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Investigation of Slow Molecular Dynamics using R-CODEX

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Abstract

A solid state NMR experiment is introduced for probing motions on the millisecond time scale, based on dephasing and refocusing ^1H - ^{13}C or ^1H - ^{15}N dipolar couplings. The method is related to the previously described Centerband-Only Detection of Exchange or CODEX experiment. The use of an R-type dipolar recoupling sequence takes advantage of the strong ^1H - ^{13}C or ^1H - ^{15}N dipolar coupling, while suppressing the effect of ^1H - ^1H homonuclear coupling. This approach paves the way to detect both the correlation time and reorientational angle of the dynamics in fully protonated samples. The performance of this pulse sequence is demonstrated using imidazole methyl sulfonate.

Keywords

Millisecond Dynamics; Solid state NMR; CODEX; R-CODEX; Reorientation angle; $\text{R}18^\circ_1$; ^1H homonuclear coupling; imidazole

Introduction

Molecular and conformational dynamics on the millisecond scale play important roles in protein function [1–3]. NMR methods provide the opportunity to probe the correlation time and the amplitude of a dynamical process in a site-specific fashion, which has the possibility to provide significant insights into protein function. For example, solution NMR studies, such as CPMG and $\text{R}^1\rho$ experiments, can provide site-specific correlation times and populations describing the dynamics of proteins in solution [4, 5]. In some cases, the amplitude of the motion can also be obtained quantitatively from for example RDC (residual dipolar coupling) experiments, and insights about the structures of the two sites can be obtained through interpreting chemical shifts [6].

Solid state NMR methods can significantly broaden the range of systems amenable to this kind of analysis, and can be sensitive on a range of timescales. On the scale of milliseconds seconds for example, a family of experiments known as CODEX (Centerband-Only detection of Exchange Experiments) [7–11] exploit anisotropic interactions such as the CSA

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(chemical shift anisotropy), to encode the orientation of a site and provide the means to detect changes in the orientation, and obtain both the correlation time and reorientational angle. To interpret the data it is necessary to know the principal values and orientations of the CSA, which can be complex for systems like proteins [12, 13]. In addition, most of the CSA tensors are non-uniaxial, which also complicates the analysis.

Therefore, experiments based on heteronuclear dipolar coupling are an attractive alternative. The heteronuclear dipolar coupling is strictly uniaxial, and has a relatively fixed coupling constant with the principal component D_{zz} along the chemical bond. Schaefer et al. used CODEX with and without ^{13}C - ^{19}F dipolar cancellation and demonstrated the presence of a large amplitude motion of a fluorinated aromatic ring [14]. Recently, Dipolar CODEX experiments [15] based on recoupling ^{13}C - ^{15}N dipolar couplings using a REDOR (rotational echo double resonance) [16] pulse sequence element, have been reported, and other experiments that utilize ^1H - ^{15}N dipolar couplings have been implemented with applications on a protein [17]. The ^{13}C - ^{15}N Dipolar CODEX requires a long dephasing time due to the weak ^{13}C - ^{15}N dipolar interaction, resulting in potential loss of signal. The ^1H - ^{15}N Dipolar CODEX experiments had potential artifacts from ^1H homonuclear coupling and therefore require perdeuteration of the protein.

In this paper, we report a R-CODEX experiment, which recouples the ^1H - ^{13}C or ^1H - ^{15}N dipolar couplings via the R18°_1 [18] recoupling element, a R-type sequence based on symmetry principles [19]. The π flip motion of the imidazolium ring in imidazolium methyl sulfonate was studied with this method to demonstrate properties of the pulse sequence. The results are promising for the detection of protein dynamics in the future. We discuss the advantages of this scheme in terms of experimental results, numerical simulations and theoretical considerations.

Theory

The pulse sequences and experiments shown schematically in Figure 1 are a CODEX like experiment using an R-based recoupling element to develop heteronuclear dipolar coupling; overall the sequence detects reorientation of a ^1H -X dipolar bond vector. The advantages of this scheme will be discussed in light of experimental results, numerical simulations and theoretical considerations. Throughout this section, we assume a conformational exchange process for a single ^{13}C - ^1H pair, that can be described using a two-site jump model, as shown in Scheme 1, and we assume that there is no exchange during the dephasing and refocusing periods (although for numerical simulations the exchange during those periods was included). The normalized populations of the two exchange sites are P_1 and P_2 . The exchange rates k_1 and k_{-1} satisfy $k_1 \times P_1 = k_{-1} \times P_2$.

