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Complete Relaxation and Conformational Exchange Matrix (CORCEMA) Analysis of Saturation Transfer Difference (STD) NMR Spectra of Ligand-Protein Complexes

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Abstract: An interesting recent application of intermolecular NOE experiment is the saturation transfer difference NMR (STD-NMR) method that is useful in screening compound libraries to identify bio-active ligands. This technique also identifies the group epitopes of the bound ligand in a reversibly forming protein-ligand complex. We present here a complete relaxation and conformational exchange matrix (CORCEMA) theory (Moseley et al., *J. Magn. Reson.* **B, 108,** 243-261 (1995)) applicable for the STD-NMR experiment. Using some ideal model systems we have analyzed the factors that influence the STD intensity changes in the ligand proton NMR spectrum when the resonances from some protons on the receptor protein are saturated. These factors will be discussed and some examples of its application in some model systems will be presented. This CORCEMA theory for STD-NMR and the associated algorithm are useful in a quantitative interpretation of the STD-NMR effects, and are likely to be useful in structure-based drug design efforts. They are also useful in a quantitative characterization of protein-protein (or protein-nucleic acid) contact surfaces from an intermolecular cross-saturation NMR experiment.

INTRODUCTION

Intermolecular NOE (INOE) experiments on interacting systems in which the signals from one molecule are perturbed and the changes due to intermolecular dipolar relaxation in the signals from a second molecule are observed have been described several years ago.[1-4] Interesting

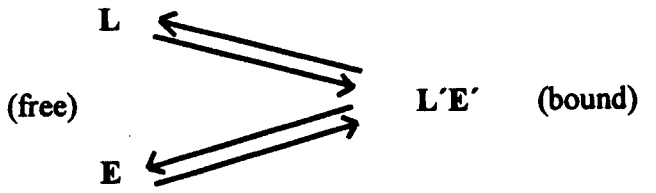
recent applications of INOE experiments are (i) the Saturation Transfer Difference NMR (STD-NMR) method [5] to screen the compound mixtures in the presence of a receptor and to characterize ligand's binding epitope and (ii) the intermolecular cross-saturation NMR (ICS-NMR) for identifying the protein-protein[6] contact surface or protein-nucleic acid [7] contact surface in a complex.

In the STD-NMR experiment, one generally works with a solution containing an excess of a single ligand or a library of ligands in the presence of a macromolecule.[5] These measurements work best under weak binding conditions. The NMR spectra of the ligand with and without irradiation of a region within the broad resonance envelope of the macromolecule are collected and a difference spectrum obtained. In the STD-NMR experiment only those protons within the ligand which are at the interface with the protein are generally expected to exhibit significant intensity changes and hence the ligand surface epitopes easily identified.

In this work, we present a complete relaxation and conformational exchange matrix (CORCEMA) theory [4,8] for quantitative analysis of the STD-NMR experiment. We also have identified and analyzed some of the factors that influence the ligand proton intensity changes in the STD-NMR experiments using some hypothetical model systems and also a typical application involving a hypothetical trisaccharide-protein complex.

THEORY

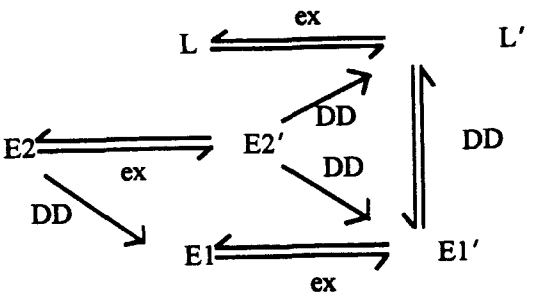
As in our previous work [4,8] we will assume a 2-state kinetic model that involves the molecular species in their free (**L, E**) and bound (**L′, E′**) states according to the following scheme.



