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ARTICLE

Resonance assignment of an engineered amino-terminal domain of a major ampullate spider silk with neutralized charge cluster

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Abstract Spider dragline fibers are predominantly made out of the major ampullate spidroins (MaSp) 1 and 2. The assembly of dissolved spidroin into a stable fiber is highly controlled for example by dimerization of its amino-terminal domain (NRN) upon acidification, as well as removal of sodium chloride along the spinning duct. Clustered residues D39, E76 and E81 are the most highly conserved residues of the five-helix bundle, and they are hypothesized to be key residues for switching between a monomeric and a dimeric conformation. Simultaneous replacement of these residues by their non-titratable analogues results in variant D39N/E76Q/E81Q, which is supposed to fold into an intermediate conformation between that of the monomeric and the dimeric state at neutral pH. Here we report the resonance assignment of *Latrodectus hesperus* NRN variant D39N/E76Q/E81Q at pH 7.2 obtained by high-resolution triple resonance NMR spectroscopy.

Keywords Spider silk · Amino-terminal domain · Major ampullate spidroin 1 · Dimerization · *Latrodectus hesperus* · Acidic charge cluster

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Biological context

The terminal domains of major ampullate spidroins control the assembly of dragline spider silks intermolecular protein contacts (Hinman and Lewis 1992; Xu and Lewis 1990). Upon lowering the pH and decreasing the sodium chloride concentration of the protein solution along the spinning duct, the amino-terminal domain forms a homodimer and, thereby, initiates spidroin assembly (Gaines et al. 2010; Hagn et al. 2010; Landreh et al. 2010). The sequence of NRN is highly conserved between different silk types and spider species, underlining the crucial role of NRN for controlling spidroin assembly (Bini et al. 2004; Chen et al. 2012; Garb et al. 2010; Rising et al. 2006). X-ray crystal and NMR solution structures of *Euprosthenops australis* NRN revealed separation of acidic and basic amino acids, prearranging the dimer in antiparallel (Askarieh et al. 2010). The negative charge cluster is composed of three highly conserved residues—D39, E76 and E81—that are hypothesized to control the pH-dependent dimerization and the simultaneous conversion into a tighter conformation, as evidenced by NMR and crystals structures. Neutralization of the acidic charge cluster suppresses the electrostatic repulsion between helices 2 and 3, which leads to their rearrangement and subsequent flattening of the dimerization interface (Bini et al. 2004; Kronqvist et al. 2014). To study the role of the acidic cluster during the

conformational change, the clustered aspartic and glutamic acid residues were simultaneously substituted by their non-titratable analogues asparagine and glutamine, resulting in the NRN variant D39N/E76Q/E81Q. This variant mimics a protonated state of NRN at low pH, which reflects conditions as found close to the end of the spider's spinneret. The majority of published research was done on *E. australis* MaSp1. For NRN from the black widow spider *Latrodectus hesperus*, solely monomeric wild type resonance assignments, but no structural coordinates or NMR distance restraints were published [Hagn et al. 2011, BioMagResBank (BMRB) accession code 17131]. Here we collected three-dimensional NMR data of D39N/E76Q/E81Q and assigned backbone as well as sidechain resonances.

Methods and experiments

Protein expression and purification

The variant *L.h.* MaSp1 NRN D39N/E76/E81Q was obtained by cloning MaSp1-NRN cDNA—mutated by using QuikChange® Site-Directed Mutagenesis Kit (Agilent, Santa Clara, CA, US)—together with a His_6-SUMO-tag in vector pET28a (Novagen, Merck, Darmstadt, Germany). Genes were transformed to and expressed in *Escherichia coli* BL21 (DE3). Before IPTG induction the cells were grown to an $OD_{600} = 0.7$ in M9 minimal medium at 37 °C containing kanamycin, ^{15}N-ammonium sulphate and ^{13}C-glucose as exclusive nitrogen and carbon source, respectively. After 5 h of protein production, the cells were harvested by centrifugation for 12 min at 12,100*g* and 4 °C. Cells were opened using a Microfluidizer M-110S (Microfluidics, Westwood, MA, US) at 6.5 bar twice. Proteins were purified by Ni-NTA chromatography (HisTrap FF, GE Healthcare, Little Chalfont, UK) and size exclusion chromatography (HiLoad™ 26/60 Superdex™ 75 pg, GE Healthcare). After SUMO protease cleavage another Ni-NTA chromatography was performed to separate the tag from the protein. The protein was freeze-dried and stored at −20 °C.

