ARTICLE

Characterization of slow conformational dynamics in solids: dipolar CODEX

Wenbo Li · Ann E. McDermott

Abstract A solid state NMR experiment is introduced for probing relatively slow conformational exchange, based on dephasing and refocusing dipolar couplings. The method is closely related to the previously described Centerband-Only Detection of Exchange or CODEX experiment. The use of dipolar couplings for this application is advantageous because their values are known a priori from molecular structures, and their orientations and reorientations relate in a simple way to molecular geometry and motion. Furthermore the use of dipolar couplings in conjunction with selective isotopic enrichment schemes is consistent with selection for unique sites in complex biopolymers. We used this experiment to probe the correlation time for the motion of ^{13}C, ^{15}N enriched urea molecules within their crystalline lattice.

Keywords Dynamics · Solid-state NMR · CODEX · Chemical exchange · Magic angle spinning · Urea

Introduction

Slow molecular and conformational dynamics (on the millisecond to second timescale) have important effects on the functions of proteins, for systems ranging from enzymes to molecular motors (Benkovic and Hammes-Schiffer 2003; Vale and Milligan 2000; Noji et al. 1997). A variety of challenges surround the NMR-based detection of slow conformational exchange processes, whether they are accessed through relaxation rates, lineshapes, or other means. Sensitivity or detection time is frequently an issue

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for biopolymer dynamics measurements, particularly when they are combined in multidimensional experiments with measurements of isotropic shifts to achieve site specific resolution. Moreover, very high frequency magic angle sample spinning, utilized to achieve good resolution and sensitivity of detection, averages many of the interactions that are crucial for obtaining dynamical information. Other challenges concern the interpretation of the data in terms of the system of interest. For many dynamical studies, unambiguous extraction of information characterizing the dynamical process can be difficult. For example, for many relaxation experiments, the parameters describing the motional model are hard to obtain uniquely because the fitting parameters are covariant and there are multiple fits compatible with the data. In recent years, Schmidt-Rohr and coworkers described a method called centerband-only detection of exchange, or CODEX (DeAzevedo et al. 1999, 2000), which has been used to determine the correlation time and to constrain the amplitude (or geometry) of exchange processes. This method alleviates the problems listed above. CODEX and some "post CODEX" methods (Reichert and Pascui 2003, 2008), are based on refocusing magnetization that was previously dephased under the effect of the chemical shift anisotropy (CSA). The yield of this refocusing depends on the time of storage (usually longitudinal storage), and on the motion, specifically the rate constant, and the geometry of the hop. For these applications it is important to know the principle values and orientations of the CSA tensor. The principle values and directions of CSA tensors depend strongly on the environment of the functional group (Sitkoff and Case 1998; Havlin et al. 1997). It is possible to compute the CSA values, or measure them for the limiting structures in favorable cases, although for high energy or relatively poorly populated states in a conformational exchange

process this might not always be practical. Most CSA tensors for biopolymers are non-uniaxial, which also complicates the simulations of these experiments.

Here we describe a new method which we call Dipolar CODEX that makes use of heteronuclear dipolar coupling interactions to probe dynamical processes. The dipolar interaction is useful in this context because both the strength and the orientation relate in a simple and direct way to the molecular structure. Changes in the orientation of the uniaxial dipolar interaction, which cause failure of refocusing, can be related quantitatively to the conformational exchange process. Selective detection of heteronuclear dipolar pairs can be performed in this method, thereby providing an avenue for studies of very complex biopolymers. In this paper, the Dipolar CODEX method will be demonstrated with experimental studies of crystalline urea alongside simulations performed using SPIN-EVOLUTION 3.3.3 (Veshtort and Griffin 2006).

Experimental

The $^{13}C(99\%)$, $^{15}N(98\%)$ isotopically enriched urea (Cambridge Isotope Laboratories Inc.) was recrystallized from water and crushed into powder form.

