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Theoretical NMR study of the chemical exchange of amide protons of proteins

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SUMMARY

A model is proposed to evaluate the rate of exchange between the amide protons of proteins and the solvent water molecules. Using this model we determined the extent of the error for the chemical exchange rate constant when cross relaxation was neglected; both selective inversion and saturation-transfer techniques were evaluated. Furthermore, the fluctuations in the NOE intensities were determined when the exchange rate was varied.

INTRODUCTION

The amide protons of proteins and glycoproteins undergo exchange reactions with solvent protons such as water. Although the precise nature of the structural fluctuations which give rise to exchange are in dispute (Woodward et al., 1982), dynamical studies of amide proton exchange are an essential element for elucidation of the structures of biomolecules (Linderstorm-Lang and Schellman, 1959; Henry and Sykes, 1990). The exchange rates of the amide protons which are hydrogen-bonded or in a fully folded protein are thought to be considerably slower than those which are not hydrogen-bonded or those found in unfolded proteins (Henry and Sykes, 1990; Udgaonkar and Baldwin, 1988). Amide proton exchange rates in fully folded proteins are reduced by factors of as much as 10^9, because these protons either participate in hydrogen-bonded secondary structure (Englander and Kallenbach, 1984) or are inaccessible to solvent or both. Hydrogen-bonded structure directly inhibits exchange because hydrogen bonds must be broken for exchange to occur (Englander et al., 1972). Thus, amide proton exchange dynamical studies provide a tool

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for probing hydrogen bonds and obtaining the detailed structural information about early events that occur during the folding of proteins (Udgaonkar and Baldwin, 1988).

FT-NMR spectroscopy is a time-domain method especially suited to study the dynamics of the hydrogen exchange processes. For exchange processes, the rate constants in the range of 10^{-2} to $10^{2}\ s^{-1}$, are generally well suited for studies using the magnetization-transfer NMR method (Led et al., 1989). The exchange reaction of amide protons is relatively complex. In order to obtain simple analytical solutions, one usually makes some simplifications. For example, one often neglects the cross-relaxation term. Sometimes, this simplification is acceptable and other times it may cause large errors depending on the relative amplitudes of exchange rate constants and relaxation rates.

In NMR studies of proteins, the NOE intensities of amide protons are very important for obtaining the structures of proteins (Kaptein et al., 1988; Wright, 1989). Since amide proton exchange affects the amplitude of the NOE, it is necessary to study the effect of neglecting chemical exchange in order to obtain the correct structure of the protein.

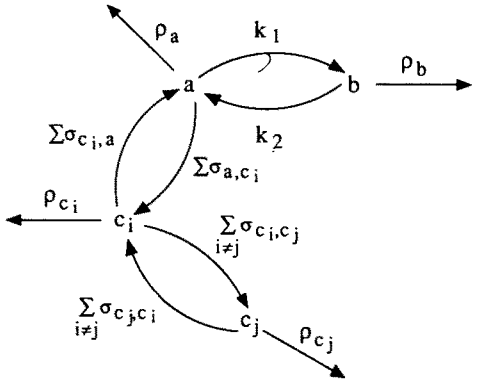
In this report, we shall demonstrate that neglect of the cross-relaxation term will cause significant errors when using magnetization-transfer NMR techniques in measuring the rate constants for the exchange of amide protons of proteins. Furthermore, we compare the extent of the error in the chemical exchange rate constant using selective inversion and saturation-transfer techniques. Lastly, we determine the effects of the exchange of the amide proton on the NOE intensities of neighboring nonlabile protons.

EXPERIMENTAL

For the general exchange reactions of amide protons of proteins and glycoproteins in water, the magnetization relaxation and proton exchange may be described as shown in Scheme I. An article related to this work was published earlier (Landy and Rao, 1989).

