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Spectral density mapping at multiple magnetic fields suitable for ^{13}C NMR relaxation studies

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ABSTRACT

Standard spectral density mapping protocols, well suited for the analysis of ^{15}N relaxation rates, introduce significant systematic errors when applied to ^{13}C relaxation data, especially if the dynamics is dominated by motions with short correlation times (small molecules, dynamic residues of macromolecules). A possibility to improve the accuracy by employing cross-correlated relaxation rates and on measurements taken at several magnetic fields has been examined. A suite of protocols for analyzing such data has been developed and their performance tested. Applicability of the proposed protocols is documented in two case studies, spectral density mapping of a uniformly labeled RNA hairpin and of a selectively labeled disaccharide exhibiting highly anisotropic tumbling. Combination of auto- and cross-correlated relaxation data acquired at three magnetic fields was applied in the former case in order to separate effects of fast motions and conformational or chemical exchange. An approach using auto-correlated relaxation rates acquired at five magnetic fields, applicable to anisotropically moving molecules, was used in the latter case. The results were compared with a more advanced analysis of data obtained by interpolation of auto-correlated relaxation rates measured at seven magnetic fields, and with the spectral density mapping of cross-correlated relaxation rates. The results showed that sufficiently accurate values of auto- and cross-correlated spectral density functions at zero and ^{13}C frequencies can be obtained from data acquired at three magnetic fields for uniformly ^{13}C-labeled molecules with a moderate anisotropy of the rotational diffusion tensor. Analysis of auto-correlated relaxation rates at five magnetic fields represents an alternative for molecules undergoing highly anisotropic motions.

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1. Introduction

Nuclear magnetic resonance (NMR) not only serves as an important source of structural data, but also provides insight into molecular motions on a time scale ranging from 10^{-12} to 10^{3} s. As fluctuations of local magnetic fields are the only sources of relaxation for spin-1/2 nuclei, stochastic motions causing the magnetic fields to fluctuate can be effectively studied by measuring and analyzing the NMR relaxation rates, most typically of ^{15}N, ^{13}C, and ^{2}H nuclei. Fluctuations of individual magnetic fields and their interference are described by auto-correlation and cross-correlation functions, respectively. The semi-classical theory of spin relaxation in isotropic liquids [1–5] shows that relaxation rates are given by linear combinations of Fourier-transformed correlation functions, known as *spectral density functions*, evaluated at specific *eigenfrequencies*. Hence, the measured relaxation rates directly reflect stochastic dynamics of the molecule.

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Numerous NMR experiments have been designed in order to measure relaxation of various quantities. In principle, values of spectral density functions at individual eigenfrequencies can be extracted from the relaxation rates, such a process is known as *spectral density mapping* [6,7]. From a practical point of view, each measured relaxation rate represents one known experimental value and each spectral density value represents an unknown parameter to be determined. Obviously, the procedure requires the number of measured relaxation rates to be equal to or greater than the number of spectral density values contributing to them. The major challenge of the spectral density mapping is the fact that various types of interactions contribute to relaxation in real systems. As a consequence, values of multiple auto- and cross-correlated spectral density functions at several eigenfrequencies contribute to the measured relaxation rates, which greatly increases the number of unknown parameters in the spectral density mapping analysis. Therefore, the practical analysis of relaxation rates requires a certain level of simplification.

One possibility is to a priori define *particular functional form(s)* of the spectral density function. Such an approach is the essence of various versions of the *model-free* analysis [8–12]. The aim is to choose a form described by fewer parameters than the number of spectral density values contributing to the relaxation rates. The experimental relaxation rates are then used to determine the parameters describing the assumed functional form of the spectral density function instead of calculating its individual values. This elegant idea made the NMR relaxation a widely used method of motional analysis of biomacromolecules.

In spite of numerous successful applications, the model-free analysis has its limitation. From the statistical point of view, the correct choice of the number of relevant motional modes is a difficult problem, considering the low amount of experimental data and high non-linearity of the target function. From the physical point of view, the model-free approach is well applicable to relatively rigid molecular fragments whose internal motions can be described reliably by a single motional mode. However, higher motional richness requires more complex functional forms of the spectral density functions described by more parameters and the simplifying potential of the model-free approach disappears. More importantly, the assumptions of the statistical independence and symmetry of the individual motions are not valid in general, and an unjustified use of the model free approach may lead to incorrect results. Various advanced approaches have been proposed [13–19] to overcome the limitations, but they are considerably more computationally demanding.

