Site-Directed Spin Labeling W-Band Study of Local RNA Base Dynamics in the Glycine Riboswitch Kink-Turn Motif using HiPER

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Introduction

RNA riboswitches are regions of mRNA transcripts that exhibit the ability to independently and selectively bind a cognate ligand to induce genetic regulation without the obligate aid of any protein counterpart. Binding of the cognate ligand induces RNA secondary and tertiary structural rearrangement that is necessary for regulation of downstream genes associated with the bound ligand. Investigation of changes in local dynamics upon conformational rearrangement, due to RNA folding and ligand binding, is well suited for study by site-directed spin labeling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy. Nitroxide spin labels used in SDSL exhibit EPR lineshapes that are sensitive to changes in local dynamics and offer a method for monitoring site-specific variations within a biomolecule. Continuous wave (CW) EPR at both X-Band (9.5 GHz) and W-band (94 GHz) are complementary experiments that provide sensitivity to a broad range of motional time scales [1]. W-band spectra have greater sensitivity to dynamic motions in the “fast-motion” regime (rotational correlation times <2 ns) and X-band spectra are more sensitive to varying rotational correlation times greater than 1 ns. In the current study, X-Band EPR was utilized to probe dynamics within an important RNA structural motif of the glycine riboswitch, known as the kink-turn motif. The resultant spectra suggest fast motion dynamics where W-band EPR is expected to exhibit greater sensitivity. To further complement the characterization of local dynamic changes upon RNA folding in the glycine riboswitch, experiments utilizing W-band HiPER spectrometer were performed.

Experimental

The kink-turn motif of the glycine riboswitch is shown in Fig. 1A. Two nucleobases, U(2) and U(3) (also indicated in Fig. 1A), are expected to exhibit differential dynamics. The former is predicted to participate in base stacking and the latter to undergo base flipping upon RNA folding in the presence of salts and glycine ligand. To characterize differences in base dynamics at these sites, SDSL, shown in Fig. 1B, was performed for each independent site and CW EPR spectra were collected at both X- and W-band. Fig. 1C shows W-band spectra collected using HiPER for the U(2) site and similar experiments for the U(3) site are forthcoming. To monitor changes in local dynamics, four RNA folding states were probed by the presence or absence of 100 mM KCl, 5 mM $MgCl_2$, and 5 mM glycine.

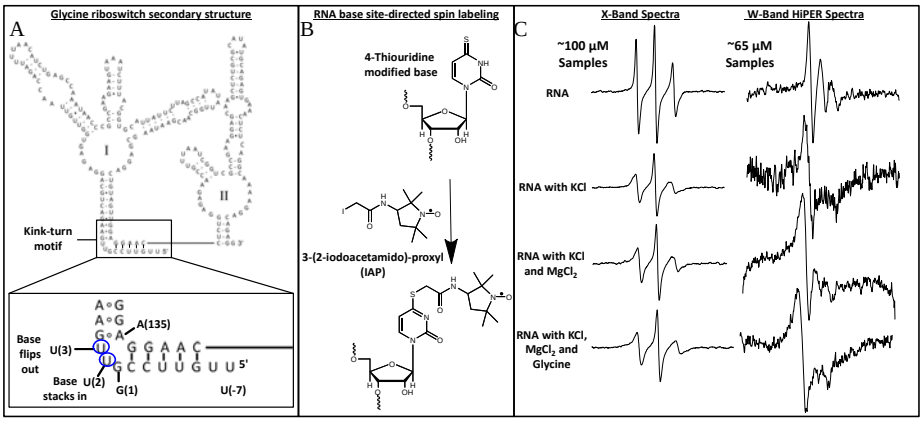

Fig. 1. (A) RNA glycine riboswitch secondary structure with kink-turn motif boxed and labeled sites indicated with blue circles (B) RNA site-directed spin labeling strategy (C) X-band and W-band HiPER spectra for the U(2) site shown in panel A

Results and Discussion

The hypothesis that base mobility will decrease due to base stacking at site U(2) is supported by the collected X-band and W-band (HiPER) spectra which show line shape broadening upon RNA folding in the presence of salts and ligand and complementary characterization of site U(3) with HiPER is in progress.

Acknowledgements

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References

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