The pulse sequence for the Dipolar CODEX experiment is shown in Fig. 1. Experiments were performed on a Varian Infinity 400 MHz triple resonance instrument, using a T3 triple resonance, MAS probe in a $^{1}H/^{13}C/^{15}N$ configuration with 4 mm rotor. The spinning speeds ranged from 6 to 9 kHz (± 3 Hz). The temperature ranged from $-10°C$ to $10°C$ ($\pm 0.1°C$). Typical RF π pulse's lengths were 4.9 μs for ^{13}C, 5.6 μs for ^{15}N, and 6.2 μs for ^{1}H. The carbon's initial signal was enhanced by the adiabatic passage Hartmann-Hahn cross polarization. The constant ^{1}H RF field had an amplitude of 45 kHz for all the experiments. The ^{13}C channel's tangential RF field followed Eq. 1 (Detken et al. 2001). The ω^{HH} ranged from 36–39 kHz according to different spinning speeds. The tangential parameters Δ and β

were 24 and 13 kHz. The contact time was 1.3 ms. About 60 kHz CW ^{1}H decoupling was applied during dephasing and refocusing parts and about 60 kHz TPPM ^{1}H decoupling was applied during acquisition.

$$\omega^C(t) = \omega^{HH} + \beta \times \tan\left[\left(\frac{2t}{\tau} - 1\right) \times \arctan\left(\frac{\Delta}{\beta}\right)\right]. \quad (1)$$

During the dephasing period, with a REDOR (rotational echo double resonance) pulse sequence element (Gullion and Schaefer 1989), under the influence of the dipolar interactions, spins develop an antiphase coherence (C_yN_z) with a phase that depends on the structure and crystallite orientation. The two conformers involved in the conformational exchange process therefore develop different phases, here denoted ϕ_1 and ϕ_2. The subsequent 90-degree pulse converts C_yN_z to C_zN_z for longitudinal storage. A relatively long mixing time is introduced during which the conformational exchange process redistributes the magnetization between two exchange sites, i.e., a site that had Hamiltonian 1 (and developed a phase angle ϕ_1) during the dephasing period will experience Hamiltonian 2 during the refocusing period. If these two Hamiltonians are different, the refocusing will fail in a mixing time-dependent manner. The final intensity can therefore be represented by

$$S \propto e^{-t_m/T_1}[(\text{Sin}\phi_1 - \text{Sin}\phi_2)^2 e^{-k_{ex}t_m} + (\text{Sin}\phi_1 + \text{Sin}\phi_2)^2]/2. \quad (2)$$

Felicitously, the decay of the refocussed signal intensity is simply an exponential decay as a function of the mixing time t_m. Therefore, the exchange rate k_{ex} can be obtained from fitting this decay curve by a simple exponential decay function. The amplitudes convey geometric information; the difference between ϕ_1 and ϕ_2 which determine the magnitude of exponential decay are functions of crystallite orientation, coupling strength, dephasing time and the hop geometry.

As is done for CODEX experiments, a reference spectrum is taken by exchanging the mixing time and z-filter t_z (usually with a length of 1 rotor period). Then the normalized signal $(S_{ref} - S)/S_{ref}$ removes the effect of T_1 processes, as well as effects of spurious signals from other immobile sites.
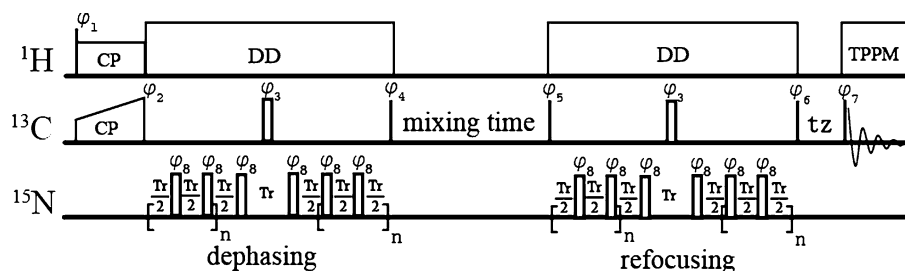

Fig. 1 Dipolar CODEX pulse program: 90° pulses are denoted using filled black lines. 180° pulses are denoted using hollow square symbols. CP adiabatic cross polarization. DD dipolar decoupling. (The detail information is in the experimental secetion) Tr: one rotor period. A REDOR (rotational echo double resonance) pulse program element was used for recoupling the ^{13}C–^{15}N dipolar coupling. The phase cycles (φ) are in the appendix (Table 2)

