Threonine side chain conformational population distribution of a type I antifreeze protein on interacting with ice surface studied via ^{13}C–^{15}N dynamic REDOR NMR

Yougang Mao [a], Myongho Jeong [a], Tieli Wang [b], Yong Ba [a,*]

[a] Department of Chemistry and Biochemistry, California State University Los Angeles, 5151 State University Drive, Los Angeles, CA 90032, USA
[b] Department of Chemistry, California State University, Dominguez Hills, Carson, CA 90747, USA

A B S T R A C T

Antifreeze proteins (AFPs) provide survival mechanism for species living in subzero environments by lowering the freezing points of their body fluids effectively. The mechanism is attributed to AFPs' ability to inhibit the growth of seed ice crystals through adsorption on specific ice surfaces. We have applied dynamic REDOR (Rotational Echo Double Resonance) solid state NMR to study the threonine (Thr) side chain conformational population distribution of a site-specific Thr ^{13}C$_\gamma$ and ^{15}N doubly labeled type I AFP in frozen aqueous solution. It is known that the Thr side chains together with those of the 4th and 8th Alanine (Ala) residues commencing from the Thrs (the 1st) in the four 11-residue repeat units form the peptide ice-binding surface. The conformational information can provide structural insight with regard to how the AFP side chains structurally interact with the ice surface. χ-squared statistical analysis of the experimental REDOR data in fitting the theoretically calculated dynamic REDOR fraction curves indicates that when the AFP interacted with the ice surface in the frozen AFP solution, the conformations of the Thr side chains changed from the anti-conformations, as in the AFP crystal structure, to partial population in the anti-conformation and partial population in the two gauche conformations. This change together with the structural analysis indicates that the simultaneous interactions of the methyl groups and the hydroxyl groups of the Thr side chains with the ice surface could be the reason for the conformational population change. The analysis of the theoretical dynamic REDOR fraction curves shows that the set of experimental REDOR data may fit a number of theoretical curves with different population distributions. Thus, other structural information is needed to assist in determining the conformational population distribution of the Thr side chains.

Published by Elsevier Inc.

1. Introduction

Antifreeze proteins (AFPs) were found existing in the body sera of fish, insects, and plants living in subzero environments due to their ability to depress the freezing point of water [1–13]. The mechanism of action for the freezing point depression is attributed to the growth inhibition of seed ice crystals arising from AFPs' ability to adsorb on specific ice surfaces [14–16]. The same mechanism is also responsible for the inhibition of recrystallization of ice crystals where bigger ice crystals grow with the sacrifice of smaller ones [17]. The HPLC6 isoform of type I AFP has an α-helical secondary structure and contains four 11-residue repeat units commencing with Thr residues [18]. This AFP has the following sequence: Asp–Thr–Ala–Ser–Asp–Ala–Ala–Ala–Ala–Ala–Ala–Leu–Thr–Ala–Ala–Asn–Ala–Lys–Ala–Ala–Ala–Glu–Leu–Thr–Ala–Ala–Asn–Ala–Ala–Ala–Ala–Ala–Ala–Thr–Ala–Arg. Previous studies of side chain mutations by Baardsnes et al. suggested that the AFP ice-binding side chains comprise mutations of the Thr residues and the conserved 4th and 8th Ala residues starting from the Thrs (the 1st residues the repeat units) [19]. Afterwards, ^{13}C spin lattice relaxation NMR of site-specific ^{13}C labeled AFPs provided direct structural information to verify the AFPs' ice-binding side chains [20,21]. In the relaxation NMR experiments, methyl group ^{13}C labeled type I AFP at the Ala$_{17}$ and Ala$_{21}$ side chains [20], at the Ala$_8$, Ala$_{19}$ and Ala$_{30}$ side chains [20] and at the Thr$_{13}$ and Thr$_{24}$ side chains [21] were individually used. The dynamics of the methyl group's C–C chemical bond rotations and that of the molecular reorientation of water in contacting the methyl groups in the ice surface directly revealed which surface of the α-helical structured peptide interacted with the ice surface. The use of the site-specific ^{13}C labeled AFPs are due to two reasons: Firstly, the line shapes and the chemical shift of the methyl ^{13}C peaks belonging to different residues did not give any resolution to identify the

