B = 1.65\ 10^5$ rad s^{-1}
$\tau_R^A = 4\ 10^{-6}$ s	$\tau_R^B = 1\ 10^{-10}$ s
$\omega_0^A = 9.45\ 10^7$ Hz	$\omega_0^B = \omega_0^A$
$P_A = 0.5$	$P_B = 0.5$
$\tau_A = P_A\tau_{ex}$	$\tau_B = P_B\tau_{ex}$
$B_0 = 1.334T$	

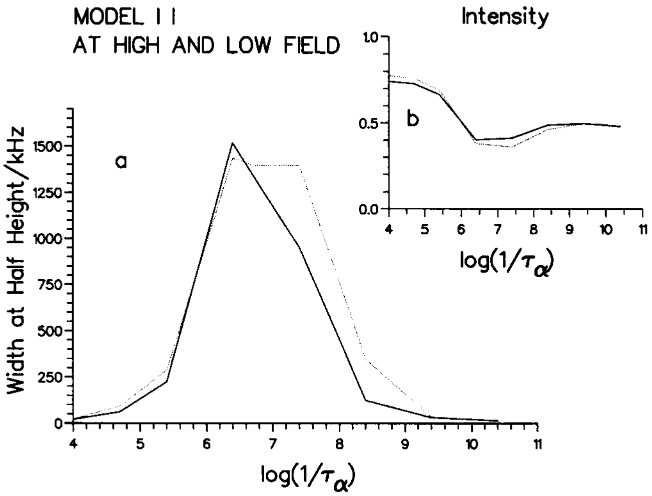

FIGURE 5 Plots of the width at half height (*a*) and the relative intensity (*b*) as functions of log $(1/\tau_\alpha)$, $\alpha = A$ or B. Model II at high field ($B = 10\ T$), i.e., in the Redfield regime, but out of extreme narrowing, is illustrated by a solid line. Model II at low-field ($B = 0.002\ T$), i.e., out of the Redfield regime, but in extreme narrowing is illustrated by a dashed line. The parameters not mentioned here are the same as in Table 2.

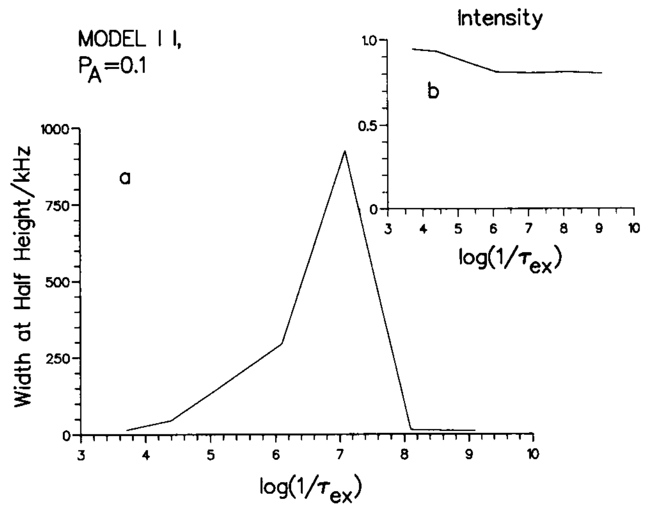

FIGURE 6 The width at half height (*a*) and the relative intensity (*b*) of model II as functions of log $(1/\tau_{ex})$. The populations are $P_A = 0.1$, $P_B = 0.9$, and the other parameters are the same as those used in Table 2. The intensity is integrated over the $-1.4 \cdot 10^6 - 1.4\ 10^6$ Hz interval.

$\omega_0\tau_{A,\mathrm{eff}} > 1$, valid for the bound site throughout the whole range of chemical exchange rates. In the low-field case extreme narrowing conditions, $\omega_0\tau_{A,\mathrm{eff}} < 1$, is valid for all the range of chemical exchange rates, and this results in one broader maximum of WHH in (*a*).

In Fig. 5 *b* the relative intensity I/I_0 is shown for the high- and low-field cases. The higher limit of slow exchange and the lower limit of fast exchange have the values of 0.7 and 0.4, respectively, as in Fig. 3 *b*.

As mentioned, it is more relevant to suppose the free hydrated site to have a higher population fraction than the bound site for a real physical situation. We present such a case for model II. Qualitatively the same behavior as described in Fig. 4 *a* is observed for small fractions of ions in the bound site, for instance, $P_A = 0.1$ and $P_B = 0.9$, presented in Fig. 6. This is also valid for model I, where two peaks are visible even at small fractions in site A. The higher limit of the relative intensity I/I_0 (Fig. 4 *b*) is here shown to have the value $0.9 + 0.1 \times 0.4 = 0.94$ (i.e., $P_B + P_A \times 0.4$). However, the lower limit has a slightly higher value than expected which is due to the chosen integration interval.