Results and discussion

As demonstrated previously (Williams and McDermott 1993; Emsley and Smith 1961; Vaughan and Donohue 1952; Zussman 1973; Taylor et al. 2007; Chiba 1965; Benkovic and Hammes-Schiffer 2003), crystalline urea has two kinds of symmetry-related, thermally active movements: a flip (or 180 degree rotation) of the whole molecule, and a rotation of the protons about each of the C–N bonds (Fig. 2). In our Dipolar CODEX experiments, reorientation of the whole molecule (more specifically of the C–N bonds) was detected without interference of the rotation of the amide group. Figure 3a shows the mixing time dependence of the carbon peak intensity, which is clearly an exponential decay. The normalized signals $(S_{ref} - S)/S_{ref}$ were fit using a single exponential decay function to obtain the exchange rate describing the rotation of urea (Fig. 3b). Figure 4 shows that the logarithm of the exchange rates so extracted (Table 1) gave a straight line when plotted against inverse temperature, indicating Arrhenius behavior, consistent with previous studies (Williams and McDermott 1993).

The geometric information is another point of interest in dynamics research. As mentioned in the publication describing the CODEX experiment (DeAzevedo et al. 2000), the hop angle can be obtained by systematically varying the dephasing and refocusing times, while fixing the mixing time. Simulations presented in Fig. 5a show that the Dipolar CODEX experiment actually has poor ability to distinguish different $^{15}N-^{13}C-^{15}N$ hop angles for this case. This is because both of the two ^{15}N neighbors are connected with the same (central) carbon, and they dephase the carbon's magnetization simultaneously. More generally, however, the Dipolar CODEX experiment can identify the hop angle very well (in Fig. 5b) if there is a single isolated $^{13}C-^{15}N$ pair, which is the case for the protein's peptide bonds. The most pronounced differences are generally at short dephasing times; for this situation a modification of CODEX experiment, termed CONTRA (Reichert and Pascui 2008) can be used, wherein the π pulse during the dephasing time can be moved from 0 and $Tr/2$ in steps.

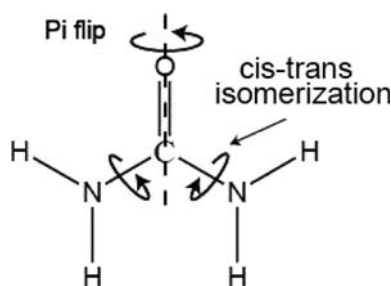

Fig. 2 The structure and reorientation process of urea

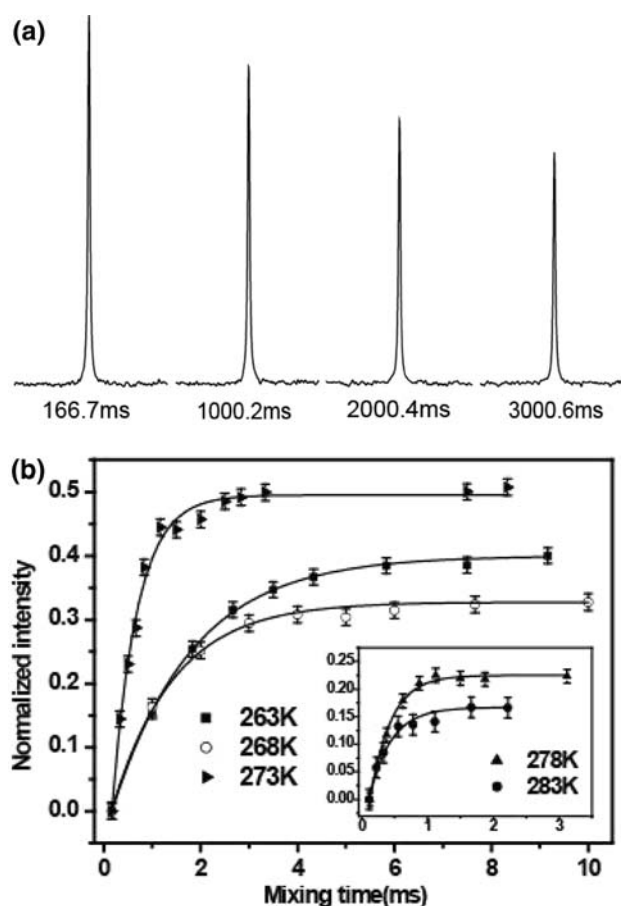Fig. 3 a The carbon center peak intensity decays with increasing the mixing time. Measurements were made at 268 K, with MAS frequency of 6 kHz, and dephasing time of four rotor periods. b The normalized center peak intensities in different temperatures were fit to a single exponential function $y = A \times e^{-k_{ex}t} + B$. The error bar was the spectrum noise divided by the reference intensity
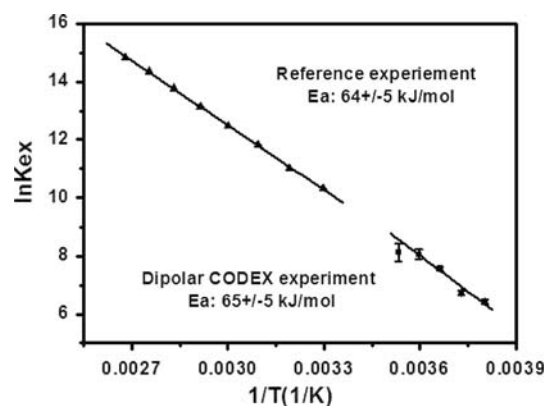