NMR experiments

The NMR samples were prepared by dissolving the freeze-dried protein in 22 mM sodium phosphate buffer at a concentration of 0.6 mM, the addition of 10 % (v/v) D_2O and final pH adjustment to 7.2. All NMR data were recorded on a Bruker Avance 700 and Avance II+ 600 MHz NMR spectrometer equipped with a 5 mm TCI cryo and TXI probe with Z-axis gradients, respectively. For sequential backbone assignment standard HNCA, HNCACB, CBCA(CO)NH and ^{15}N-resolved NOESY-HSQC spectra were recorded (Sattler et al. 1999). For side chain assignment ^{13}C-resolved aliphatic and aromatic NOESY-HSQC, as well as (H)CCH-TOCSY and NOESY spectra were acquired (Marion et al. 1989; Zuiderweg and Fesik 1989). Additionally two-dimensional ^{15}N- and ^{13}C-HSQC experiments were recorded regularly to check for protein stability (Mori et al. 1995). Spectral analysis, resonance assignment and imaging was done with the CCPNMR software package (Vranken et al. 2005), NMRViewJ (One Moon Scientific, Westfield, NJ, US), qtiplot (IONDEV SRL, Bucarest, Romania) and Adobe Illustrator CS3 (Adobe Systems, San Jose, CA, US).

Resonance assignment, secondary structure prediction and data deposition

The ^{15}N-HSQC spectrum of D39N/E76Q/E81Q showed well-dispersed signals as typical for a well-folded folded protein (Fig. 1). TALOS-N was used to perform a secondary structure estimation that indicated high helix probability for regions 12–29, 34–42, 44–54, 61–80, 85–103 and 108–125 (Shen and Bax 2013); Fig. 2). Sequential backbone assignment using standard experiments could be achieved for the majority of the chain. In sequence regions in helices 2 and 3 (39–78), where signal intensities were very weak, the assignments turned out to be more challenging. Additionally Met2, Pro8, Ile43, Pro60 and Ser61 could not be assigned. The dimerization interface could be anticipated by highlighting of region 39–78 on the homologue dimeric NRN from *E. australis* (PDB 2LTH, (Kronqvist et al. 2014). The modelled dimerization interface coincides with a sequence region of D39N/E76Q/E81Q that showed weak signal intensities in the NMR spectra, probably resulting from an intermediate chemical exchange of partial dimerization. C-terminal amino acids 134-137 neither showed inter-residual NOE nor sidechain proton signals. Through examination of all available types of spectra a total of 90.3 % of the backbone (HN, CA, N, HA) and 71.5 % of the total sidechain, 83 % of aromatic and 85.6 % of aliphatic side chain atoms could be assigned. $^{13}C^{\alpha}$ chemical shifts were compared to deposited wild type resonances (BioMagResBank accession code 17131) and summarized in Fig. 3. For most sequence positions of the protein $^{13}C^{\alpha}$ resonances of both variants were in good agreement. However some isolated residues showed unusual big shift differences. After careful re-evaluation of the assigned and deposited resonances with the recorded spectra and with the BMRB chemical shift statistics, some unusual chemical shifts could be observed for BMRB entry 17131. However, given that there is no access to the wild type spectra, a direct comparison of the wild type and variant three-dimensional data is currently not possible.

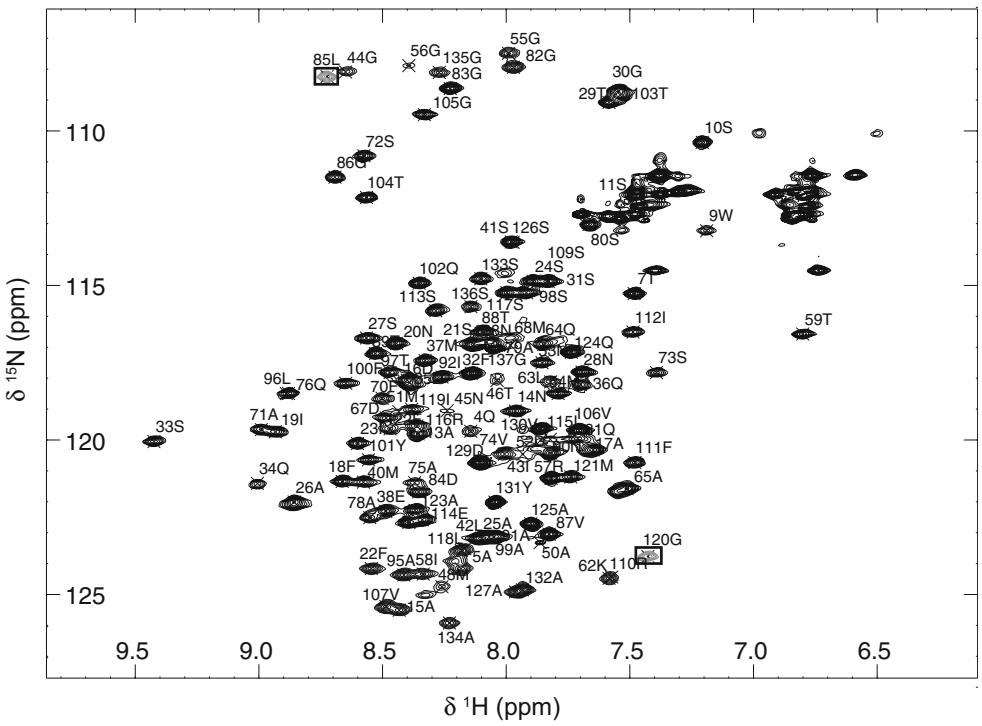

Fig. 1 ^{15}N-HSQC spectrum of NRN variant D39N/E76Q/E81Q in 20 mM sodium phosphate buffer with 10 % (v/v) D_2O at pH 7.2 and 298 K. Aliased signals are boxed

