UmuD Homodimeric Complex: Distance Calculation Using Electron Spin Resonance

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Introduction

UmuD is involved in DNA repair in Escherichia Coli and part of the Y-family polymerase [1]. Homodimers ($UmuD_2$) are formed in solution and becomes activated by latent proteolytic activity induced by interaction with RecA nucleoprotein filament, leading to the cleavage of the N-terminal first 24 amino acid. Only a partial NMR structure, crystal structure for this protein are available for the N-terminal (supposed unstructured).

Experimental

The experiments were performed on a UmuD variant with a S60A mutation, prohibiting the self cleavage of the arms and leading to a variant that mimics UmuD. The protein posses a single native cysteine at the position 24 (cleavage site), where the protein was labeled with N-(1-oxy-2,2,5,5-tetramethyl-4-piperidinyl) maleimide (MSL). The sample was supplemented by 30% Glycerol as cryoprotectant prior to snap freezing in liquid nitrogen.

Results and Discussion

One the one hand, no line broadening of the CW conventional EPR spectra confirm the absence of short distance (<20Å). But on the other hand, DEER experiments with 1 and 2µs (shown in Figure 1) evolution time show evidence of a relatively broad population (from 25 to 39 Å width) with an interspin distances from 34 to 41 Å respectively.

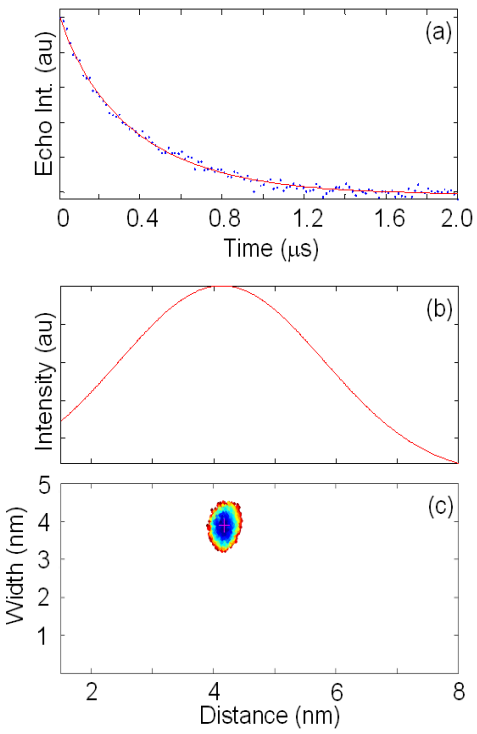

Figure 1: 2µs evolution time DEER experiment: **(a)** DEER spectrum (points) with corresponding best fit (solid line). **(b)** Gaussian distance distributions corresponding to the best fit, and **(c)** χ^2 error surface.

Conclusions

These preliminary experiments show the feasibility of our attempt to approach the N-terminal structure of this $UmuD_2$ variant in solution.

References

[1] Jarosz, D. F., *et al.* TRENDS in Microbiology **15**(2), 70 (2007).
[2] Beuning, P. J., *et al.*, Journal of biological chemistry **281**, 9633-9640 (2006).