Pulsed EPR-based Approach for Mapping Global Structures of DNAzymes

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Introduction

DNAzymes are oligomers of DNA whose sequence provides them with catalytic functionality and could serve as medical diagnostics, functional components in nanotechnologies, or even drugs [1]. However, structural determination of DNAzymes is comparatively underexplored and only very limited structural information is available for DNAzymes. The technique of SDSL-EPR (Site-Directed Spin Labeling-Electron Paramagnetic Resonance) spectroscopy has emerged as an efficient tool to elucidate the structure and the conformational dynamics of biomolecules such as proteins and nucleotides under conditions close to the native state of the system under investigation [2]. Pulsed EPR, such as double electron–electron resonance (DEER), can yield distances of 15–100 Å, which can be used to determine the global structure of a macromolecule and to monitor conformational changes. Also, SDSL-EPR distance measurements do not require crystalline samples and are not limited by the molecular weight of the system, making the method suitable for systems that are difficult to study using X-ray crystallography or NMR spectroscopy. In this research, we combined SDSL with DEER technique to measure the distances between different positions in a Zinc-specific DNAzyme.

Experimental

Spin labeling of DNAzyme was carried out in UIUC. For the pulsed-EPR measurement, each DEER sample contains 50-100 uM of labeled DNA, 50mM MES-Na, 50mM NaNO3 and 45%(v/v) glycerol, pH 5.5. X-band DEER measurements were carried out at 65 K on a Bruker E680 spectrometer equipped with a MD4 resonator, at the NHMFL. Inter-spin distance distributions were computed from the resulting dipolar evolution data using Defit software [3].

Results and Discussion

Spin labels have been successfully introduced into the DNAzyme as shown in **Fig.1**. DEER traces were recorded and analyzed. Analysis suggests that the mean distances obtained from the DEER experiment are in general consistent with those from previous crystal structure. The distance between 18G and 27T shows a broader distribution than the distance between 11T and 18G, is likely due to different conformations adopted by the spin label introduced at 27T.

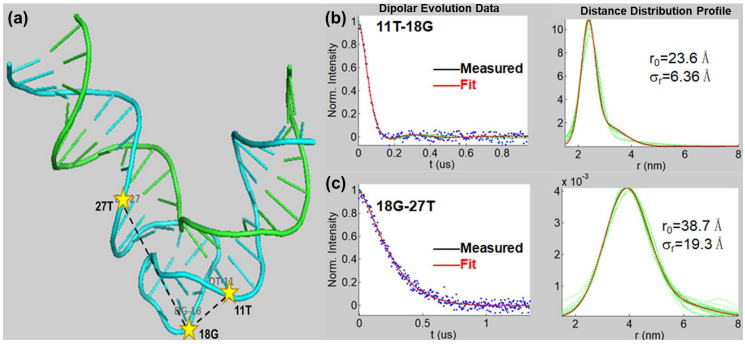

Fig.1 DEER measurement on spin-labeled DNAzyme. (a). Structure of the DNAzyme in this research with asterisks showing the labeling sites; (b). DEER measurement of distance between site 11T and 18G; (c). DEER measurement of distance between site 18G and 27T.

Conclusions

Altogether, our results confirmed that SDSL-EPR measurements can be successfully applied to study the topology of DNAzymes. Following research will focus on distance measurement between different sites on a new DNAzyme in order to get a clearer view of the structure and deeper insights into its catalytic mechanism.

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