Scheme (I)

We assumed that the receptor protons can be subdivided into two classes, **E1** (and **E1′**) and **E2** (and **E2′**), where **E2** and **E2′** stand for protons that experience rf irradiation directly. The primes indicate protons within the complex. **E1** and **E1′** stand for all the remaining protons in the receptor in its free and bound states respectively. Thus, in our model, the saturation originates from **E2** and **E2′** and spreads to other protons according to the following scheme (DD stands for

exchange through dipole-dipole relaxation):



Scheme (II)

Under these conditions, the expression for the observable magnetization in an INOE experiment in which the **E2** and **E2'** protons are *instantaneously* saturated by rf irradiation is given by [8]

$$\mathbf{I}(t) = \mathbf{I}_0 + [\mathbf{1} - \exp\{-(\mathbf{R}+\mathbf{K})t\}](\mathbf{R}+\mathbf{K})^{-1}\mathbf{Q} \qquad [1]$$

In this paper, all quantities in bold letters (such as **I, R, K, Q** etc) stand for matrices or group of protons. The generalized kinetic matrix **K**, relaxation matrix **R**, intensity matrix **I** and **Q** are defined in reference. [8]

METHOD

CORCEMA-STD protocol shown in Fig. 1 of reference,[8] employs Eq. [1] to compute the absolute ($I-I_0$) and the fractional $\{(I-I_0)/I_0\}$ STD-1DNMR intensity spectra. A program employing this protocol using MATLAB (The Mathworks, Inc., Natick, MA) was written for use on a personal computer. The starting point of the program is reading the pdb coordinates for the free ligand, free protein, and the ligand-protein complex either for existing models from the protein data bank (PDB) or from proposed and/or modified models. For methyl groups, a 'Model-Free' calculation of spectral densities with order parameters (S^2) and internal (τ_m) and overall (τ_r) correlation times was performed, as in reference.[4,8,9] For intra-methyl proton-proton interactions, S^2 was set to 0.25. For methyl-to-other proton interactions, the S^2 value is in the range of 0.6 to 0.9. [4,8,9] The details of the program are discussed in reference.[8] In the final step, the "predicted" STD changes are calculated and compared with the experimental values using the NOE R-Factor. [3,7]

We first did some simulations using two hypothetical models consisting of 5 ligand protons and 5 protein protons with the configurations shown in Fig. 1 (the Asymmetric model). The k_{on} rate was assumed to be 10^8 s^{-1} M^{-1}. A uniform leakage factor of 0.3 s^{-1}, a

correlation time of 10^{-7} s for the protein and complex, the free ligand correlation time of 2.966×10^{-10} s corresponding to null NOE at 600 MHz were also assumed.

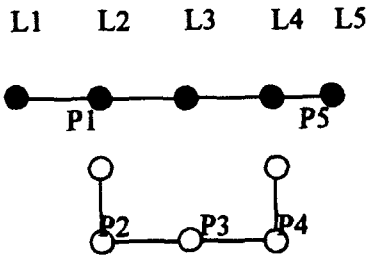


Fig. 1. Asymmetric model of ligand-protein complex. All the distances between the nearest neighbour protons in the complex are assumed to be 2.5Å, with the L4-L5 distance set at 1.8Å. In the symmetric model L4 - L5 distance is set at 2.5Å

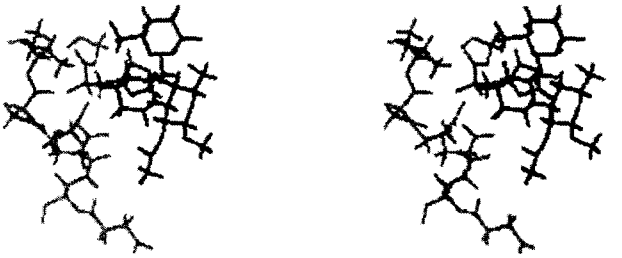


Fig. 2. Stereo view of a hypothetical trisaccharide, Gal-MAG-Fuc bound to the mannose-binding protein (MBP). The hydrogens were omitted for clarity.We retained three sugars (Gal, MAG (α-methyl-N-acetyl-D-glucosamine), and Fuc), and 8 residues (D184, E185, N187, H189, G190, N205, D206, and I207) in the MBP binding pocket from the pdb file 2kmb for sLex/MBP.[10]

For simulating a typical experimental application, we also took the pdb coordinates (ID# 2kmb) for the complex of sLex/MBP(mannose-binding protein)[10] and constructed a hypothetical trisaccharide protein complex shown in Fig. 2. All exchangeable hydrogens (OH and NH) were excluded in the calculation to simulate measurements in D_2O. The methyl internal correlation time was set at 10^{-11}s.