different methyl groups. This was especially worse for the frozen AFP solution. Secondly, the low natural abundance of ^{13}C (1.108%) was not feasible to give good enough signal-to-noise ratio within a reasonable NMR experimental time. Thus, site-specific isotope labeled AFP is essential for this study. To gain more detailed structural information with regard to how the Thr side chains interact with the ice surface, we have carried out dynamic ^{13}C–^{15}N REDOR (Rotational Echo Double Resonance) NMR [22,23] on a site-specific Thr $^{13}C_\gamma$ and amide ^{15}N doubly labeled type I AFP. Ideally, dynamic REDOR NMR is able to provide the information of conformational population distribution due to the variation of the internuclear distance of weakly coupled spin pair ^{13}C–^{15}N. The population distribution of the Thr side chains can in turn provide certain insight into how the Thr side chains structurally interact with the ice surface. We hope that this study together with our previous relaxation NMR results will provide useful information for theoreticians to model the nature of the interaction of the AFP with the ice surface.

2. Theory

To calculate the theoretical REDOR curves corresponding to the dynamic average of the dipolar couplings due to the conformational population distribution, in the following we will briefly review the dynamic REDOR theory and then derive the dynamic REDOR formulae for our specific case. The dipolar coupling angular frequency (rad s^{-1}) of an unlike spin pair of I and S with half integer magnetic quantum numbers under magic angle spinning (MAS) is given as [22]

$$\omega_D(\alpha,\beta,\tau) = \pm \frac{1}{2}D(\sin^2\beta\cos2(\alpha+\omega_r\tau)-\sqrt{2}\sin2\beta\cos(\alpha+\omega_r\tau)) \quad (1)$$

where α and β are the azimuthal angle and the polar angle, respectively, as defined by the internuclear vector with respect to the MAS axis, ω_r is the angular frequency of MAS, τ is the evolution time, $D=(\gamma_I\gamma_S h/2\pi r^3)(\mu_0/4\pi)$ being the dipolar coupling constant (rad s^{-1}) in which γ_I, and γ_S denote the gyromagnetic ratios of I

spin and S spin, respectively, h is Plank's constant, μ_0 is the permeability constant and r is the internuclear distance. The average dipolar coupling frequency (s^{-1}) under the REDOR pulse sequence and MAS for a full rotor period is given as

$$\bar{v}_D(\alpha,\beta) = \pm \frac{\sqrt{2}}{\pi}D\sin2\beta\sin\alpha \quad (2)$$

Eq. (2) is applicable to the REDOR pulse sequence where all the π pulses are placed in the half and full rotor period. The three conformers of a Thr side chain are shown in Fig. 1(a)–(c), and (d)–(e) as well. The internuclear distance of the anti-conformer is different from those of the gauche conformers. Thus, the dynamically averaged dipolar coupling frequency is the population weighted average of them as below

$$\bar{v}_D(\alpha_1,\beta_1,\alpha_2,\beta_2,\alpha_3,\beta_3) = \pm \frac{\sqrt{2}}{\pi}\sum_{i=1}^{3}P_iD_{Sli}\sin2\beta_i\sin\alpha_i \quad (3)$$

where the index i labels the three conformations and P_i denotes the corresponding population (where $P_1+P_2+P_3=1$). This equation is valid when the correlation time of the C_α–C_β chemical bond rotation is much shorter than the inverse dipolar coupling frequency. The I spin (here ^{13}C) observable I_{xobs} along the x axis in the rotating frame of reference due to the dipolar dephasing by the S spin (here ^{15}N) is given as