A Simple Description of R-CODEX

During the pulse sequence shown in Figure 1 (a), after cross polarization, the carbon magnetization (S^x) accumulates phase φ_A under the action of the recoupled single quantum ^1H - ^{13}C dipolar coupling ($I^+S_z + I^-S_z$) during the dephasing time.

$$S_x \xrightarrow{I^+S_z + I^-S_z} S_x \cos \varphi_A + 2I_x S_y \sin \varphi_A \quad (1)$$

A subsequent 90° pulse flips the in-phase magnetization (S_x) to generate the stored longitudinal magnetization. Remaining anti-phase magnetization will disappear because of the choice of phase-cycle. During the mixing time, the exchange process redistributes the magnetization between the two exchange sites. Subsequently a 90° pulse flips the magnetization back to the transverse plane, and the magnetization evolves during the refocusing period under the same recoupling condition as was used for the dephasing period previously, which yields again an accumulated phase ϕ_B . The final amplitude of the signal after a z-filter is proportional to

$$S \propto e^{-t_m/T_1} \langle [P_1 P_2 (\cos\phi_A - \cos\phi_B)^2 e^{-k_{ex} t_m} + (P_1 \cos\phi_A + P_2 \cos\phi_B)^2] \rangle \quad (2)$$

where k_{ex} is the sum of the forward and reverse rate constants k^1 and k^{-1} , and the angle brackets $\langle \rangle$ represent a powder average over all molecular (“crystallite”) orientations. The accumulated phases ϕ_A and ϕ_B will be different if the ^1H - ^{13}C bond reorients during the mixing time. Due to this effect, the final total (powder averaged) signal will exhibit in a single exponential decay as a function of mixing time t_m . The exchange rate k_{ex} can be obtained from fitting the decay curve. Moreover, when the dephasing (refocusing) time is long enough, the equation (2) can be approximately simplified as (see details in the supplemental materials)

$$S \propto e^{-t_m/T_1} [2P_1 P_2 e^{-k_{ex} t_m} + (P_1^2 + P_2^2)] \quad (3)$$

The population ratios can be obtained by simply comparing the intensities for the short mixing time ($(P_1 + P_2)^2 = 1$) and the long mixing time ($P_1^2 + P_2^2$).

The pulse sequence designed to extract the reorientation angle (Figure 1 (b)) is in principle similar to the pulse sequences to extract the $\text{NH}_i\text{-NH}_{i+1}$ projection angle [20]. The length of the heteronuclear dipolar recoupling time increases gradually to form a dephasing curve, which in this case records the information about the projection angle between two exchanging orientations for the ^1H - ^{13}C (^1H - ^{15}N) pairs. A constant dephasing (and refocusing) time is used to minimize the effect of T_2 . The mixing time is long enough as compared with the correlation time of dynamics to make the conformers fully exchanged. Meanwhile, reference experiments with a very short mixing time are carried out as described by Schmidt-Rohr et al. [7]. The normalized intensity is given by

$$\frac{S_{\text{short}} - S_{\text{long}}}{S_{\text{short}}} = 2P_1 P_2 \times \left(1 - \frac{\langle \cos\phi_A \cos\phi_B \rangle}{\langle \cos^2\phi_A \rangle}\right) \quad (4)$$

Comparison of the dephasing curve with numerical simulations will yield the reorientation angle. Measurement of the intensity changes at short dephasing times is crucial for determining the reorientational angle, and so the increment time step used during the dipolar recoupling period should be short, due to the strong dipolar coupling of ^1H - ^{13}C . The $\bar{R}\bar{R}$ sub-cycle in R18^7_1 allows for taking data points at one ninth of a rotor period [18], making the reorientation angle detection particularly successful with this sequence.

The choice of the coherence used for dynamics detection

In the R-CODEX experiments we stored only the in-phase magnetization, rather than anti-phase part [15] or a combination of both [7–9]. The main concern is that if the ^1H spin lattice relaxation time is short, the anti-phase component $C_z H_z$ or $N_z H_z$ will lose significant magnetization during mixing time. Moreover, the proton spin lattice relaxation process will modulate some protons' spin states. This flip of the spin state will change the sign or

direction of the dipolar coupling, and spurious decays for the anti-phase magnetization will be exhibited even if the ^1H - ^{13}C or ^1H - ^{15}N bond does not reorient. The in-phase component C_z or N_z does not have this problem. It usually develops as the cosine function of the phases as shown in equation 1, which only depends on the absolute value of the dipolar coupling. Furthermore, the in-phase, anti-phase or a combination of them will have similar results when the dephasing time is long enough compared to the inverse of the recoupled anisotropic interaction (as shown in the supplemental materials). In addition, the anti-phase magnetization developed under the recoupled Hamiltonian $I^+S_z + I^-S_z$ by R18^{71}_1 is a mixture of zero and double quantum coherence, which is difficult to store efficiently. Therefore, only the in-phase component was kept in these experiments.

