CONFORMATIONAL CHANGES IN CALMODULIN PROTEIN UPON CALCIUM BINDING

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RESULTS & DISCUSSION

1. Intact HDX

Changes in calcium binding protein, calmodulin, utilizing a Waters nanoACQUITY UPLC® System with Protein Lynx Global Server™ (PLGS) and Waters HDX Browser software was used to link the labeled ion data with peptide information in a chromatogram to identify conformational changes. No visible change and minor change were observed upon various conditions for stability experiments.

2. Peptide HDX

We report a recent HDX MS study of conformational changes in important intracellular protein, calmodulin with calcium bound (holo) and without calcium (apo). The analytical column was an ACQUITY UPLC® BEH C18 1.7 µm 5 x 2.1 mm. ESI-MS in positive mode was used. Capillary/Cone: 3.0 kV/37 V, Source/Detector temperature: 120 °C/300 °C. Deconvoluted intact mass was determined for all peaks by running fast intact HDX screening.

3. HDX results in 3D structure and Heat map

Figure 3. Deuterium uptake information represented in CaM apo and holo 3D protein structure. (A) apo CaM and (B) holo CaM. The orange color region represents the same region of CaM were compared in black curves for apo and holo. Because of conformational change in apo CaM, increased deuterium incorporation was found.

CONCLUSIONS

- The nanoACQUITY UPLC System with HDX Technology was very useful as a robust HDX MS platform for studying protein conformation.

- Global and local conformation of CaM was analyzed. The peptide-level comparison revealed the location of conformational changes. No visible change and minor change were observed upon various conditions for stability experiments.

- This HDX information is useful to better understand biological properties of the biomolecules.

REFERENCES


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