$$I_{xobs} \propto \cos(\bar{v}_D(\alpha_1,\beta_1,\alpha_2,\beta_2,\alpha_3,\beta_3)N_rT_r)$$
$$\propto \cos\left(N_rT_r\frac{\sqrt{2}}{\pi}\sum_{i=1}^{3}P_iD_{SIi}\sin2\beta_i\sin\alpha_i\right) \quad (4)$$

where N_r and T_r denote the number of rotor periods and the length of one rotor period, respectively. The dipolar dephasing time τ at the end of N_r rotor cycles is $\tau=T_rN_r$. For a solid powder, the integrated signal intensity over the entire spatial orientations after normalization is

$$S(\tau)/S_0 = \frac{1}{8\pi^2}\int_{\psi=0}^{2\pi}\int_{\theta=0}^{\pi}\int_{\phi=0}^{2\pi}\cos\left(N_rT_r\frac{\sqrt{2}}{\pi}\sum_{i=1}^{3}P_iD_{Sli}\sin2\beta_i\sin\alpha_i\right)$$
$$\times d\phi\sin\theta d\theta d\psi \quad (5)$$

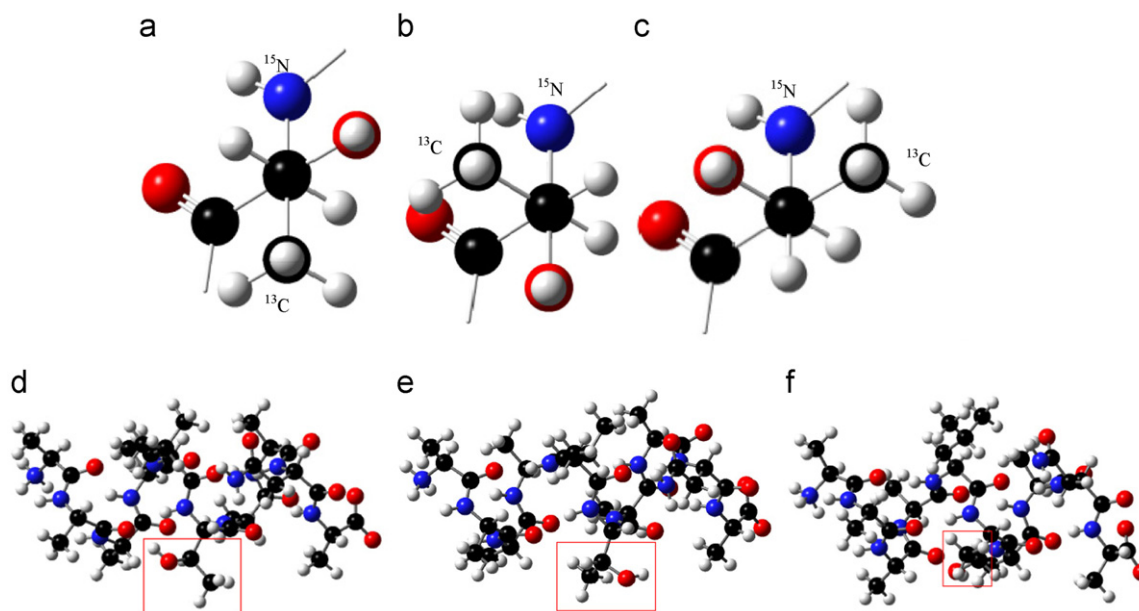**Fig. 1.** (a), (b), and (c) show the Thr side chain conformations, where the β carbon atom is over the α carbon atom in each of the diagrams. The Thr-13 side chains inside the given squares in (d), (e), and (f) show the relative orientations with respect to the local structure in a section of the Type I AFP. (Nitrogen is shown in blue color, oxygen red, carbon black, and hydrogen gray: (a) Anti, (b) Gauche 1, (c) Gauche 2, (d) Anti, (e) Gauche 1 and (f) Gauche 2) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).