Spontaneous Calcium-Independent Dimerization of the Isolated First Domain of Neural Cadherin

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Introduction

Cadherins are calcium-dependent, transmembrane adhesion molecules that assemble through direct noncovalent association of their N-terminal extracellular modular domains. As the transmembrane component of adherens junctions, they indirectly link adherent cells' actin cytoskeletons. Here, we investigate the most distal extracellular domain of neural (N-) cadherin (NCAD1), a protein required at excitatory synapses, the site of long-term potentiation. This domain is the site of the adhesive interface, and it forms dimer spontaneously without binding calcium.

Results and Discussion

Proteolytic mapping of NCAD1 with protease XIII was performed with NHMFL's 14.5 T FT-ICR mass spectrometer, to determine sequence coverage for hydrogen/deuterium-exchange mass spectrometry (HDX-MS) experiments. The proteolytic susceptibility to protease XIII was determined for samples of the NCAD1 monomer (Figure 1) and dimer (not shown). Our results support the idea that NCAD1 is missing critical contacts that facilitate the rapid exchange of the βA-strand. Furthermore, monomer and dimer have equivalent and exceptionally high intrinsic stability for a 99-residue Ig-like domain with no internal disulfides (Tm = 77 °C; ΔH= 85 kcal/mol). The studies reported here improve our understanding of the essential biophysics of an atypical Ig-like domain that is the site of the adhesive interface of N-cadherin.

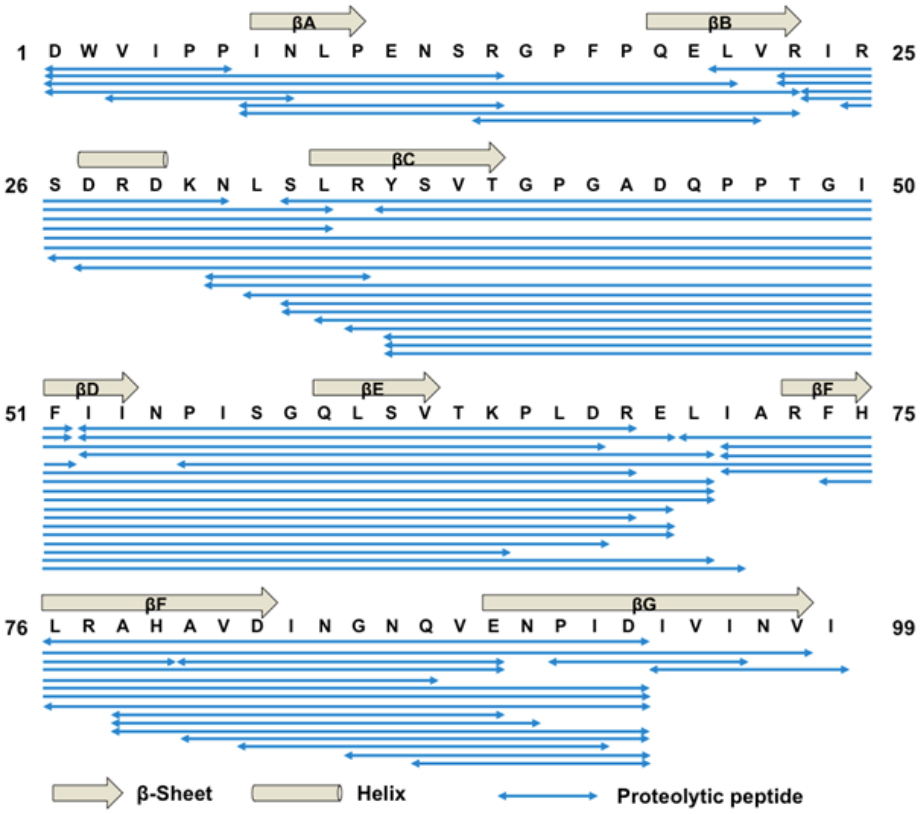

Fig. 1. 14.5 T FT-ICR-MS-derived sequence coverage map for NCAD1 monomeric stock by protease XIII. Blue arrow bars below the primary sequence of NCAD1 denote proteolytic peptide coverage from the digestion experiment. The secondary structure above the sequence represents the βA- through βG-strands throughout the sequence.

Acknowledgments

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References

[1] Davila, S.; *et al.*, Biochemistry, **57**, 6404-6415 (2018).