Experimental

^{15}N (98%) isotopically enriched imidazole (Cambridge Isotope Laboratories Inc.) was mixed with equimolar methyl sulfonate acid (diluted to 15%, to minimize the release of heat) and crystallized at room temperature. It was crushed into powder form and center-packed in the 4 mm rotor.

All experiments were performed on a Chemagnetics Infinity 300 MHz double resonance instrument, using an APEX double resonance MAS probe in $^1\text{H}/^{13}\text{C}$ or $^1\text{H}/^{15}\text{N}$ configuration with a 4 mm rotor. The sample temperature was maintained at 20°C ($\pm 0.1^\circ\text{C}$). For the ^{13}C R-CODEX experiment, the R18^{71}_1 element (180° phase shift relative to R18^{71}_1) in Figure 1(a) was replaced with 100 kHz CW decoupling because one rotor period was sufficient to fully dephase the ^{13}C magnetization. In the angle detection experiment (Fig 1b), the total time of dephasing (refocusing) element was 2 rotor periods. The CW decoupling time was decreased incrementally with increasing the number of R18^{71}_1 blocks. The magic angle sample frequency was 11 kHz (± 3 Hz). The ^1H field strength for the R18^{71}_1 element was 99 kHz, and the ^{13}C field strength for all the 90° and 180° pulses was 100 kHz. The initial ^{13}C signal was enhanced using adiabatic passage through the Hartmann-Hahn condition [21]. The constant ^1H RF field during cross polarization had an amplitude of 52 kHz. The ^{13}C tangential RF field followed equation in the supplemental materials [22], where ω^{HH} was 41 kHz and the tangential parameters Δ and β were 12 kHz and 7 kHz, and the contact time was 3 ms. 60 kHz TPPM ^1H decoupling was applied during acquisition.

For the ^{15}N R-CODEX experiment, the magic angle sample frequency was 8.889 kHz, and the dephasing and refocusing time was 4 rotor periods (450 μs). The ^1H field strength for the R18^{71}_1 element was 80 kHz, and the ^{15}N field strength for all 90° and 180° pulses was 40 kHz. The initial ^{15}N signal was enhanced using a flat Hartmann-Hahn cross polarization element, with a ^1H RF field had amplitude of 40 kHz and a ^{15}N RF field amplitude of 32 kHz. The contact time was 3 ms. 60 kHz TPPM ^1H decoupling was applied during acquisition.

The ^{13}C chemical shift was referenced to the ^{13}C adamantane's methylene peak at 40.26 ppm [23]. The ^{15}N chemical shift was calibrated indirectly.

All simulations were performed using SPINEVOLUTION 3.3.4 [24].

Results

Correlation times and population ratios of the two exchange sites

The 180° flip of the imidazolium ring [25, 26] was previously well characterized by ^{13}C and ^{15}N R-CODEX experiments at 20°C . Spectra from experiments with different mixing times are shown in Figure 2 (a) and Figure 3 (a)). The peak intensities for carbon and

nitrogen lines generally decay markedly with increasing mixing time as expected (peak near 122 ppm in Figure 2 (a) and the peak in Figure 3(a)). The peak intensity of the immobile carbon in the rotational axis has only a small amplitude decay with increasing mixing time (peak near 139 ppm in Figure 2 (a)), which can be attributed to the contribution of the motion of the dipolar vector connecting the immobile carbon with remote protons (i.e. the imidic ^1H directly bonded to ^{15}N). As shown in Figure 2 (b) and Figure 3 (b), the signal decay was fit well to a single exponential function, and the extracted exchange rates are comparable to previous results from line-shape analysis [25]. Analogous results from the ^{13}C and ^{15}N R-CODEX experiments indicate that the ^{13}C - ^1H and ^{15}N - ^1H motions are concerted (or at least have the same time constant) as expected. Moreover, the plateau values in Figure 2 (b) and Figure 3 (b) are 0.5, consistent with the two site jump model proposed with equal populations (Equation 2). We did not need to correct for the T_1 of ^{13}C and ^{15}N apparently because they are longer than 5 seconds, i.e. very long as compared with millisecond ring reorientation. The peak intensities of both carbon and nitrogen with the shortest mixing time are about 30% and 20% relative to cross polarization spectrum. This signal loss results from the intrinsic 50% loss of CODEX type experiments [7] and relaxation during the dephasing and refocusing periods.