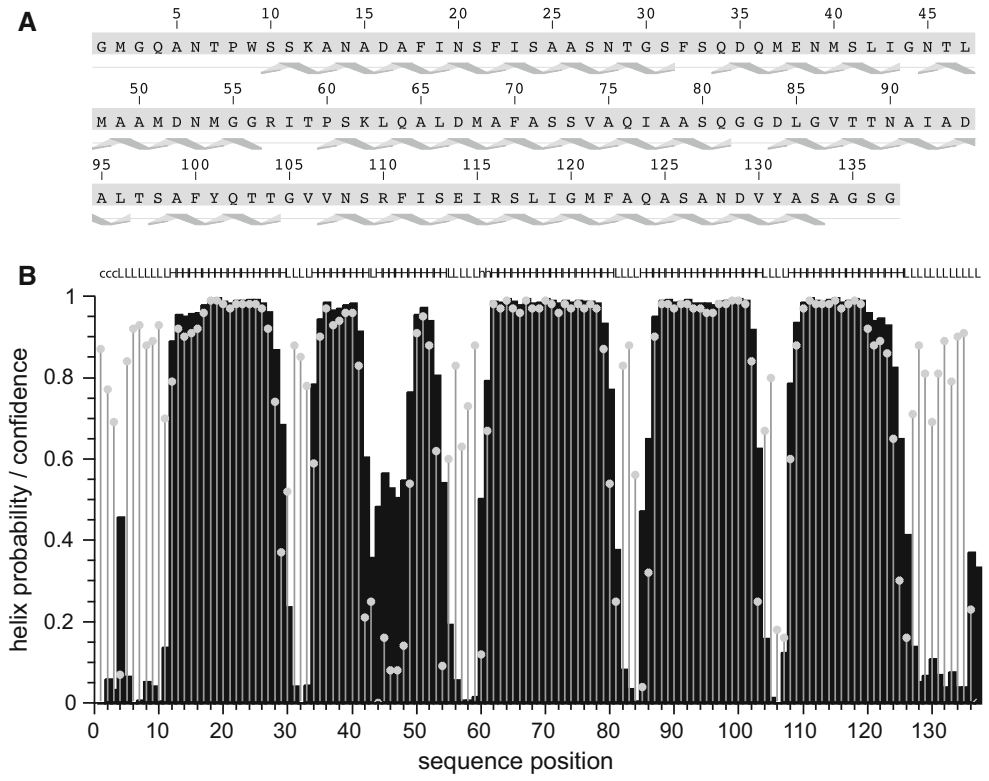

Fig. 2 **a** Amino acid sequence of NRN variant D39N/E76Q/E81Q with predicted secondary structure from a chemical shift index using assigned HA, HN, CA resonances. **b** TALOS-N predicted helix probabilities (black bars) and secondary structure (top bar c = coil, L = loop, H = helix), estimated from assigned chemical shifts with associated confidence levels as grey vertical lines (Shen and Bax 2013)

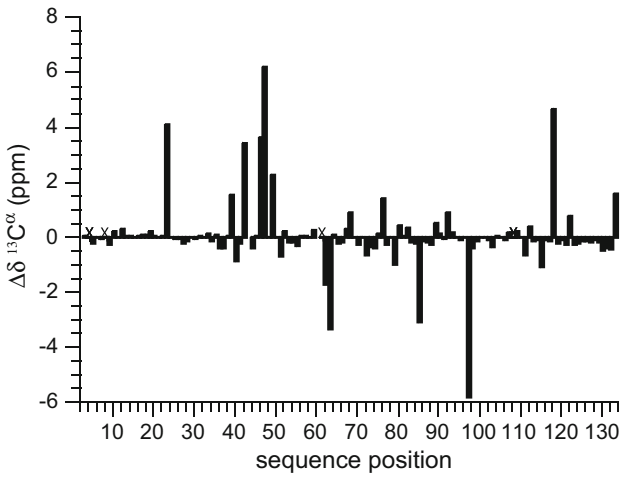

Fig. 3 Chemical shift differences $\Delta\delta^{13}C^{\alpha}$ of deposited NRN wild type and variant D39N/E76Q/E81Q as a function of the sequence position. Unusual chemical shift differences are observed for individual residues and are addressed in the main text. Unassigned residues are marked with X

The resonances and assignments of NRN D39N/E76Q/E81Q have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession code 25643.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the experiments in this study comply with the current laws of the country in which they were performed.

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