In order to get an explicit solution, it was necessary to simplify the scheme to a three-spin system, i.e. let $\Sigma_{i\neq j}\sigma_{c_i,c_j}=\Sigma_{i\neq j}\sigma_{c_j,c_i}=0$ and $\Sigma\sigma_{a,c_i}=\Sigma\sigma_{c_i,a}=\sigma$, and cross relaxation between water

Scheme I



a represents the amide proton, b the water proton, and c_i and c_j are any nonlabile protons (e.g. α-proton)

protons and nonlabile protein protons was neglected. The modified Bloch equations for longitudinal magnetization values of the components in Scheme I are given by

$$da_z/dt = -\rho_a(a_z - a_z^{\infty}) - \sigma(c_z - c_z^{\infty}) - k_1 a_z + k_2 b_z \tag{1}$$

$$db_z/dt = -\rho_b(b_z - b_z^{\infty}) + k_1 a_z - k_2 b_z \tag{2}$$

$$dc_z/dt = -\rho_c(c_z - c_z^{\infty}) - \sigma(a_z - a_z^{\infty}) \tag{3}$$

where a_z, b_z, and c_z are the instantaneous values of the z-magnetization for spins a, b, and c, and a_z^{∞}, b_z^{∞}, and c_z^{∞} are the equilibrium values for spins a, b and c.

When b $\gg$ a in the above system (very dilute sample conditions), Eq. (1) can be further simplified to

$$da_z/dt = -(\rho_a + k_1)a_z - \sigma(c_z - c_z^{\infty}) + \rho_a a_z^{\infty} + C \tag{4}$$

where C is a constant. It follows then that the general solutions for Eqs. (3) and (4) are given by

$$a_z = A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} + a_z^{\infty} \tag{5}$$

$$c_z = B_1 e^{\lambda_1 t} + B_2 e^{\lambda_2 t} + c_z^{\infty} \tag{6}$$

where

$$\lambda_1 = -1/2\{(\rho_a + \rho_c + k_1) - [(\rho_a + \rho_c + k_1)^2 - 4\rho_c(\rho_a + k_1) + 4\sigma^2]^{1/2}\} \tag{7}$$

$$\lambda_2 = -1/2\{(\rho_a + \rho_c + k_1) + [(\rho_a + \rho_c + k_1)^2 - 4\rho_c(\rho_a + k_1) + 4\sigma^2]^{1/2}\} \tag{8}$$

$$A_1 = \{(a_z^0 - a_z^{\infty})(\lambda_2 + \rho_a + k_1) + (c_z^0 - c_z^{\infty})\sigma\}/(\lambda_2 - \lambda_1) \tag{9}$$

$$A_2 = \{-(a_z^0 - a_z^{\infty})(\lambda_1 + \rho_a + k_1) - (c_z^0 - c_z^{\infty})\sigma\}/(\lambda_2 - \lambda_1) \tag{10}$$

$$B_1 = \{(c_z^0 - a_z^{\infty})(\lambda_2 + \rho_c) - (a_z^0 - a_z^{\infty})\sigma\}/(\lambda_2 - \lambda_1) \tag{11}$$

$$B_2 = \{(c_z^0 - c_z^{\infty})(\lambda_1 + \rho_c) - (a_z^0 - a_z^{\infty})\sigma\}/(\lambda_2 - \lambda_1) \tag{12}$$

and a_z^0 and c_z^0 are initial z-magnetization values for spins a and c at t = 0.