Fig. 4 Arrhenius plot of the natural log of the rate constant for urea whole body reorientation as a function of inverse temperature. The data at left top (triangle) are the prior literature data (Williams and McDermott 1993) taken at relatively high temperatures (30–100°C). The data at right bottom (square) are based on our Dipolar CODEX experiments

Table 1 Exchange rates obtained from single exponential decay fits of the peak intensities are given for different temperatures

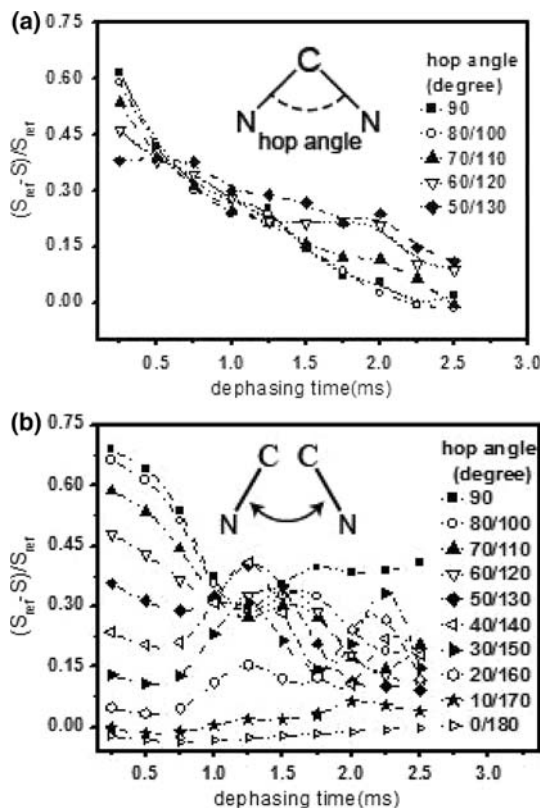| Temperature (K)	263	268	273	278	283
k_{ex} (kHz)	0.62 ± 0.05	0.84 ± 0.08	2.0 ± 0.1	3.2 ± 0.5	3 ± 1

Fig. 5 The simulation of Dipolar CODEX data for different hop angles, prepared using SPINEVOLUTION 3.3.3. **a** The urea's nitrogen atoms' 2-site jump motions. The ^{13}C–^{15}N bond length used was 1.335A (based on X-ray data in reference Vaughan and Donohue 1952). **b** The reorientation of a hypothetical ^{15}N–^{13}C isolated pair with a bond length of 1.335A. Note the strong dependence in this case on the hop angle. The simulation parameters for both models were MAS frequency of 8 kHz, an exchange rate of 800 s^{-1}, a mixing time of 5 ms and T_2 relaxation during the dephasing and refocusing period of 3.7 ms (based on our measurements at −5°C). The field strengths for both channels were 100 kHz in the simulations, similar to our experiment

Conclusion

We developed a new pulse sequence, Dipolar CODEX, for characterization of slow molecular conformational dynamics. The experimental and simulation results demonstrate its ability to detect both exchange rates and hop angle. In principal, ^{13}C–^{15}N, ^{15}N–^1H and ^{13}C–^1H couplings can be used which could provide multiple constraints on the motional mode. The flipping motion of urea molecules within a crystalline lattice was probed with this method.

