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^{15}N CEST data and traditional model-free analysis capture fast internal dynamics of DJ-1

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ABSTRACT

Previous studies have shown that relaxation parameters and fast protein dynamics can be quickly elucidated from ^{15}N-CEST experiments [1]. Longitudinal R_1 and transverse R_2 values were reliably derived from fitting of CEST profiles. Herein we show that ^{15}N-CEST experiments and traditional modelfree analysis provide the internal dynamics of three states of human protein DJ-1 at physiological temperature. The chemical exchange profiles show the absence of a minor state conformation and, in conjunction with ^{1}H-^{15}N NOEs, show increased mobility. R_1 and R_2 values remained relatively unchanged at the three naturally occurring oxidation states of DJ-1, but exhibit striking NOE differences. The NOE data was, therefore, essential in determining the internal motions of the DJ-1 proteins. To the authors' knowledge, we present the first study that combines ^{15}N CEST data with traditional model-free analyses in the study of a biological system and affirm that more 'lean' model-free approaches should be used cautiously.

Introduction

NMR spectroscopy is a powerful tool for the study of protein structures and dynamics in the solution state. Over the years, many NMR methods have been developed to observe protein dynamics for a range of timescales [2]. In which, fast timescale dynamics have been traditionally studied using two-dimensional (2D) ^{1}H-^{15}N HSQC R_1, R_2, and heteronuclear NOE experiments with the Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion approach [3]. The T_1, T_2 and NOE data obtained from these experiments are routinely used to characterize sub-nano to millisecond protein dynamics with modelfree formalism [4,5]. The CPMG approach has also been extended to the study of conformational exchange due to its sensitivity to chemical shift differences between ground and excited states [6]. However, CPMG relaxation dispersion fails for proteins undergoing slow conformational exchange or for lowly populated excited states [7].

Recent advances employing saturation transfer, such as chemical exchange saturation transfer (CEST) and dark-state exchange saturation transfer (DEST), have enabled the detection of these previously invisible protein states [7,8]. Several studies have already reported the use of CEST to study the invisible conformers of slowly exchanging proteins on the millisecond to second timescale [1,9–12]. Additionally, the fitting of CEST profiles have been shown to reliably extract R_1 and R_2 parameters that can be used for modelfree analysis of fast timescale dynamics (ps to ns). Thus, the simultaneous measurement of both fast and slow timescale dynamics is possible with the CEST experiment. The extraction of the R_1 and R_2 parameters is particularly advantageous due to the fact that CEST and CPMG experiments can be acquired in a similar amount of experimental time [12]. To date, however, no study has combined CEST-derived R_1 and R_2 parameters with ^{1}H-^{15}N NOE data to establish the picosecond to nanosecond dynamics of a protein. Instead, leaner versions of modelfree have been applied without the NOE data [1].

The NOE is a sensitive measure of the high frequency motions as it reports directly on the structure of the protein and is strongly associated with its correlation time (τ_c) [13]. Therefore, the heteronuclear NOE experiment has been essential to traditional dynamics analyses in conjunction with R_1 and R_2 values. The importance of the NOE is strengthened by the fact that, at the expense of precise R_1 measurements, only precise NOE and R_2 values are necessary to calculate a reliable S^2 [14]. Additionally, the NOE is more sensitive than the R_1 parameter for capturing internal dynamics [10]. The significance of the NOE to the understanding of fast protein dynamics is considerable and we present further evidence to substantiate the use of NOE data for