The Geometry of the Motion

The reorientation angle of the basal ^1H - ^{13}C bond was characterized as shown in Figure 4. The intensities of both mobile and immobile carbons decay, but the intensities of mobile carbon decay much more in the long mixing time as compared with that in the short mixing time. The build-up curve of the normalized intensities $(S_{\text{short}} - S_{\text{long}})/S_{\text{short}}$ are fit using the corresponding simulations to obtain the reorientational angle as 82° with a 95% confidence interval $[70^\circ, 90^\circ]$ (The RMSD plot is in the supplemental materials). This agrees well with the reorientation angle of 81° determined from the neutron diffraction [27] (Cambridge Structural Database Entry: IMZMAL11). The relatively wide confidence interval partly results from limited S/N (the measurements were performed using natural abundance ^{13}C materials). Also, the build-up curve depends on a sine function of reorientation angle [7], and thus is relatively insensitive at large angles as shown in Figure 4c. Meanwhile, the increment unit of the $\text{R}18^\circ_1$ pulse in the dephasing and refocusing times is an incomplete cycle, which also will introduce small errors [28]. Use of an accurate dipolar coupling is important for determining the angle accurately in these simulations. An internally consistent method to extract the dipolar coupling constant for these simulations is to detect the ^{13}C - ^1H dipolar coupling constant on a similar static molecule using a 2D DIPSHIFT [29] experiment, in which the heteronuclear dipolar coupling is reintroduced during the first dimension using the same sequence element [18], $\text{R}18^\circ_1$. In the current simulations, the dipolar coupling was assumed to be 23.3 ± 0.5 kHz, the value extracted from the 2D experiment on the imidazolium ring of histidine- hydrochloride (described further in the supplemental materials). This dipolar coupling constant (corresponding to 1.09 ± 0.01 Å) is the same as the previous $\text{R}18^\circ_1$ experiment [18], but is 0.02 Å longer than the neutron diffraction result [30] due to the vibrational averaging effects [31, 32].

Discussion

Dipolar recoupling via an R-type pulse sequence element

In order to detect the change of the dipolar orientation during the mixing time, the dipolar coupling, which is averaged to zero by magic angle spinning, should be reintroduced. The use of a strong ^{13}C - ^1H or ^{15}N - ^1H dipolar coupling and a sequence with a moderately good scaling factor is advantageous because the dephasing and refocusing times can be very short, thus reducing the loss of signal and increasing the maximum detection range of the dynamic rate. Compared with other heteronuclear dipolar recoupling schemes (like T-MREV [33]),

the $R18^7_1$ (and the similar $R18^5_2$) element can be applied with use of a wide range of magic angle sample spinning frequencies (~ 10 kHz–20 kHz) while efficiently suppressing the ^1H homonuclear coupling [34]. This aspect lends it to practical use in protein studies. Furthermore, the $R18^7_1$ can be used to better digitize the dephasing curve of the strong ^1H - ^{13}C or ^1H - ^{15}N dipolar coupling, because more data points can be collected during a rotor period. These features can result in more accurate determination of the reorientational angle. In addition, the $R18^7_1$ pulse sequence recouples the single quantum heteronuclear dipolar coupling ($I^+S^z + I^-S^z$), which has the property of dipolar truncation [35, 36]. Hence the pulse sequence can specifically detect the motion of the directly connected ^1H - ^{15}N (^1H - ^{13}C) bond with little interference of the motion of the remote ^1H . These features will be discussed more in the following sections.

Effective range of correlation times that can be detected

CODEX based experiments are very efficient for detecting slow motions. However, spin lattice relaxation and the magnetization exchange due to dipolar spin diffusion complicate the detection of very slow motions. It is generally impossible to distinguish magnetization transfer due to spin diffusion from that due to chemical exchange based only on a single NMR experiment. As a result, the motional rate should ideally be much faster than the spin diffusion rate. The correlation time of ^{13}C spin diffusion can be as short as 100 ms in sparsely labeled proteins [11, 37], while in natural abundance small molecules it can be approximately 1 s [38]. However, fast magic angle spinning (up to 60 kHz) can be used to quench the ^{13}C spin diffusion even in a fully isotopically enriched protein [39, 40], and a variety of R-type pulses (Table 15 and 18 in Ref [28]) can be utilized during the dephasing and refocusing periods even during high frequency magic angle spinning. ^{15}N labeling schemes can also be used to achieve minimal spin diffusion. The dipolar coupling between the closest amides ^{15}N is only 55 Hz (e.g. for α -helix), and for this case negligible spin diffusion is expected during time intervals up to 500 ms at the moderate magic angle spinning frequencies around 10 kHz [11].