If we neglect the cross-relaxation term in Eq. (4), the solution for the equation can be given as

$$a_z' = (a_z^0 - a_z^{\infty})\, e^{-(\rho_a + k_1)t} + a_z^{\infty} \tag{13}$$

Hence, the equation for the errors in the values of the z-magnetization caused by neglecting cross relaxation is

$$(A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} - (a_z^0 - a_z^{\infty})e^{-(\rho_a + k_1)t}) \tag{14}$$

and the percent error in the value of the z-magnetization is given by

$$\{(A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} - (a_z^0 - a_z^{\infty})e^{-(\rho_a + k_1)t})/a_z^{\infty}\} \times 100 \tag{15}$$

For the selective-inversion recovery experiment, $c_z^0 = c_z^\infty$ and $a_z^0 = a_z^\infty \cos(\theta)$, where θ is the flip angle. Therefore, $A_1 = \{a_z^\infty(\cos(\theta)-1)(\lambda_2+\rho_a+k_1)\}/(\lambda_2-\lambda_1)$ and $A_2 = \{-a_z^\infty(\cos(\theta)-1)(\lambda_1+\rho_a+k_1)\}/(\lambda_2-\lambda_1)$. According to Eq. (15), there is an absolute maximum value for the percent error in the value of the z-magnetization for a set of A_1, A_2, B_1, B_2, λ_1, and λ_2 values.

A more useful term may be to determine the error in the exchange rate constants derived from the calculations; Eq. (13) was used for these calculations. We can estimate the errors of exchange rates caused by neglecting the cross-relaxation term based on the errors in the values of the z-magnetization. The procedure is as follows. According to Eq. (13), we have

$$tk_1 = -\ln\{(a_z - a_z^\infty)/(a_z^0 - a_z^\infty)\} - \rho_a t \tag{16}$$

and

$$tdk_1 = -da_z/(a_z - a_z^\infty) \tag{17}$$

In this equation, da_z is defined as the error in the value of the z-magnetization for a_z and this is equivalent to

$$A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t} - (a_z^0 - a_z^\infty) e^{-(\rho_a + k_1)t}$$

Thus, the error for exchange rate constant (dk_1) depends on the relaxation time t for particular relaxation rates and rate constants.

Since $(\Delta k_1/\Delta a_z) \approx (dk_1/da_z)$, the consolidation of Eqs. (17) and (5) yields Eq. (18)

$$\Delta k = \{1 - (a_z^0 - a_z^\infty) e^{-(\rho_a + k_1)t}/(A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t})\}/t \tag{18}$$

where A_1, A_2, λ_1, and λ_2 have the same simple forms for the selective-inversion recovery experiment. The error in the exchange rate constant has a maximum value for some value of t. But since we use many data points for the nonlinear least-square analysis and these data were taken at different relaxation times (t), it is more useful to determine the average of the error.

If the rate constants are obtained by combining saturation transfer (using steady-state values) and inversion-recovery experiments, the error for the rate constant is described by Eq. (19) (Dill et al., 1991).

$$\Delta k = k_1 \sigma^2/(\sigma^2 - \rho_a \rho_c) \tag{19}$$

If spin c is selectively inverted, the NOE for spin a is given by

$$NOE = (A_1' e^{\lambda_1 t} + A_2' e^{\lambda_2 t}) \times 100 \tag{20}$$

where $A_1' = -A_2' = -2\sigma c_z^\infty / a_z^\infty$ for $a_z^0 = a_z^\infty$ and $c_z^0 = -c_z^\infty$.

The pairwise dipole–dipole interaction which occurs among I = 1/2 nuclei may dominate nuclear relaxation, provided that suitable short internuclear distances between the magnetic

moments in the molecular framework and the motion of the internuclear vectors are typical of biomolecules in solution (Bloch, 1957; Niccolai and Rossi, 1989). If we apply the mechanism to our spin system, the following equations (Heatley, 1986) can be obtained.

