STUDIES ON LONG-LIVED STATES AND COHERENCES IN LIQUID STATE NMR

by

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DEDICATED

TO

MY SISTER

Certificate

I certify that the thesis entitled ***Studies on long-lived states and coherences in liquid state NMR*** submitted by Chinthalapalli Srinivas to Indian Institute of Technology Delhi, for the award of the degree of Doctor of Philosophy is a record of bona fide research work done by him. He has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis which to my knowledge has reached the requisite standard. The results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any other degree or diploma.

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Abstract

NMR plays a vital role in determining the structure and dynamics of the molecules in liquids. Relaxation of spins is crucial in structural and dynamical studies of molecules as well as in the design of pulse sequences. It was assumed that the upper limit of the relaxation is the conventional spin-lattice (T_1) and spin-spin relaxation time (T_2). Recently, long-lived states for a chemically inequivalent two spin system, were demonstrated that have lifetimes longer than T_1. From then onwards studies have been carried out to create, sustain and detect long-lived states in a wide range of systems and to probe the structure and dynamics of molecules. In a further development it was shown that, under appropriate conditions, a coherence is created that could also have long-life time which lead to highly resolved spectra. Such coherences are termed as long-lived coherences. In this thesis we study various properties of long-lived states (LLS) and coherences (LLC's) and their applications. We explore techniques for the creation and sustenance of LLS and LLC's and exploit them for the structural and dynamical characterization of the molecules.

The thesis reports on our studies on long-lived states (LLS) and coherences (LLC's). The population in the LLS can be stored for a long time and may be exploited to follow slow processes such as flow, diffusion, and slow molecular motion. The long-lived coherences, although not as long-lived as LLS, have lifetimes that are longer than the conventional single quantum coherence. Methods to create and sustain the LLS are introduced as are also applications of LLS for structural and dynamical studies. We exploit LLC's to record high-resolution two-dimensional (2D) spectrum in inhomogeneous magnetic fields.

This thesis is composed of five chapters. In the introductory chapter, tools such as the vector model, Hamiltonians, density matrix and the product operator formalism that are used

in the rest of the chapters are discussed. LLS are observed in diamagnetic systems where the major relaxation mechanism is intramolecular dipole-dipole coupling. The relevant details of NMR relaxation due to this mechanism are provided before a detailed presentation of long-lived states and their relaxation mechanisms. Finally the forms of some relevant operators in two different bases are given.

The creation and sustenance of LLS play a prominent role for their application in structural and dynamic studies. Till now only laboratory frame methods, involving delays and pulses, have been used for their creation. In such methods, the delays are a function of chemical shift difference and scalar coupling between the spins with the efficiency being compromised if incorrectly set. It is advantageous to have methods that do not require this prior information, which is especially difficult to determine if the spin pairs undergo conformational exchange between sites with different chemical shifts and couplings. Thus, we exploited linear and tangential Adiabatic Demagnetization in the Rotating Frame (ADRF) techniques which does not require the chemical shift of the other coupling partner and coupling information. Experimental studies performed on 4-nitrophenol show that the LLS creation with these techniques, under optimal conditions, is about half of the laboratory methods with the tangential ADRF method better than the linear. The time required to create the LLS in these methods is also longer than the laboratory frame methods. Numerical simulations are in concordance with these experimental observations. We attribute the reduced efficiency to the effect of relaxation time during the creation phase. For chemically inequivalent coupled spin system LLS can be sustained by suppressing the chemical shift difference ($\Delta\delta_{12}$). It was done in two ways. The first way, which involves mechanical transport of the sample, is to temporarily remove the sample out of magnetic field. In the absence of a field the chemical shift difference which is a linear function of the field is zero. The second one, which is often the preferred method, is to use an RF field with a power that