The upper limit for the detectable correlation time depends on the effect of the dynamics during the dephasing and refocusing parts. In this paper, we have assumed that there were no reorientation events occurring during the dephasing and refocusing periods. This is of course correct only when the dephasing and refocusing periods are much shorter than the correlation time of dynamics. During the dephasing and refocusing periods, the dynamics will interfere with the evolution of the dipolar couplings. This will have two practical consequences: The first is that the reorientation angle detected by R-CODEX experiment becomes artefactually smaller due to this averaging. The second is that the amplitude of the signal becomes smaller [41, 42] as shown in Figure 5, although the time constant for the motion is still faithfully represented in this experiment. Therefore, stronger anisotropic interactions and high scaling factors for the recoupling are preferred for detecting faster dynamics, because shorter dephasing and refocusing times can be used, and therefore fewer effects are observed from dynamics during the dephasing and refocusing.

The effect of vicinal protons

In most protein samples, the ^1H - ^{15}N (or ^1H - ^{13}C) bond of interest is located in a dense ^1H network. The $R18^7_1$ pulse sequence element used in the R-CODEX experiment, which can effectively suppress the ^1H homonuclear dipolar couplings during the dephasing and recoupling time, can retain larger amplitude of signal (as shown in the simulations in the supplemental materials). Moreover, R-CODEX experiments minimize the effects from the motions of additional nearby ^1H s. The single quantum dipolar spin operators generated in this R-CODEX experiment corresponding to various neighboring spin pairs ($H^+C_z^{\text{SITE1}} + H^-C_z^{\text{SITE1}}$ compared to $H^+C_z^{\text{SITE2}} + H^-C_z^{\text{SITE2}}$) do not commute, so the weak

dipolar interaction between a ^{13}C and a *remote* ^1H is truncated by the directly bonded ^1H - ^{13}C coupling. Therefore, in a R-CODEX the artefactual decay due to the motion of the remote ^1H has a very small amplitude (as shown in the Figure 6).

In summary, in this study we used an R element to dephase and rephase ^{13}C or ^{15}N magnetization via the H-X heteronuclear dipolar coupling, in the context of a CODEX like pulse sequence for characterization of slow dynamics. This scheme has a number of useful features. The use of a strong ^{13}C - ^1H or ^{15}N - ^1H dipolar coupling and a sequence with a moderately good scaling factor is advantageous because the dephasing and refocusing times can be very short, thus reducing the loss of signal and increasing the maximum detection range of the dynamic rate (elaborated further in the supplemental material). Compared with other heteronuclear dipolar recoupling schemes (like T-MREV [33]), the R18^{71}_1 (and the similar R18^{52}_2) can be applied with use of a wide range of magic angle sample spinning frequencies (~ 10 kHz–20 kHz) while efficiently suppressing the ^1H homonuclear coupling [34]. This aspect lends it to practical use in protein studies. Furthermore, the R18^{71}_1 can be used to better digitize the dephasing curve of the strong ^1H - ^{13}C or ^1H - ^{15}N dipolar coupling, because more data points can be collected during a rotor period. These features can result in more accurate determination of the reorientational angle. In addition, the R18^{71}_1 pulse sequence recouples the single quantum heteronuclear dipolar coupling ($I^+S_z + I^-S_z$), which has the property of dipolar truncation [35, 36]. Hence the pulse sequence can specifically detect the motion of the directly connected ^1H - ^{15}N (^1H - ^{13}C) bond with little interference of the motion of the remote ^1H .

Conclusions

We have developed and demonstrated a new pulse sequence, R-CODEX, for characterization of slow molecular conformational dynamics. As for other dipolar based CODEX type sequences, the uniaxial dipolar coupling tensor and relatively fixed dipolar coupling constant provide a convenient probe for the reorientation angle analysis. The use of a strong heteronuclear coupling (e.g. ^1H - ^{13}C or ^1H - ^{15}N) is advantageous, because less signal loss occurs during the sequence, making faster motions amenable to study and making angle determination potentially more accurate. We demonstrate that with this sequence quantitative measurement can be carried out with fully protonated material. All of these properties make it a convenient method to detect slow dynamics in proteins. A model motional system, the π flipping of imidazolium ring was characterized in imidazolium methyl sulfonate. The motional rate constants and reorientational angle were consistent with previous results and models [25, 26].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. An approach to detect millisecond-second dynamics
2. Both correlation time and reorientation angle can be detected
3. The effect of vicinal protons is suppressed in the dense ^1H environment.