$$\rho_a = D[J(\omega_a - \omega_c) + 3J(\omega_a) + 6J(\omega_a + \omega_c)] \tag{21}$$

$$\rho_c = D[J(\omega_c - \omega_a) + 3J(\omega_c) + 6J(\omega_a + \omega_c)] \tag{22}$$

$$\sigma_{ac} = D[6J(\omega_a + \omega_c) - J(\omega_a - \omega_c)] \tag{23}$$

$$D = 1/4(\mu_0/4\pi)^2\gamma_a^2\gamma_c^2(h/2\pi)^2r^{-6}$$

where μ_0 is the permeability of vacuum ($4\pi \times 10^{-7}$), h is Planck's constant, γ_a and γ_c are the gyromagnetic ratios of spin a and c, r is the internuclear distance between spin a and c, and $J(\omega)$ is a spectral density function, which is described by

$$J(\omega) = 2/5\tau_c/(1 + \omega^2\tau_c^2) \tag{24}$$

RESULTS AND DISCUSSION

Figure 1 shows the 3D plot of the percentage error for the values of the z-magnetization versus the correlation time (τ_c) and exchange rate constant (k_1), using an internuclear distance of 2.5 Å (selective inversion). The results show that the errors increase with an increase in the absolute value of the cross-relaxation rate σ, and the sign of σ does not affect the sign of the error. This result was obtained from the use of Eqs. (7) and (8). For different internuclear distances, the shapes of 3D

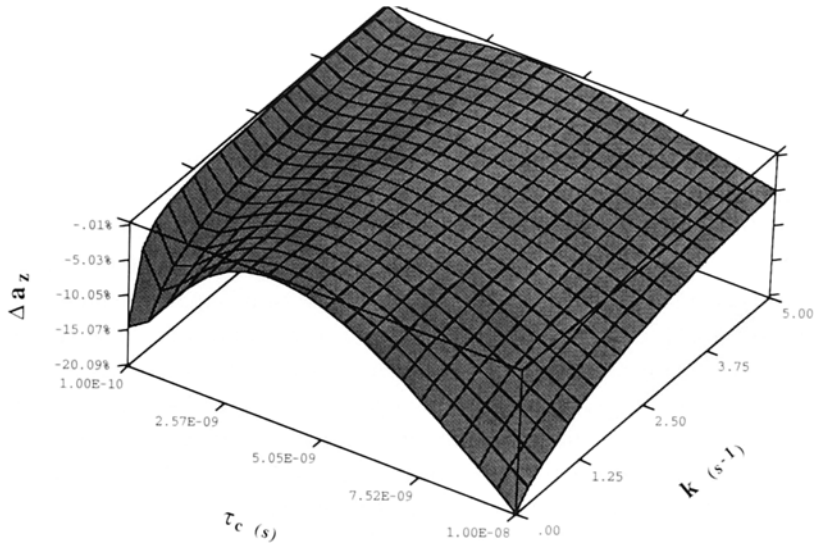


Fig. 1. Three-dimensional plot of the percentage error for Δa_z vs. correlation time (τ_c) and the exchange rate constant (k_1) using a distance of 2.5 Å to the nearest nonlabile proton (selective-inversion experiment). For each τ_c and k_1 pair, the % error in Δa_z was calculated.

