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Communication

^{15}N spin diffusion rate in solid-state NMR of totally enriched proteins: The magic angle spinning frequency effect

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Abstract

As demonstrated by means of the one-dimensional solid-state MAS exchange experiment (CODEX), the rate of the proton driven spin diffusion between backbone ^{15}N nuclei in totally enriched protein depends strongly on the magic angle spinning (MAS) frequency: spin diffusion at MAS frequency 16 kHz is about 4–5 times slower as compared to that at MAS frequency 1 kHz which is due to the averaging of the homo- and hetero-nuclear dipolar interactions by MAS. It is important that even at the highest MAS frequencies used in our experiments the spin diffusion rate is comparable or larger than typical values of the spin–lattice relaxation rates of backbone nitrogens in solid proteins. Thus, the precise quantitative analysis of ^{15}N T_1's in totally enriched solid proteins may lead to wrong quantitative results. On the other hand, the effectiveness of the ^{15}N–^{15}N correlation and structure determination experiments making use of ^{15}N–^{15}N distances can be increased by decreasing the MAS frequency as far as possible, which is counter intuitive to the commonly applied fast MAS conditions in order to reduce the dipolar-broadened line widths for increased spectral resolution.

Keywords: Proton driven spin diffusion; Magic angle spinning; CODEX; Proteins; Structure; Dynamics; Isotope enrichment

Proton driven spin diffusion between chemically inequivalent X nuclei like ^{13}C and ^{15}N is a process driven by dipolar interactions in nuclear magnetic resonance (NMR) which can be used to assign solid-state NMR spectra and support the structure determination of such complex molecules as proteins [1–4]. On the other hand, in molecular dynamics studies, it is an interfering effect: spin diffusion equalizes the relaxation times of different nuclei, and the selectivity of dynamic information that can be obtained from the relaxation experiments is lost. Solid-state exchange experiments [5,6], which are sensitive to motions in the millisecond to second time scale, suffer from spin diffusion as well: both spin diffusion and molecular motions result in a change of the orientation dependent resonance frequency of the nucleus under consideration and thus produce the same effect in the exchange spectra. Their separation by experimental means is difficult and time consuming at least, if possible at all [7]. Methods to separate the two effects past-acquisition were proposed but do not solve the problem completely, since the spin diffusion still determines the upper limit of the experimentally accessible dynamic range [8,9].

The spin diffusion rate between chemically inequivalent nuclei can be decreased by suppressing either homonuclear ^{15}N–^{15}N (^{13}C–^{13}C) or heteronuclear ^{13}C(^{15}N)–^{1}H dipolar interactions. Homonuclear interaction can be suppressed for instance by diluting the spin system, i.e., working with natural abundance or even isotopically depleted materials, or using selective isotopic enrichment. This may resolve the spin diffusion problem; however, in addition to the loss of signal intensity, it deteriorates the abundance of the molecular dynamics information and makes correlation experiments difficult to perform. The heteronuclear X–^{1}H dipolar interaction can be suppressed by ^{1}H

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decoupling, however, this is not feasible for practical reasons: the delays in the relaxation experiments or the mixing periods in exchange sequences can be on the time scale of seconds, during which strong RF irradiation for such a long time can be dangerous for both sample and spectrometer. Additionally, in the case of exchange between nuclei with the same isotropic chemical shift (which is however a rare instance for proteins), ^{1}H decoupling even increases the spin diffusion rate [10]. A feasible way to average the dipolar interaction is fast magic angle spinning (MAS) which is a prerequisite for many experiments in any way. However, in the case of ^{13}C nuclei, fast MAS may affect spin diffusion only for non-protonated carbons and has a vanishingly small influence for aliphatic carbons only [7]. This is obviously due to the much stronger ^{13}C–^{1}H interaction of aliphatic carbons (around 20 kHz) which can not be averaged out by MAS. In the case of ^{15}N, however, both the ^{15}N–^{1}H and ^{15}N–^{15}N interactions are much smaller and thus MAS could have a much stronger effect on the spin diffusion rate between ^{15}N nuclei. Still, to our knowledge, the MAS effect on ^{15}N spin diffusion has not been verified experimentally until now.