RESULTS AND DISCUSSION

Effect of i) Saturation time and ii) the bound ligand conformation

Even if a given set of protein proton signals (**E2** and **E2′** protons) are instantaneouly saturated, the saturation will take a finite time to spread to other protein protons (**E1** and **E1′** sets of protons) and the bound ligand protons (**L′**) through dipolar networks, and through chemical exchange. Fig. 3 shows the calculated ligand STD values (free + bound) for the models in Fig.1. The time-dependent intensity changes in the initial regions (< 0.1 s in Fig.3A and 3B) reflect the spatial proximity of the ligand protons to the enzyme protons in the bound state. In Fig.3B, L2 and L4 have substantially different STD values with L4 showing significantly smaller effect, *even though these two protons are equidistant* from the P3' proton. This is a simple consequence of the differences in the relaxation rates for these L2 and L4 protons due to differences in their 'local environments' (e.g., in the asymmetric model, the L4-L5 distance is shorter than the L3-L2 distance). These observations suggest that caution is needed in qualitative attempts to relate the magnitudes of steady state STDs to spatial proximity of ligand protons to the protein protons.

Effect of the relative position of saturated proton(s) at the active site

Figs. 3B, 4A and 4B show the STD effects when the P3, P2 and P4 protons are saturated respectively. The dramatic difference in the magnitudes of the STD effects between Figures 3B, 4A and 4B is self evident. This is a reflection of the fact, that even for long correlation times of 10^{-7}s, with all the protein protons separated by 2.5Å each (and a leakage of 0.3 s^{-1}) the saturation of protein protons due to spin diffusion is not 100% efficient in the complex as a result of exchange-mediated leakage.[8] Thus, some caution is needed in interpreting STD-NMR results based on the assumption of near 100% saturation of entire protein resonance envelope due to irradiation at a single frequency.

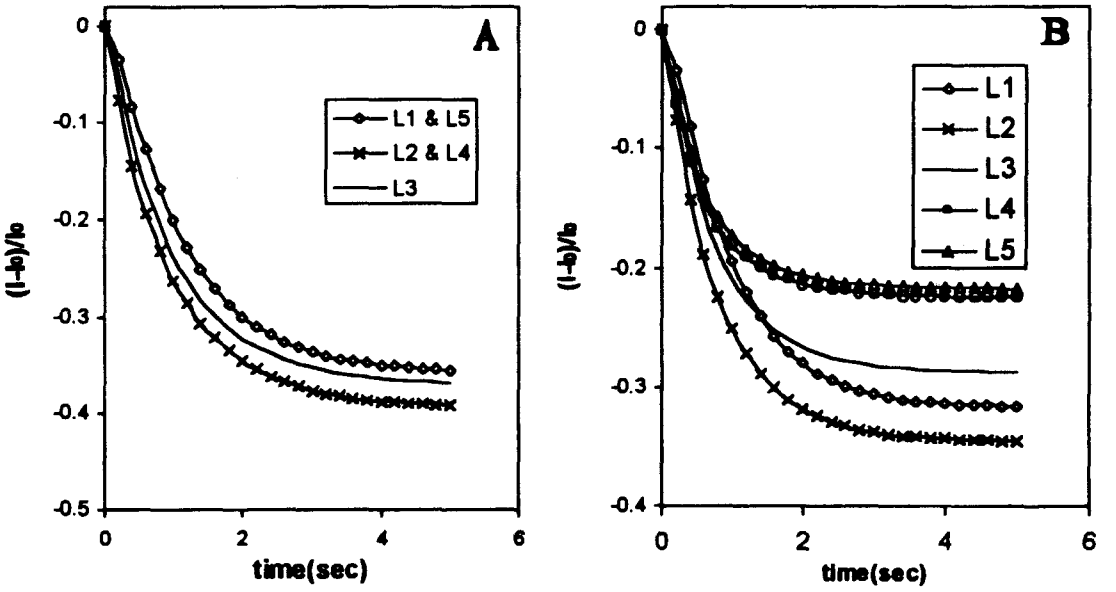


Fig. 3. (A) STD curves for the symmetric ligand-protein complex. (B) STD curves for the asymmetric ligand-protein complex in Fig. 1. The P3' and P3 protrons are saturated. A spectrometer frequerncy of 600 MHz and the free ligand correlation time of 2.966×10^{-10} s corresponding to null NOE at 600 MHz were assumed. The protein correlation time was 10^{-7}s. Lt/Et=10:1. Leakage rate=$0.3s^{-1}$.

