Backbone Resonance Assignment of a Transmembrane Peptide in a Biological Environment

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Introduction

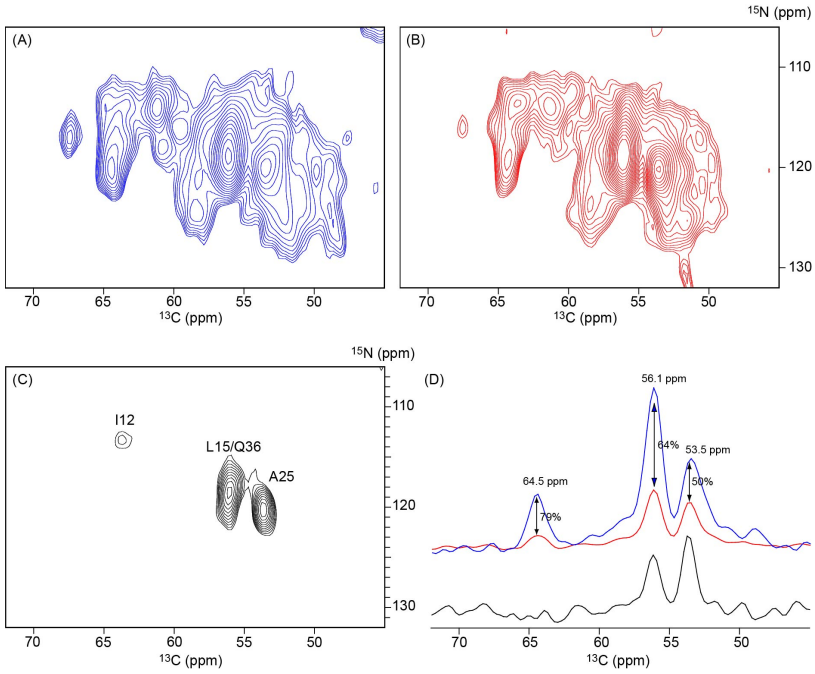
In our current *in situ* NMR spectra of membrane proteins in native *E. coli* membrane vesicles, the ^{13}C resonance linewidth is ~1 ppm, while the ^{15}N linewidth suffers from heterogeneous broadening and is ~2 ppm [1-3]. The limited spectral resolution, which is typical for non-crystalline samples including membrane proteins reconstituted into liposomes, impedes resonance assignment and structural studies. Amino acid selective labeling, commonly used to reduce the number of observed resonances, is not effective in situations like this since ^{13}C chemical shifts of a particular amino acid usually overlaps in the sample spectral region. Amino acid ^{13}C-^{15}N pair labeling is useful but expensive. We have overcome this difficulty for resonance assignment by using a combination of the reverse amino acid labeling strategy and spectral editing techniques.

Experimental

NMR data were collected on Bruker 600 and 800 MHz spectrometers on a ^{13}C, ^{15}N-labeled, Pro and Phe unlabeled LR11 transmembrane (TM) in native *E. coli* inner membrane vesicles.

Results and Discussion

Figures (A)-(C) show 2D ^{15}N-$^{13}C_\alpha$ correlation spectra of a ^{13}C, ^{15}N-labeled, Pro and Phe unlabeled LR11 TM in *E. coli* inner membrane vesicles. **Figure (A)** acquired with the FDR dephasing-off [4], is similar to conventional NCA correlation spectrum. Due to limited spectral resolution, resonances are highly overlapped. **Figure (B)** was acquired with FDR dephasing-on to selectively attenuate signals from $^{13}C'$-^{15}N, but not $^{12}C'$-^{15}N, spin pairs. Thus resonance intensity from residues following unlabeled Pro and Phe remains about the same to that in (A), while intensity from others is greatly reduced. **Figure (C)** is the difference of spectra (A) and (B), which selectively detected ^{15}N-$^{13}C_\alpha$ cross-peaks from residues that immediately following the unlabeled Pro and Phe residues. Based on their characteristic $^{13}C_\alpha$ chemical shifts, we were able to achieve residue specific assignments. **Figure (D)** 1D slices along ^{13}C dimension at ^{15}N=120 ppm from 1(A) to 1(C) highlight differential reductions in resonance intensity from FDR dephasing, and the resulting difference spectrum.



Conclusions

We have successfully *de novo* assigned ~75% of $^{13}C_\alpha$ chemical shifts of LR11 TM in native *E. coli* membrane vesicles using this strategy.

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