Sensitivity Comparison of W-band EPR Instruments and Dynamics Investigation of Spin-labeled IA_3 by HiPER

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Introduction

IA_3, an intrinsically disordered protein (IDP), is a potent inhibitor for yeast proteinase A (YprA), which undergoes a disorder-to-α-helix transition when binding with YprA or when exposed to the secondary structure stabilizer 2,2,2-trifluoroethanol (TFE). Consequently, the overall rotational diffusion and backbone dynamics change during the structural evolution. Site-directed spin labeling Electron Paramagnetic Resonance (SDSL-EPR) is a versatile technique capable of characterizing these rotational diffusion dynamics. Among the different mw-frequency regimes, W-band EPR spectra provide a "sweet spot" for charactering this IDPs [1]. Our aim in this study is to demonstrate the enhanced sensitivity of a new quasi-optical W-band EPR instrument known as HiPER, specifically in comparison to the commercially available Bruker Elexsys 680 W-band EPR instrument. We aim to show that with the heightened sensitivity afforded by the technical advantages of the HiPER instrument we can obtain a more detailed characterization of structural transitions in IDPs such as IA_3.

Experimental

In order to determine the sensitivity limit of the HiPER W-Band EPR spectrometer, we studied solutions of spin-labeled IA_3 (E10P1) constructs having protein concentrations ranging from 10 to 266 μM. We set the measurement to be in similar data acquisition conditions for HiPER and W-band Bruker respectively. Representative EPR spectra are shown in the inset of Fig. 1a and the corresponding signal to noise ratio (SNR) is shown in Fig. 1a. We define SNR as the ratio of the peak-to-peak intensity of the largest feature to the standard deviation of the background noise. HiPER measurements on IA_3 E10P1 with different TFE fractions are shown in Fig. 1b.

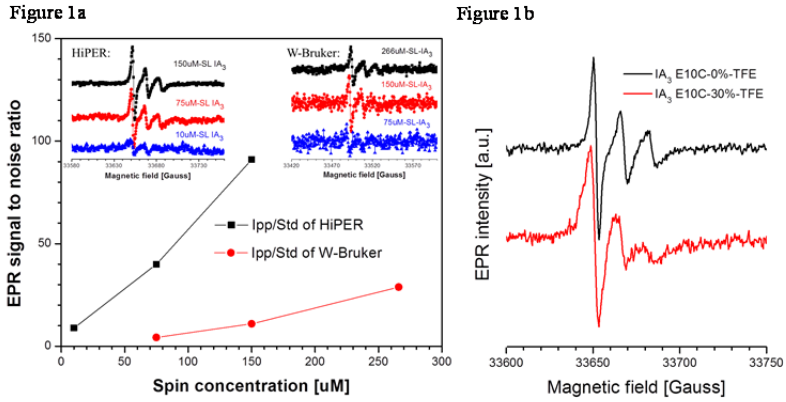

Fig. 1. HiPER and W-band Bruker EPR spectra of spin-labeled IA_3 with different concentration (1a inset), signal to noise ratio of HiPER and W-band Bruker with different spin concentration (1a), HiPER measurements of E10P1 with 0 and 30% TFE fraction (1b).

Results and Discussion

The results in Fig. 1a suggest that HiPER offers significantly higher SNR when compared with the Bruker Elexsys 680. With this, HiPER can allow for characterization of protein dynamics at concentrations down to 10 μM, previously inaccessible with commercially available instrumentation. The improvement enables us to do the EPR study of the protein dynamics with low protein concentration which is essential for our future research. At the lower concentrations, issues such as protein aggregation and lineshape broadening due to dipolar coupling are minimized. Fig. 1b shows HiPER measurements of IA_3 with 0% and 30% TFE fraction in which line shape parameter experiences a dramatically change when we increase to 30% TFE. The results suggest that introduction of 30%-TFE slows down the protein dynamics dramatically, which confirms the structural transition with the structural stabilizer – TFE.

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References

[1] Casey, T.M., *et al.*, Biochem. Bioph. Res. Comm., **450**, 723-728 (2014).