Another possibility is to find a setup of the relaxation experiments and/or analysis when the number of the spectral density values to be determined does not exceed the number of available experimental values. It can be achieved by (i) assuming higher symmetry of molecular motions, (ii) reducing the number of eigenfrequencies (*reduced spectral density mapping* [20,21]), or (iii) extending the set of relaxation rates measured. The first option is frequently used but applicable only to certain types of motions (usually isotropic). The second possibility is typically exploited in ^{15}N relaxation studies, when the large difference between ^{15}N and ^{1}H relaxation rates permits to replace high-field values of the given spectral density function with a single effective value without introducing a too large error. The third route follows the original approach of Peng and Wagner [6,7], who used two-spin relaxation rates and proton longitudinal relaxation rates to extend the set of experimental data. This approach, extended to the ^{13}C–^{1}H spin system by Allard, Jarvet, Ehrenberg, and Gräslund [22,23], is experimentally more demanding (the additional relaxation rates may be more difficult to obtain without systematic errors, as described by Peng and Wagner in their seminal study [7]), but it has a potential to overcome limitations of the first two approaches. It can include both new types of experiments and measurements repeated at multiple magnetic fields.

In this paper, we examine the potential of extending the amount of experimental data for reduced spectral density mapping by using multiple magnetic fields and including cross-correlated relaxation rates. We focus on relaxation of protonated ^{13}C nuclei, where some simplifications routinely applied to peptide-bond ^{15}N are not well justified (large difference of ^{15}N and ^{1}H magnetogyric constants, collinearity of the ^{15}N–^{1}H bond and the main axis of the ^{15}N chemical shift tensor). Several methods of a combined analysis of relaxation rates obtained at multiple fields are described. Systematic errors are simulated and propagation of random experimental errors is analyzed in order to estimate when application of individual methods may be beneficial. Finally, analyses of relaxation data of two real molecules are presented. The tested samples included a uniformly ^{13}C,^{15}N-labeled pppGGCA-CUUCGGUGCC, an RNA hairpin containing the UUCG tetraloop, and methyl β-D-glucopyranosyl-(1 → 6)-α-D-[6-^{13}C] mannopyranoside, a β-(1 → 6)-linked disaccharide with a selectively ^{13}C labeled bridging methylene group. The RNA sample documents that the multiple-field mapping is applicable to relatively large uniformly labeled molecules. Also, it allowed us to test the influence of a significant conformational or chemical exchange. The disaccharide served as an example of a molecule exhibiting fast and highly anisotropic motions. The selective labeling allowed us to collect a large set of relaxation rates at seven magnetic fields, and to compare the performance of various methods of spectral density mapping in a systematic manner.

2. Methods

2.1. Sample preparation

2.1.1. UUCG hairpin

Fully ^{13}C- and ^{15}N-labeled sample of RNA oligomer pppGGCA-CUUCGGUGCC was synthesized in vitro from ^{13}C and ^{15}N-labeled NTPs. T7 RNA polymerase was used for the transcription from a DNA template. The synthesized oligomer was purified by a gel electrophoresis [24,25]. A 3.0 mM oligomer sample was prepared in 99.95% D_2O at pH* 6.7 (uncorrected reading). The sample contained 0.2 mM EDTA and 10 mM sodium phosphate buffer. A small amount of sodium azide was added to the sample. A detailed description of the sample preparation was published elsewhere [26].

2.1.2. β-D-Glcp-(1 → 6)-α-D-Manp-OMe

The sample was prepared by dissolving 9.5 mg of freeze-dried methyl β-D-glucopyranosyl-(1 → 6)-α-D-[6-^{13}C] mannopyranoside [27] in 367 μl DMSO-d_6 (99.96% ^{2}H, Euriso-Top) and 204 μl D_2O (99.96% ^{2}H, Aldrich). The solution in a 4 mm NMR tube was degassed by three cycles of freezing, application of a mild vacuum, and melting. Then the evacuated NMR tube was heat-sealed.

2.2. NMR experiments

2.2.1. UUCG hairpin

NMR experiments were carried out at the temperature of 298.2 K on the 400 MHz Varian Unity spectrometer with a double resonance (^{1}H, ^{13}C) room-temperature probe, and on 500 MHz and 600 MHz Varian Unity spectrometers with triple resonance (^{1}H, ^{13}C, ^{15}N) room-temperature probes. The temperature was calibrated based on the chemical shift differences of pure methanol peaks. Delays of the polarization transfer periods were set for a

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