5. CONCLUSIONS

In this work we have studied the lineshapes and the relative intensities obtained from two different chemical exchange models. Both models are valid in the fast chemical exchange limit but differ in the influence of the exchange process. For studies of Na$^+$ ions in intracellular environments, we suggest that the model allowing for randomization of the EFG by Marshall (1970) and Vasavada et al. (1985) is the most appropriate to use when the spin changes site. The other model (Eliav et al. [1988]), treating the orientation of the molecular fixed interaction frame as unchanged when the spin changes site, is a more appropriate model for intramolecular determined relaxing spins.

A careful analysis of both WHH and I/I_0 may help to discriminate between different models. The WHH studied as a function of chemical exchange rate (or temperature) may exhibit two maxima or one maximum depending on the dynamic parameters describing the system. From the experimental point of view the observation of the two maxima in the WHH variation is neither sufficient nor necessary condition for the existence of a slow-motion site. The variation of WHH is sensitive to the chosen chemical exchange model, whereas I/I_0 is not. However, the relative intensity is very sensitive to the effect of a slow-motion site.

APPENDIX

Matrix elements for one site will be given in the quantum levels of the space coordinates $|LKM)$, and in the spin coordinates $|\Sigma\sigma)$. Let the Hamiltonians of section 2 and the additional operator Γ_{ex} operate on the matrix element $(L'K'M'(\Sigma'\sigma'|, |L, K, M)\Sigma\sigma)$.

(*a*) The diffusion-rotation part, depending on the space coordinates, only contributes to the diagonal elements.

$$(L'K'M'|\Gamma_\Omega|LKM) = -\delta_{\mathrm{L'L}}\delta_{\mathrm{K'K}}\delta_{\mathrm{M'M}}[D_{\mathrm{R}}L(L+1) + 1/\tau].$$

We have here assumed isotropic diffusion.

$$D_{\mathrm{R}} = {}^1\!/\!_6\,\tau_{\mathrm{R}}^{-1}.$$

τ_{R} is the rotational correlation time and τ the lifetime of the site.

(*b*) The quadrupolar Hamiltonian consists of a field-gradient tensor (space coordinates) and spin operators. The quadrupole Liouville superoperators are off-diagonal in the representation used.

$$(L'K'M'(\Sigma'\sigma'|L_{\mathrm{Q}}(\Omega)|\Sigma\sigma)LKM) = \beta\sum_{m,n}(-1)^n$$
$$\cdot\ V_m^2(L'K'M'|D^2_{-m,-n}(\Omega)|LKM) \times (\Sigma'\sigma'|A_n^2|\Sigma\sigma).$$

The index *n* indicates the coordinate system in the laboratory frame and *m* the molecular coordinate system. In the high-field approximation, the only value of importance of the index *m* is $m = 0$, whereas in the low field limit the values $m = \pm 2$ are of importance as well. We rewrite this equation in low field case:

$$\begin{aligned}&(L'K'M'(\Sigma'\sigma'|L_{\mathrm{Q}}(\Omega)|\Sigma\sigma)LKM)\\ &\quad= \beta\sum_n V_{\mathrm{o}}^2\{(L'K'M'|D^2_{0,-n}(\Omega)|LKM)\\ &\qquad+ \eta[(L'K'M'|D^2_{-2,-n}(\Omega)|LKM)\\ &\qquad+ (L'K'M'|D^2_{2,-n}(\Omega)|LKM)]\}\\ &\qquad\cdot(\Sigma'\sigma'|A_n^2|\Sigma\sigma).\end{aligned}$$

V_0^2 is the quadrupolar strength constant, and η an asymmetrical parameter, i.e., in the high-field approximation $\eta = 0$.

The Wigner, $3j$ and $6j$, symbols are used in evaluating the factors of the quadrupolar Hamiltonian. The field gradient factor is:

$$(L'K'M'|D^2_{-m,-n}(\Omega)|LKM) = (-1)^{K'-M'}\sqrt{(2L'+1)(2L+1)} \cdot \begin{pmatrix} L' & 2 & L \\ -K' & m & K \end{pmatrix} \times \begin{pmatrix} L' & 2 & L \\ -M' & n & M \end{pmatrix}.$$

The spin super-matrix elements are:

$$(\Sigma'\,\sigma'|A_n^2|\Sigma\,\sigma) = \delta_{\Sigma,\Sigma\pm1} R_{\Sigma'2\Sigma}\sqrt{(2\Sigma+1)}\,(-1)^{\Sigma+\sigma}\begin{pmatrix} \Sigma & 2 & \Sigma' \\ \sigma & -n & -\sigma' \end{pmatrix},$$

where the reduced matrix elements $R_{\Sigma'2\Sigma}$ are given by

$$R_{\Sigma'2\Sigma} = \sqrt{(2\Sigma+1)5}\;2\begin{Bmatrix} \Sigma' & 2 & \Sigma \\ S & S & S \end{Bmatrix}(-1)^{\Sigma}\,tr\{(A_n^2)^2\}^{1/2}.$$

(A_n^2) is the spherical spin tensor in the Liouville space of rank two. The trace of this tensor is a numerical factor according to Halle and Wennerström (1981).