Figure 1.

The R-CODEX pulse sequences used in these studies to detect conformational exchange rates (a) and reorientation angles (b). 90° pulses are denoted using black lines, and 180° pulses are represented by hollow rectangles. The 180° pulse in the middle of dephasing and refocusing periods refocuses the dephasing due to the isotropic chemical shift. CP: cross polarization. Tr: Rotor period. $*\text{R}18_1^7$: $\text{R}18_1^7$ with 180° phase shift to prevent averaging of the recoupled dipolar coupling by 180° pulse. The mixing time should be an integral number of rotor periods [7]. The dephasing and refocusing periods should be even multiples of the rotor periods to reduce net evolution of other anisotropic interactions. In (b), the mixing time was fixed while the number of $\text{R}\bar{\text{R}}$ blocks (denoted by one hollow (R) and one solid ($\bar{\text{R}}$) square) of the $\text{R}18_1^7$ pulse was increased systematically. All phases represented by a-g are given in the supplemental materials.

Figure 2.

(a) ^{13}C - ^1H R-CODEX spectra with different mixing times (black: 0.09 ms, blue: 1.36 ms, green: 2.73 ms, magenta: 4.55 ms, red: 27.27 ms). The peak near 139 ppm is the apical carbon in the imidazolium ring. The peak near 122 ppm is due to the two basal carbons. The two basal carbons' peaks are merged and unresolved. (b) Peak intensity vs. mixing time for apical and basal carbons. The black lines are single exponential fits with an exchange rate k_{ex} of 0.32 ± 0.03 kHz.

Figure 3.

(a) ^{15}N - ^1H R-CODEX spectra with different mixing times (black: 0.09 ms, blue: 1.12 ms, green: 2.81 ms, magenta: 6.19 ms, red: 28.12 ms). The two ^{15}N peaks are merged and unresolved. (b) Peak intensities (black squares) are plotted vs. mixing time. The black line is a single exponential fit with exchange rate k_{ex} of 0.27 ± 0.02 kHz.

Figure 4.

^{13}C - ^1H R-CODEX spectra with different R18 7_1 recoupling times during the dephasing (and refocusing) period. (black: 0 μs , blue: 20.2 μs , green: 40.4 μs , magenta: 60.6 μs , red: 80.8 μs). The mixing times are 0.18 ms in (a) and 27 ms in (b). In (c), the normalized intensities change as the R18 7_1 recoupling times are simultaneously increased (i.e. both the dephasing and refocusing time). Squares are experimental data for the immobile apical ^{13}C while triangles are experimental data for the basal mobile ^{13}C . The single lines are simulations of ^{13}C - ^1H bond exchanging with different reorientation angles, which vary from 10° to 90°. The exchange rate in the simulation is 0.3 kHz.

Figure 5.

Intensity loss during R-CODEX experiments due to exchange during dephasing is illustrated with simulations that assume different dipolar couplings and motional rate constants. In (a) simulations assumed a two-site jump of a single ^{13}C - ^1H dipolar pair (with a coupling of 23.3 kHz), a reorientation angle of 120° and equal site populations. The solid lines are the single exponential fits to the discrete simulation results; rate constants so derived were perfectly consistent with the exchange rates assumed in the simulations. In (b) the parameters were the same as those used in (a) except that the dipolar coupling was reduced to 4.6 kHz. Note that the amplitude of magnetization is reduced, particularly for the faster motions, but the simulated decay constants are again perfectly consistent with the exchange rates assumed in the simulations.

Figure 6.

The effect of motion of a remote ^1H on ^{13}C - ^1H R CODEX and Dipolar CODEX (which uses a REDOR element to recouple the ^1H - ^{13}C dipolar interaction). The simulation system contains the apical carbon, the ^1H bonded to it and the imidic ^1H bonded to ^{15}N (depicted as red in the right figure). We assume the imidazolium ring has a 180° flip with k_{ex} of 0.5 kHz. The left figure shows the remote ^1H s (imidic ^1H bonded to ^{15}N) have a substantial effect for dipolar CODEX and a modest one for R-CODEX.