Solid-State NMR Reveals the Transmembrane Structure and Dynamics of Microsomal Cytochrome P450 Reductase

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Introduction

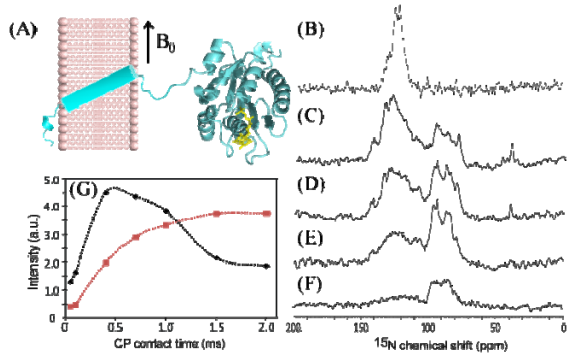
Approximately 75% of the pharmaceuticals in use today are metabolized by a group of heme-containing monooxygenases known as the cytochrome P450 (cyt P450) superfamily. These enzymes catalyze a variety of chemical transformations by activating stable carbon-hydrogen bonds of alkanes using oxygen and electrons from their redox partners. Cytochrome P450 reductase (CPR), an essential redox partner of cyt P450, is a membrane-bound protein responsible for shuttling electrons from NADPH to cyt P450. Additionally, CPR plays an indispensable role in the development of high-level organisms; it is also able to reduce cytochrome c, cytochrome b_5 and heme oxygenase, as well as a variety of therapeutic prodrugs. Rat CPR is a multi-domain membrane protein composed of a soluble 70 kDa carboxyl terminal catalytic domain, a 5 kDa amino terminal transmembrane (TM) segment, and a 2 kDa linker connecting these two domains. A complete understanding of the physiological function of the TM domain requires a thorough characterization of its structure and dynamics in a lipid membrane environment. Here, we present the first solid-state NMR investigation on the structural and dynamics features of the amino terminal FMN binding domain of CPR – including the TM domain and linker region – in a native-like membrane environment.

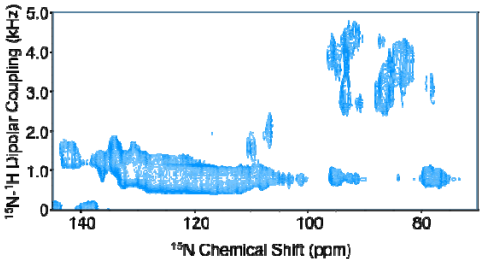
Experimental

All 1D ^{31}P and ^{15}N spectra were obtained from a 600 MHz Bruker NMR spectrometer using a low-E bicelle coil probe at the NHMFL. 2D Separated Local Field (SLF) spectrum was obtained using a SAMPI4 sequence on a 720 MHz Varian NMR spectrometer All experiments were recorded at 20 °C.

Results

In this study, experiments were performed on a uniformly ^{15}N-labeled membrane-bound FMN binding domain (MFBD) in bicelles composed of DLPC/DHPC/cholesterol, the schematic of which is shown in Fig. 1. 2D separated local field (SLF) spectra, which correlate ^{15}N chemical shift with ^{1}H-^{15}N dipolar coupling, were obtained to characterize the structure and further investigate the dynamics of the TM domain (Fig. 2).





(Left) FIGURE 1 (A) A schematic of MFBD with its N-terminal TM domain incorporated in lipid bilayers. ^{15}N NMR spectra were obtained using RINEPT (B) and ramped cross-polarization (CP) with a contact time of 2.0 ms (C), 1.0 ms (D), 0.4 ms (E), 0.1 ms (F). The RINEPT spectrum (B) shows spectral intensity only in the 100-140 ppm region, indicating the high mobility of the soluble domain. Resonances in the 70-100 ppm region in the CP spectra (C-F) are assigned to the immobile TM domain. Buildup curves (G) were measured for resonances at 93.5 ppm (black) and 128.4 ppm (red) representing the two chemical shift regions corresponding to two different domains.

(Right) FIGURE 2 2D ^{15}N separated-local-field solid-state spectrum of uniformly ^{15}N labeled MFBD incorporated in DLPC/DHPC/cholesterol bicelles. This spectrum was collected with a 0.5 ms CP contact time and 25 kHz SPINAL16 decoupling.

Conclusions

In conclusion, we have shown the first structural and dynamic study on the FMN binding domain of CPR including the TM helix and the linker region in the protein's native-like membrane environment using solid-state NMR. We have provided the first experimental evidence of an α-helical conformation for the rigid TM anchor of CPR.

Acknowledgements

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