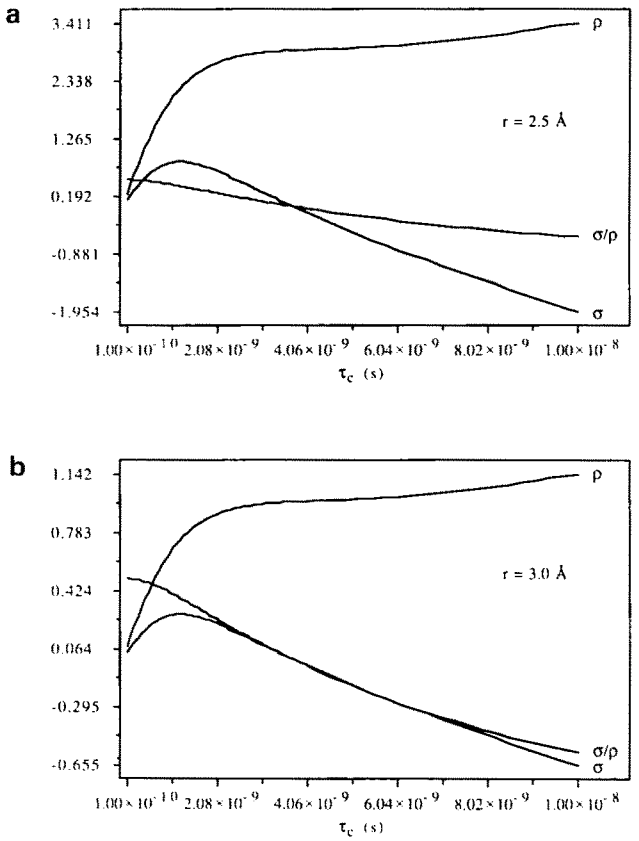


Fig. 2. Plots of the relaxation rates (ρ and σ) and ρ/σ vs. correlation time. The plots are based on Eqs. (21)–(23) in the text with (a) r = 2.5 Å and (b) r = 3.0 Å.

plots are the same except for the magnitude of the error changes. This is because the distance only affects the magnitude of ρ and σ, but not the ratio of σ/ρ. When $k_1 \neq 0$ and the correlation time is in the range of 10^{-10} to 3.7×10^{-9} s^{-1}, the percent error for the z-magnetization has a maximum value with $\tau_c \approx 1.2 \times 10^{-9}$ s^{-1}. Although the maximum value of the error depends on the value of k_1, it always occurs at the same value of τ_c. The reason is that σ, which is independent of the internuclear distance (Fig. 2), has a maximum value at that value of τ_c. The absolute magnitude of the error decreases quickly as the value of k_1 increases, especially when τ_c is short.

The 3D plot of the average errors for the exchange rates vs. τ_c and k_1 has the same shape as depicted in Fig. 1, but the plot of the maximum error for k_1 is different (compare Figs. 3 and 4). Although for a given correlation time the average error for k_1 will decrease with increasing exchange rate constant, the maximum error for k_1 will increase. At first glance this would appear to be an odd phenomenon but it can be explained. With increasing k_1, the rate of recovery from the nonequilibrium magnetization to the thermal equilibrium value increases and the time required to reach the maximum error for k_1 decreases. This can be seen in Eq. (18). Figures 3 and 4 also show that although the maximum error for k_1 varies greatly over the range of the exchange

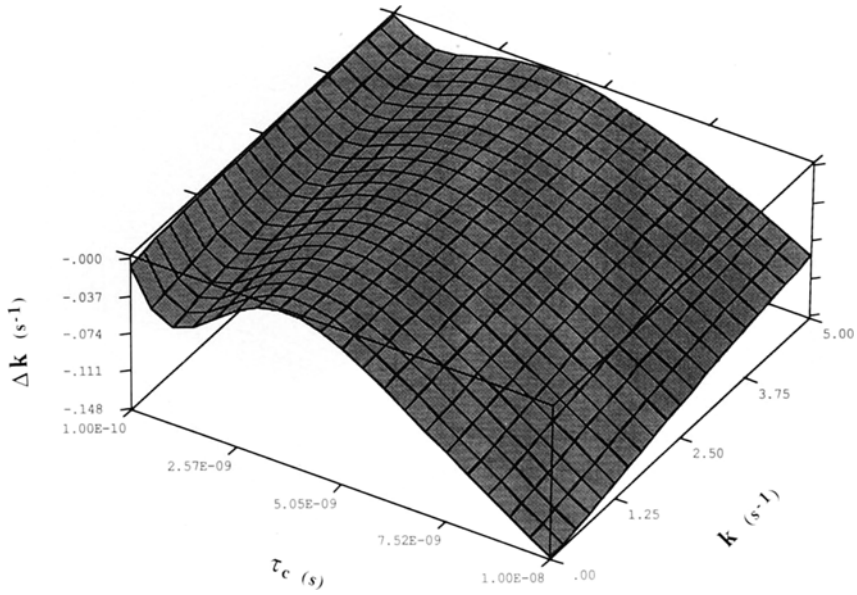


Fig. 3. Three-dimensional plot of the average error in the exchange rate constant vs. τ_c and k_1 using a distance of 3.0 Å to the nearest nonlabile proton (selective-inversion experiment).

rate constants studied, the average value of the error is small, even for relatively large negative values of σ (longer correlation times).