Appendix

First, we will describe the recoupling of the heteronuclear dipolar coupling under MAS condition. The average Hamiltonian dominant in the evolution during an interval (t_0, t) is

$$\overline{H}(t_0, t) = \frac{\phi(t_0, t)}{t - t_0} 2\vec{I}_z \vec{S}_z \tag{3}$$

where $\phi(t_0, t)$ is the phase developed by the dipolar coupling during the interval,

$$\phi(t_0, t) = \int_{t_0}^{t} \frac{1}{\sqrt{6}} \Lambda_{20}^{Lab}(\omega_R t) dt. \tag{4}$$

The laboratory frame tensor $\Lambda_{20}^{Lab}(\omega_R t)$ can be obtained by transforming the dipolar coupling's uniaxial tensor from the principal axis frame to the rotor frame through the Euler angles $\{\alpha, \beta, \gamma\}$, and then to the laboratory frame, which causes a time-dependent due to magic angle spinning. For the REDOR pulse sequence element used during the dephasing and refocusing parts, π pulses invert the sign of the dipolar coupling Hamiltonian for every other half rotor period. Therefore, the phase accumulated in one rotor period (Tr) is

$$\phi_{Tr} = \int_{t_0}^{t_0+Tr/2} \Lambda_{20}^{Lab}(\omega_R t) dt - \int_{t_0+Tr/2}^{t_0+Tr} \Lambda_{20}^{Lab}(\omega_R t) dt$$

$$= 2 \int_{t_0}^{t_0+Tr/2} \Lambda_{20}^{Lab}(\omega_R t) dt = 2\phi(t_0, t_0 + Tr/2) \tag{5}$$

where t_0 defines the initial rotor phase $\omega_R t_0$. Therefore, after the N rotor periods during the dephasing or refocusing time, the total phase which the magnetization evolves is

$$\phi = 2 \times N \times \phi(t_0, t_0 + Tr/2)$$

$$= 2N \times \frac{-\mu_0 \hbar \gamma_i \gamma_j}{4\pi r_{ij}^3} \times \frac{2\sqrt{2}}{\omega_R} Sin2\beta \times Sin(\omega_R t_0 + \gamma) \tag{6}$$

which shows that the phase is scaled by a sine function of the Euler angle β. Thus the two exchange sites, which have different Euler angles $\{\alpha, \beta, \gamma\}$ transforming their dipolar

coupling tensor from principal to rotor frame, will develop different phases under the dominant of the dipolar coupling Hamiltonian. In the following, the product operator formalism (Sørensen et al. 1983) and an exchange matrix description are applied to illustrate how this phase difference can detect the dynamics of the two exchange sites.

The dynamical model we used is a two-site exchange model for a single ^{13}C–^{15}N pair (Fig. 5b), and we assume that there is no exchange during the dephasing and refocusing parts (although for numerical simulations the exchange during those periods was included). After cross polarization between proton and carbon, the resulting × magnetizations of the two exchange sites evolve under the action of ^{13}C–^{15}N recoupled heteronuclear dipolar coupling during the dephasing time.

$$\vec{C}_x^{1(2)} \overset{\text{dipolar coupling}}{\rightarrow} \vec{C}_x^{1(2)}\text{Cos}\phi_{1(2)} + 2\vec{C}_y^{1(2)}\vec{N}_z\text{Sin}\phi_{1(2)}. \quad (7)$$