(*c*) The Zeeman part of the Liouville superoperator only contributes to the diagonal elements, and is only spin- and frequency-dependent:

$$(L'K'M'(\Sigma'\sigma'|L_{\mathrm{Zeeman}}|\Sigma\,\sigma)LKM) = \delta_{\mathrm{L'L}}\delta_{\mathrm{K'K}}\delta_{\mathrm{M'M}}\delta_{\Sigma'\Sigma}\delta_{\sigma'\sigma}\omega_{\mathrm{o}}(\Sigma'\sigma'|A_0^1|\Sigma\,\sigma).$$

The spin operator above, expressed in the Wigner, $3j$ and $6j$, symbols becomes:

$$(\Sigma'\,\sigma'|A_0^1|\Sigma\,\sigma) = (-1)^{\Sigma-1-\sigma'}\sqrt{(2\Sigma'+1)}\,R_{\Sigma'1\Sigma}\begin{pmatrix} \Sigma & 1 & \Sigma' \\ \sigma & 0 & -\sigma' \end{pmatrix},$$

where the reduced matrix elements of rank one are given by

$$R_{\Sigma'1\Sigma} = \sqrt{(2\Sigma+1)3}\;2\begin{Bmatrix} \Sigma' & 1 & \Sigma \\ S & S & S \end{Bmatrix}(-1)^{\Sigma}\,tr\{(A_0^1)^2\}^{1/2}.$$

To supplement formulas given here, the full expression of a nonzero diagonal and an off-diagonal element of the density matrix, for one site, respectively, will be given.

Diagonal element:

$$\begin{aligned}(L'K'M'(\Sigma'\,\sigma'|i(-\omega + L_{\mathrm{Zeeman}} &\\ + i\Gamma_{\Omega})|\Sigma\,\sigma)LKM) &= i\{\{-\omega + (-1)^{\Sigma-1+\sigma'} \\ &\cdot \sqrt{(2\Sigma+1)}\,R_{\Sigma'1\Sigma} \times \begin{pmatrix} \Sigma & 1 & \Sigma' \\ \sigma & 0 & -\sigma' \end{pmatrix}\omega_{\mathrm{o}}\} \\ &+ D_{\mathrm{R}}L(L+1) + 1/\tau.\end{aligned}$$

Off-diagonal element:

$$\begin{aligned}&(L'K'M'(\Sigma'\,\sigma'|i(L_{\mathrm{Q}}(\Omega))|\Sigma\,\sigma)LKM) \\ &= i\beta V_0^2(-1)^{L'-M'+\Sigma+\sigma}\sqrt{(2L'+1)(2L+1)(2\Sigma+1)} \\ &\times \delta_{\Sigma,\Sigma'\pm1}R_{\Sigma'2\Sigma}\begin{pmatrix} L & 2 & L \\ -M' & n & M \end{pmatrix}\begin{pmatrix} \Sigma & 2 & \Sigma' \\ -\sigma & n & \sigma' \end{pmatrix} \\ &\cdot \left\{\begin{pmatrix} L' & 2 & L \\ -K' & 0 & K \end{pmatrix} + \eta\begin{pmatrix} L' & 2 & L \\ -K' & -2 & K \end{pmatrix} + \eta\begin{pmatrix} L' & 2 & L \\ -K' & 2 & K \end{pmatrix}\right\}.\end{aligned}$$

The analytical expressions used to calculate the density matrix are now complete, and an investigation of the basis set spanning the system space of interest is desirable. The quantum numbers are restricted as follows: $L = 0, 1, 2, \ldots; -L \le K \le L; -L \le M \le L; \Sigma = 1, 2, 3\ (I = 3/2); -\Sigma \le \sigma \le \Sigma$. Some more restrictions on the quantum numbers and their mutual coupling are found in the formulas above and in the rules of the $3j$-symbols. The Wigner-Eckart theorem states: $|\Sigma - \Sigma'| = 1$.

The basis set of interest is a set where all elements, of any rank are coupled to the lineshape determining element, ((11(000|,|000)11)). This element will restrict the σ-quantum number, so that $\sigma = 1 - \mathrm{M}$ for all elements of interest. The rank of coupling needed may be evaluated while calculating the lineshape. The matrix may be truncated when the elements of the next rank do not contribute appreciably to the lineshape.

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