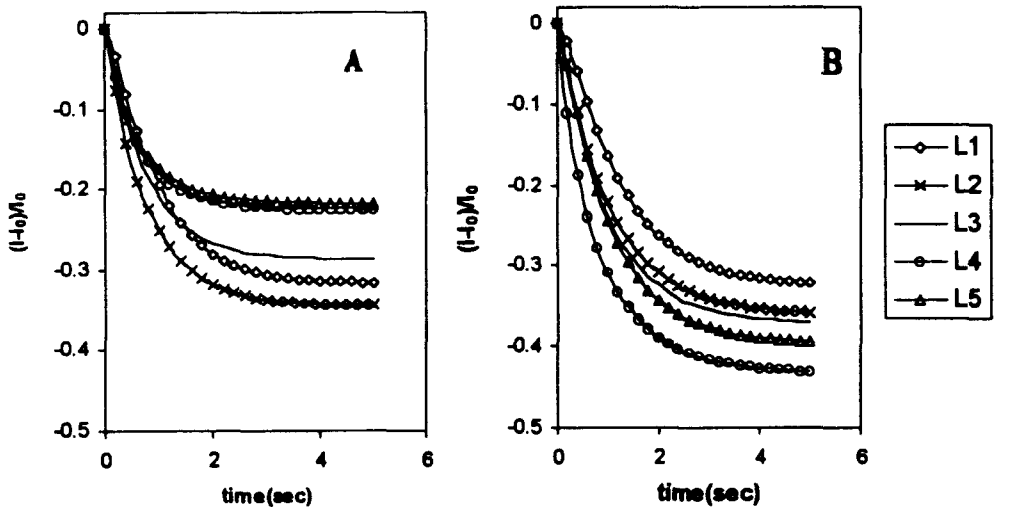


Fig. 4. Same as in Figure 3, but (A) with P2' and P2 protons saturated (B) with P4' and P4 protons saturated.

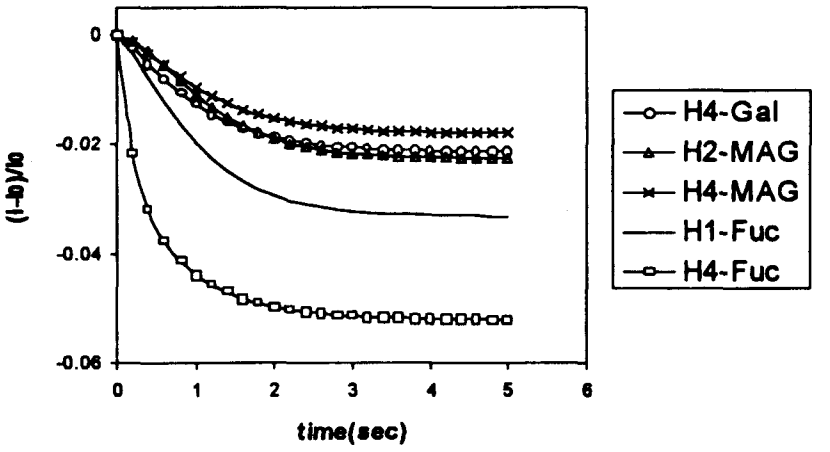


Fig. 5. The calculated STD curves for Gal-H4, MAG-H2, MAG-H4, Fuc-H1, and Fuc-H4 as a function of time when the methyl protons on I 207 are saturated. S^2 was set at 0.85 and a methyl internal correlation time of 10^{-11} s.

Trisaccharide/MBP Complex

Fig. 5 shows the STD-NMR spectra for the trisaccaharide/MBP model shown in Fig.2. In general those protons which are close to the saturated protein protons show the largest steady-state STD values (Fig. 5), but the order of magnitudes of STD do not necessarily reflect the order of spatial proximity of ligand protons to the ILE methyl protons in the complex. As pointed out earlier using the asymmetric 5-proton model, the steady state STD values are significantly influenced by the intra-ligand dipolar relaxation behaviour of different proton pairs. Protons, such as the geminal protons in a ligand which can have a very strong intra-dipolar relaxation, are likely to show smaller steady-state STD effects even when they are closer to a saturated protein proton, than a relatively isolated ligand proton which is a bit farther.

CONCLUSIONS

The CORCEMA theory presented here for the STD-NMR method is useful for quantitative analysis of STD-NMR effects in ligand-protein complexes. We have performed simulations for more interesting and realistic (trisaccharide-protein complex) models. The sensitivity of the method to changes in bound ligand conformation and position of the saturated protein protons is illustrated. Thus the CORCEMA-STD theory is likely to be useful in characterising the binding epitopes of the ligand and for optimizing the ligand in structure-based drug design protocols. This theory is also applicable in a quantitative data interpretation in ICS-NMR method.

Acknowledgments

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