is an order of magnitude larger than the $\Delta\delta_{12}$. In this method, various heteronuclear decoupling or isotropic mixing pulse schemes are used to sustain LLS over a wide range of offsets. Windowless multiple pulse sequences, which involve a continuous sequence of RF pulses of well-defined lengths and phases cause sample heating for large $\Delta\delta_{12}$. An alternative that is less investigated are sequences which utilize iterative Carr-Purcell (CP) trains and Uhrig's Dynamic Decoupling (UDD). These schemes are multiple-pulse schemes with windows, that is RF pulses interspersed with periods of free evolution. Although these two classes of sequences have been used to minimize environmental decoherence, they have not been considered as an option for sustaining LLS, which forms the focus of our study. These two schemes are applied on the LLS created and compared with that of the continuous wave (CW) RF irradiation. Experiments were carried out on two samples 2, 3, 6-trichlorobenzaldehyde (small $\Delta\delta_{12}$, 76 Hz) and p-nitro phenol (large $\Delta\delta_{12}$, 356 Hz). It has been observed that LLS can be sustained using CP and UDD for large $\Delta\delta_{12}$ without sample heating with a marginal drop in the T_{LLS}. Our results indicate that the CP and UDD can be used for the sustenance of LLS in case of large $\Delta\delta_{12}$. Simulations also reveal that the leakage of LLS content for CP and UDD is lesser than the CW.

^{2}H-labeling provides the dual advantage of spectral simplification and higher resolution of one dimensional ^{1}H NMR spectra. In this report, we combine these advantages of ^{2}H labeling with the longer lifetimes and high sensitivity to the environmental effects of the LLS to study the structure and dynamics of a small protein, Ubiquitin. All residues except the six glycines at 10, 35, 47, 53, 75, and 76 positions were ^{2}H-labeled for this study. This molecule is ideal for our study since the glycine residues are present both at outer surface and inner core of the molecule and, in addition, form a simple two spin system in which LLS creation and sustenance is straightforward. The T_1 and T_{LLS} for Gly-76, 75, 47, 35 and Gly-10 residues were calculated over temperatures ranging from 280K to 315K at two different

magnetic field strengths of 500 and 600 MHz. We have observed that there is a perceptible difference in the lifetimes of the LLS between inner core and outer surface residues, while such difference was not seen for conventional relaxation time constant over the range of temperatures studied at both the magnetic fields leading us to conclude that the combination of deuterium labeling with LLS forms an effective tool to study the structure and dynamics of proteins.

The relaxation properties of scalar-coupled two-spin systems subjected to intramolecular dipolar relaxation is a well-studied problem but it had escaped the attention of researchers that the lifetimes of zero-quantum coherences in the rotating frame are 3 to 9 times longer than single-quantum coherences. Such coherences, called long-lived coherences (LLC's), were exploited for accurate determination of the coupling constants even in inhomogeneous magnetic fields. In this chapter we present a method to address the challenging problem of recording the NMR spectrum in inhomogeneous magnetic fields which exploits these coherences. The method involves the broadband excitation and detection of LLC's and allows one to record two-dimensional NMR spectra with chemical shift differences in one dimension and scalar couplings in the other dimension. Spectra with unprecedented resolution ($\Delta\nu/\nu_0 = 4\cdot10^{-11} = 4\cdot10^{-5}$ ppm) in magnetic fields with a homogeneity worse than $\Delta B_0/B_0 = 10^{-5} = 10$ ppm are obtained in a mixture of bromothiophene carboxylic acid and alanine-glycine. In contrast to other techniques, the present method does not require the use of sophisticated gradients or adiabatic frequency-swept pulses for measuring high-resolution NMR spectra in inhomogeneous magnetic fields.

Bispidine (3,7-diazabicyclo[3.3.1]nonane) derivatives find wide range of applications ranging from the synthesis of organo-metallic compounds to inducing secondary structure in the peptides necessitating structural and dynamical studies of these derivatives. Boc protected

bispidine leucine is one such derivative which in $CDCl_3$ exists in *syn* and *anti* forms due to restricted rotation around N-CO bonds. Two-dimensional NMR experiments were performed to confirm the structure of the molecule and spectral assignment. With the help of selective and non-selective inversion-recovery experiments the exchange rate constant of the compound in the slow-exchange limit was determined over a range of temperatures. Arrhenius and Eyring plots gave the activation energy ($\Delta E^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) of activation to be 140 kJ/mol, 110 kJ/mol, and 105 $JK^{-1}mol^{-1}$ respectively. We attribute the high value of $\Delta E^{\ddagger}$and $\Delta H^{\ddagger}$ to the presence of long chains attached to the nitrogens of the bispidine ring restricting the rotation. The counter-intuitive value of $\Delta S^{\ddagger}$ is due to the large error from the extensive extrapolation required for its determination. These studies also revealed that the rate constant below 313 K was unreliable since the exchange rate was lower than the T_1. To alleviate this problem we attempted to exploit the LLS. The attempt was unsuccessful as the many protons proximal to the LLS decrease the lifetime drastically.

Contents