The main aim of the present contribution is the investigation of the MAS dependence of the spin exchange rate between ^{15}N nuclei (which are governed by dipolar interaction and thus do depend on the MAS rate [11–14]) and the determination of typical values of the spin diffusion rates in totally ^{15}N-enriched protein. It is an important parameter for the design and analysis of both structural/correlation and dynamic NMR experiments in solid proteins. To demonstrate the MAS effect on the ^{15}N spin diffusion rate we performed a series of one-dimensional solid-state MAS exchange experiments (CODEX) [15,16] in dry lyophilized powder of totally ^{15}N-enriched lysozyme from bacteriophage T4. It has been demonstrated by MAS exchange experiments that a dehydrated protein does not undergo slow backbone motions [8], thus the exchange process observed is solely due to spin diffusion. For comparison, the same experiments were performed in ^{15}N-enriched BOC-glycine (in which the NH_3 group is replaced by a NH group), which does not exhibit any slow molecular mobility either. We would like to note that the two substances are examples of (a) spin exchange between chemically inequivalent nuclei (different residues in a protein have different isotropic chemical shifts), and of (b) exchange between chemically equivalent but magnetically inequivalent nuclei (there are no resolved resonances in the BOC-Gly). More detailed information about these different groups of exchange processes and the appropriate NMR experiments to characterize them can be found in [6,10]. Fig. 1 presents exchange decays (mixing time dependencies of the amplitude of the peak corresponding to backbone nitrogens in the CODEX spectrum) measured for these substances at different MAS rates. The evolution periods were kept at 0.5 ms for BOC-Gly and 1 ms for T4 lysozyme for all MAS rates.

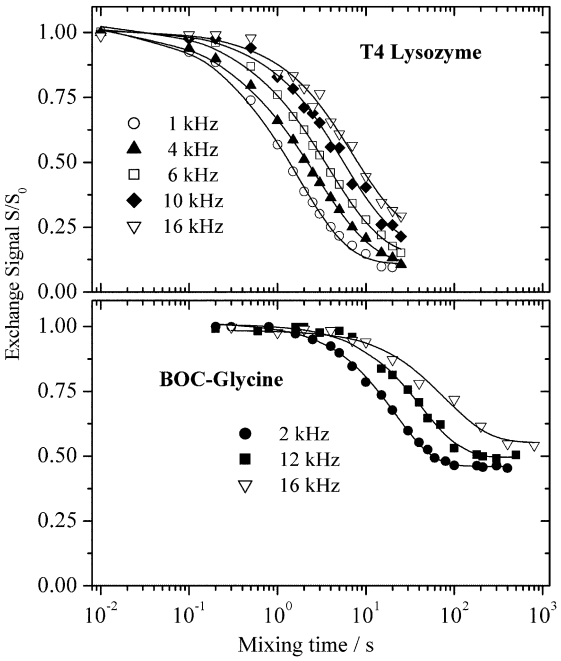

Fig. 1. Examples of the CODEX mixing time dependencies for ^{15}N-enriched BOC-Gly and T4 lysozyme at different MAS rates. To exclude the influence of spin–lattice relaxation, all exchange decays were divided by separately measured spin-relaxation decays, as described in [8]. Solid lines are best fits to Eq. (1). Experimental conditions were: resonance frequency for ^{15}N nuclei 40.5 MHz; $T = 20$ °C for BOC-Gly and $T = 10$ °C for T4 lysozyme. The exchange decays did not reveal any temperature dependence (data not shown), confirming the assignment of the exchange process to spin diffusion. Experimental errors from the spectral noise are on the order of the size of the symbols.

For a samples not exhibiting molecular dynamic processes, the spin diffusion rate dependence on the MAS frequency the exchange decays can be for all practical purposes fitted to the equation.

$$\frac{S}{S_0}(\tau_m) = \left(1 - \frac{1}{M}\right) \cdot \exp\left(-\frac{\tau_m}{\tau_{SD}}\right)^{\beta} + \frac{1}{M} \qquad (1)$$

where S/S_0 is the normalized signal intensity in a CODEX experiment [16], τ_m is the length of the mixing period and $1/M$ is the value of the normalized peak intensity for the limit $\tau_m \to \infty$. It depends on the duration of the CODEX evolution period, the value of the chemical shift anisotropy (CSA) tensors parameters and their mutual orientations during the exchange process [6]. If the evolution period is sufficiently long, M equals the number of sites connected by the exchange process [16]. τ_{SD} is the spin diffusion time constant, and β is a phenomenological parameter describing the distribution of τ_{SD} values. Experimental values of β are one for BOC-Gly and 0.75 ± 0.1 for T4 lysozyme, indicating that the protein is obviously a more complex system with an appreciable distribution of ^{15}N–^{15}N distances between magnetically inequivalent nuclei for which a single-exponential approximation cannot adequately describe the spin diffusion process. The parameter $1/M$ was

determined to 0.5 ± 0.06 for Gly-BOC and to 0.14 ± 0.05 for T4 lysozyme, meaning the number of magnetically inequivalent sites is two for BOC-Gly, and indicating that in the protein the spin exchange occurs between several amino acids. In the BOC-Gly structure, there exist two magnetically inequivalent molecules [6,17] The shortest distances between two nitrogens belonging to magnetically inequivalent molecules are 4.81 and 5.18 Å, respectively [17]. All other N–N distances involved are all larger than 10 Å. At the same time, the distances between nitrogens of the successive aminoacids in the T4 lysozyme structure is between 2.65 Å and 3.65 Å, which can be readily estimated from the available Protein Data Bank files. Thus, the protein structure makes relayed spin diffusion between many nitrogen nuclei in T4 lysozyme possible, whereas in the BOC-Gly crystal structure, spin exchange may effectively occur only between two neighboring nitrogen nuclei.