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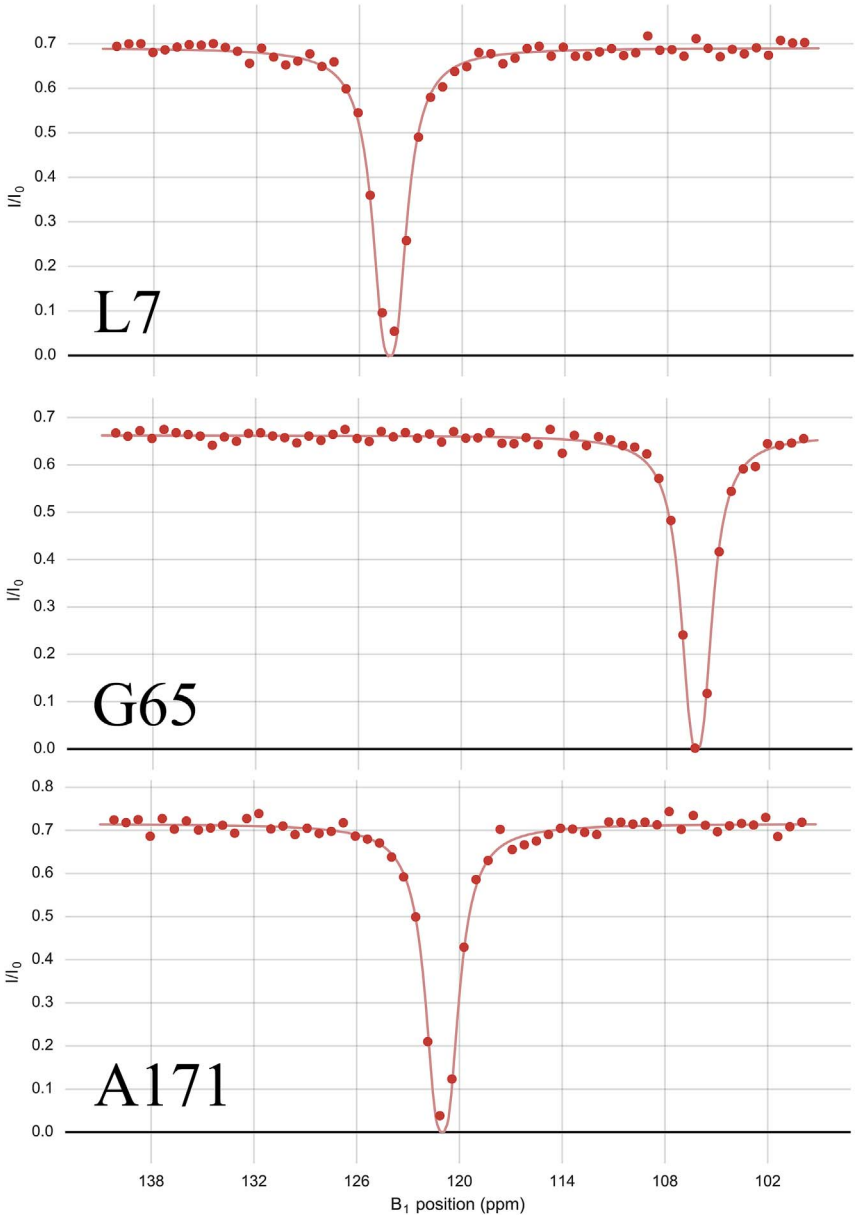

Fig. 1. Representative CEST profiles for 3 residues in DJ-1 Cys106-SH at 35 °C. The profiles show the proper fitting of the dip in intensity and the lack of a noticeable minor state conformation. Residues were chosen based on position in the primary sequence to highlight the consistency of the fitting of the profiles.

Table 1
Average R_1, R_2, NOE, and S^2 values from DJ-1 Cys106-SH at 35 °C.

Exp. Type	Protein	Observed R_1[a]	R_1 Error[b]	Observed R_2[a]	R_2 Error[b]	Observed NOE[a]	NOE Error[b]	Calculated S^2[a]	S^2 Error[b]
Traditional (35 °C)	DJ-1, Cys106-SH	0.78 (0.10)	0.06	19.30 (2.30)	0.7	0.79 (0.11)	0.15	0.92 (0.10)	0.02
CEST (35 °C)	DJ-1, Cys106-SH	0.72 (0.21)	0.06	19.33 (3.50)	2.1	0.79 (0.15)	0.11	0.88 (0.12)	0.07

[a] Standard deviations are in parenthesis.
[b] The reported errors are the standard error of the mean.

Table 2
Average R_1, R_2, NOE, and S^2 values from different physiological states of DJ-1.

Exp. Type	Protein	Observed R_1[a]	R_1 Error[b]	Observed R_2[a]	R_2 Error[b]	Observed NOE[a]	NOE Error	Calculated S^2[a]	S^2 Error[b]
CEST (37 °C)	DJ-1, Cys106-SH	0.72 (0.22)	0.06	19.50 (3.46)	2.1	0.79 (0.16)	0.11	0.86 (0.15)	0.07
	DJ-1, Cys106-SO_2^-	0.78 (0.13)	0.08	20.65 (2.66)	1.3	0.80 (0.16)	0.14	0.92 (0.13)	0.04
	DJ-1, Cys106-SO_3^-	0.71 (0.21)	0.06	18.47 (4.96)	1.0	0.64 (0.42)	0.10	0.76 (0.22)	0.04

[a] Standard deviations are in parenthesis.
[b] The reported errors are the standard error of the mean.