The 3D plot of Δk_1 vs. τ_c and k_1 for the saturation-transfer experiment is shown in Fig. 5. The results are noticeably different from those obtained for the selective-inversion transfer experi-

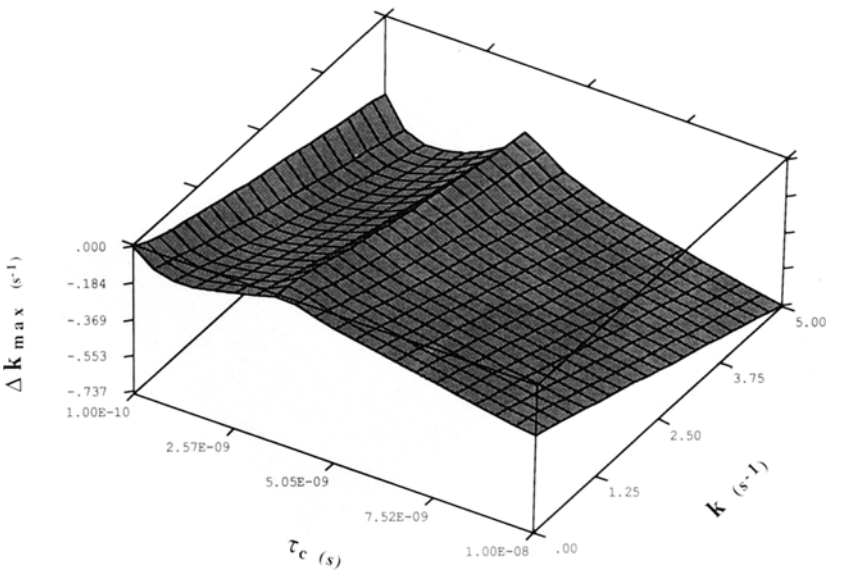


Fig. 4. Three-dimensional plot of the maximum error in the exchange rate constant vs. τ_c and k_1 using a distance of 3.0 Å to the nearest nonlabile proton (selective-inversion experiment).

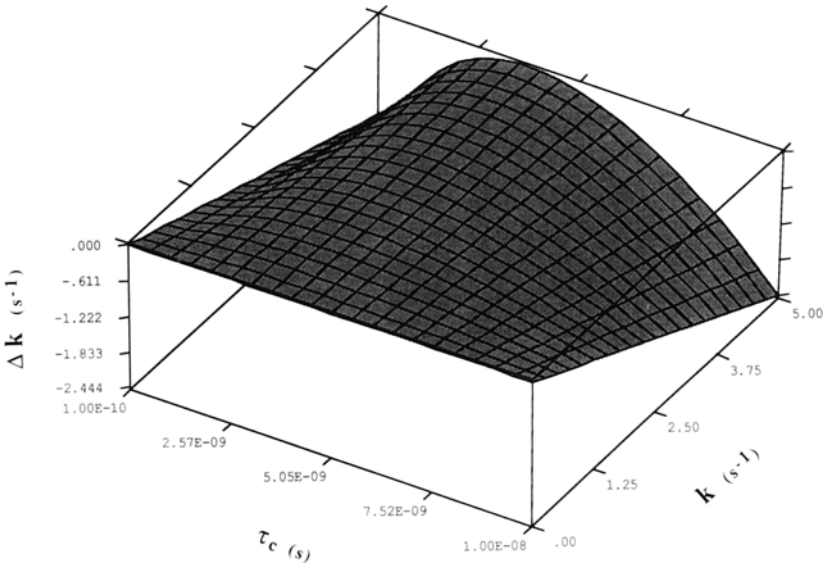


Fig. 5. Three-dimensional plot of the error in the exchange rate constant (r = 3.0 Å to the nearest nonlabile proton) vs. τ_c and k_1 for the saturation-transfer experiment (irradiation of water resonance).