Then a 90° pulse (phase cycle is indicated in Table 2) on ^{13}C flips the antiphase magnetization to generate longitudinal two spin order. (The remaining in-phase part will disappear during mixing time due to spin-spin relaxation and is therefore omitted.) Then, in the mixing time, the exchange process redistributes the magnetization between two exchange sites. After the exchange process,

$$\begin{pmatrix} 2\vec{C}_z^1\vec{N}_z(t_m) \\ 2\vec{C}_z^2\vec{N}_z(t_m) \end{pmatrix} = \frac{1}{2}\begin{pmatrix} (e^{-k_{ex}t_m}+1)e^{-t_m/T_1} & 1-e^{-k_{ex}t_m} \\ 1-e^{-k_{ex}t_m} & (e^{-k_{ex}t_m}+1)e^{-t_m/T_1} \end{pmatrix}$$
$$\times \begin{pmatrix} 2\vec{C}_z^1\vec{N}_z\text{Sin}\phi_1 \\ 2\vec{C}_z^2\vec{N}_z\text{Sin}\phi_2 \end{pmatrix} \quad (8)$$

where t_m is the length of the mixing time, k_{ex} is the exchange rate which is the summation of the forward and backward rates.

Finally, the 90 pulse on carbon flips the carbon's part back to the y direction and the refocusing part makes the antiphase part evolve back to x direction,

$$2\vec{C}_y^{1(2)}\vec{N}_z(t_m) \overset{\text{dipolar coupling}}{\rightarrow} -\vec{C}_x^{1(2)}\text{Sin}\phi_{1(2)} \quad (9)$$

Therefore, the final signal should be

$$S \propto \frac{1}{2}e^{-t_m/T_1}[(\text{Sin}\phi_1 - \text{Sin}\phi_2)^2e^{-k_{ex}t_m} + (\text{Sin}\phi_1 - \text{Sin}\phi_2)^2]. \quad (10)$$

The difference between urea model (Fig. 5a) and the single ^{13}C–^{15}N model (Fig. 5b) is that two nitrogen atoms connected with the center carbon atom simultaneously in urea. Therefore, after dephasing time, the carbon's magnetization in urea is

$$\bar{C}_x \rightarrow (2\bar{C}_y\bar{N}_{1z}\text{Sin}\varphi_1\text{Cos}\varphi_2 + 2\bar{C}_y\bar{N}_{2z}\text{Cos}\varphi_1\text{Sin}\varphi_2)$$
$$+ (\bar{C}_x\text{Cos}\varphi_1\text{Cos}\varphi_2 - 4\bar{C}_x\bar{N}_{1z}\bar{N}_{2z}\text{Sin}\varphi_1\text{Sin}\varphi_2) \quad (11)$$

where φ_1 and φ_2 are the phases developed under the two ^{13}C–^{15}N dipolar couplings separately. The terms in the first bracket of Eq. (11) are similar to the "SinSin" portion in original CODEX publication (DeAzevedo et al. 2000, 90_x or 90_{-x} pulse can flip them to the z-direction) while those terms in the second bracket are similar to "CosCos" portion (90_y or 90_{-y} can flip them to the z-direction). The reorientation process which exchanges N_1 and N_2 only affects the "SinSin" portion. Therefore, only "SinSin" portion was obtained in our experiment. The final signal of "SinSin" portion is

$$S \propto -\frac{1}{2}[\text{Sin}^2(\varphi_2 + \varphi_1) + \text{Sin}^2(\varphi_2 - \varphi_1)e^{-k_{ex}t_m}]e^{-t_m/T_1}. \quad (12)$$

Equation (12) is a little different from Eq. (10) but the signal in this equation has the same mixing time dependence with that in Eq. (10).

Table 2 Phase cycling for dipolar CODEX

	H_{90} φ_1	X_{CP} φ_2	C_{180} φ_3	C_{90} φ_4	C_{90} φ_5	C_{90} φ_6	C_{90} φ_7	N_{180} φ_8	Receiver
1	+x	+x	+x	−x	+x	−y	+y	+x	+x
2	−x	+x	+x	−x	+x	−y	+y	+x	−x
3	+x	+x	+y	−x	−x	−y	+y	+x	−x
4	−x	+x	+y	−x	−x	−y	+y	+x	+x
5	+x	+x	+y	+x	+x	−y	+y	+x	−x
6	−x	+x	+y	+x	+x	−y	+y	+x	+x
7	+x	+x	+x	+x	−x	−y	+y	+x	+x
8	−x	+x	+x	+x	−x	−y	+y	+x	−x

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