The dependence of the spin exchange time constants τ_{SD} on the MAS-spinning frequency is shown in Fig. 2. The ratio of about 10 between the protein and BOC-Gly spin diffusion rates is due to different ^{15}N–^{15}N distances in these samples, as explained above (the internuclear dipolar coupling and hence, the spin diffusion rate is proportional to $1/r^6$). A rigorous theoretical analysis of these dependencies is possible [18] but beyond the scope of this communication.

Fig. 2 demonstrates that the application of the proton driven ^{15}N–^{15}N spin diffusion in NMR experiments aimed at spectra assignment and structure determination requires the MAS frequency to be as low as possible: the slower the MAS, the more efficient the spin diffusion. However, the results presented in Fig. 2 also indicate that the precise quantitative analysis of ^{15}N T_1's of individual residues in solid totally ^{15}N-enriched proteins is bound to possible errors: the T_1's of backbone ^{15}N nuclei in solid proteins are about 10–50 s [19–21]. It is seen from Fig. 2 that at all MAS frequencies used in our experiments the typical values of ^{15}N T_1 are longer than τ_{SD}. Even taking into account that at higher MAS and resonance frequencies the spin diffusion rate can be several times slower, it is obvious that the value of the relaxation times T_1 as well as the shape of spin–lattice relaxation decays of individual residues in totally enriched proteins can be significantly distorted by the spin diffusion effect. At the same time, the spin diffusion does not affect $T_{1\rho}$ data, since the $T_{1\rho}$ relaxation times are commonly much shorter [20]. From Fig. 2 it is also clear that the spin diffusion at high MAS frequencies is sufficiently slowed-down so that it does not interfere anymore with the study of millisecond timescale motions by means of solid-state MAS exchange methods. Thus, isotopic dilution as applied in [20] can be more effectively replaced by faster MAS.

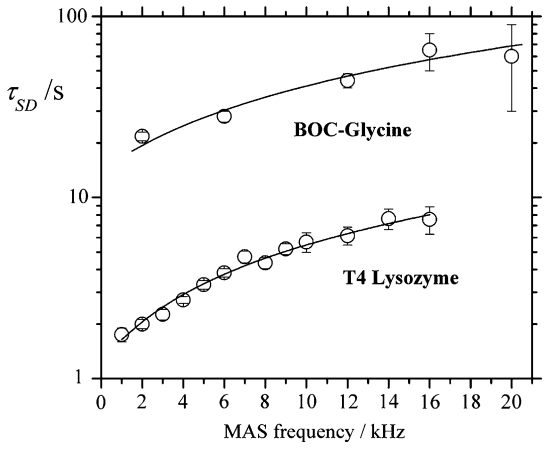

Fig. 2. MAS frequency dependencies of the spin-diffusion time constant for BOC-Glycine and T4 Lysozyme. Straight lines are best linear dependence [7,18] fits of τ_{SD} on the MAS frequency.

In addition, we would like to add some remarks about the different labeling strategies for structural investigations on one hand and experiments aimed to retrieve dynamic data on the other hand. Totally $^{13}C/^{15}N$-enriched proteins are very suitable for experiments aimed to obtain *structural* data through spin exchange processes. However, for *dynamic* investigations, such samples are less suited for the following reasons. First, in addition to the ^{15}N–^{15}N spin diffusion, the dipolar interaction between ^{15}N and the two adjacent ^{13}C nuclei in the backbone adds to the dipolar effects on the relaxation times making the analysis more complicated and ambiguous. Second, gaining spectral resolution in totally enriched proteins requires time-consuming multidimensional experiments. However, for a detailed quantitative study of the internal dynamics, performing a series of these experiments at different resonance frequencies, temperatures and some other experimental conditions is highly desirable. In this case, multidimensional experiments may have time requirements beyond reasonable limits. The issue of machine time can, in principle, be resolved by using the proton detection methods in perdeuterated proteins [22], however this technique is still far from becoming routine and will not resolve the spin diffusion problem for the T_1 experiments anyway. Note that for ^{13}C nuclei the spin diffusion problem is much more severe. Thus, in our opinion the application of the selective isotopic enrichment for detailed protein dynamics studies is still well warranted despite the fact that it provides worse sampling of the protein molecule.

Acknowledgments

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