ment. In the saturation-transfer experiment (for $\tau_c = 10^{-10}$ and 10^{-8} s^{-1}), the absolute values of Δk_1 increase with increasing values for k_1 and are equal to zero when $k_1 = 0$. However, there is a point ($\tau_c \approx 3.5 \times 10^{-9}$ s^{-1}) where Δk_1 appears to be invariant with a change in k_1 (because $\sigma = 0$). Another observation is that Δk_1 only depends on the values of k_1 and τ_c, and not on the internuclear distance. These results were obtained using Eq. (19).

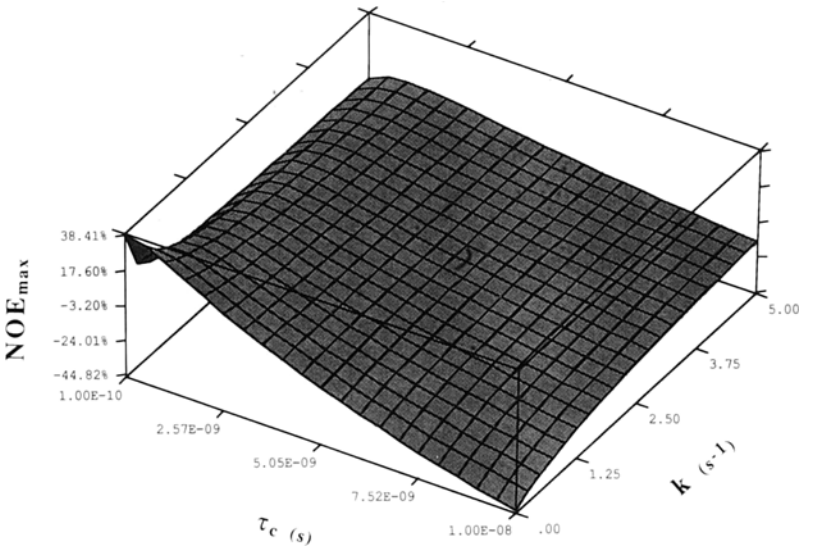


Fig. 6. Three-dimensional plot of the maximum NOE (between amide proton and nonlabile proton) vs. τ_c and k_1 (r = 3.0 Å) by selectively inverting spin c. Calculation was done using $\omega_H = 300$ MHz.

Comparison of the results from Figs. 3 and 5 clearly shows that the results from the selective-inversion magnetization transfer experiment will have smaller errors in the values of k than the saturation-transfer experiment, if we neglect cross-relaxation rates. However, when the values of k_1 are very small, the saturation-transfer method is more suitable for obtaining accurate values of k_1.

Figure 6 shows the effect of the exchange rate constant and correlation time on the magnitude of the NOE of a neighboring nonlabile proton at 300 MHz. Between the limits of k_1 investigated (0–5 s^{-1}) the NOEs increase as the ratio of σ/ρ (decreasing τ_c) increases. The NOEs reach a peak value and then decrease. This is because as the correlation time decreases (ρ decreases), k_1 makes the NOE more positive. For proteins with a correlation time of 1×10^{-8} the results are the most dramatic; when $k_1 = 0$, the intensities of NOEs are negative ($\sim -45\%$) but they approach 0 when k_1 reaches 5 s^{-1}. These results have a direct bearing on the use of amide protons to obtain internuclear distances within proteins using 1D and 2D NOE NMR experiments. Under certain conditions (correlation times and exchange rates), the NOE intensities could be off by as much as $\sim 30\%$ and this would cause a significant error in the internuclear distance calculations.

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