MICROCIN AMYLOID FIBRILS ARE A RESERVOIR OF TOXIC OLIGOMERIC SPECIES

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Supplemental Experimental Procedure

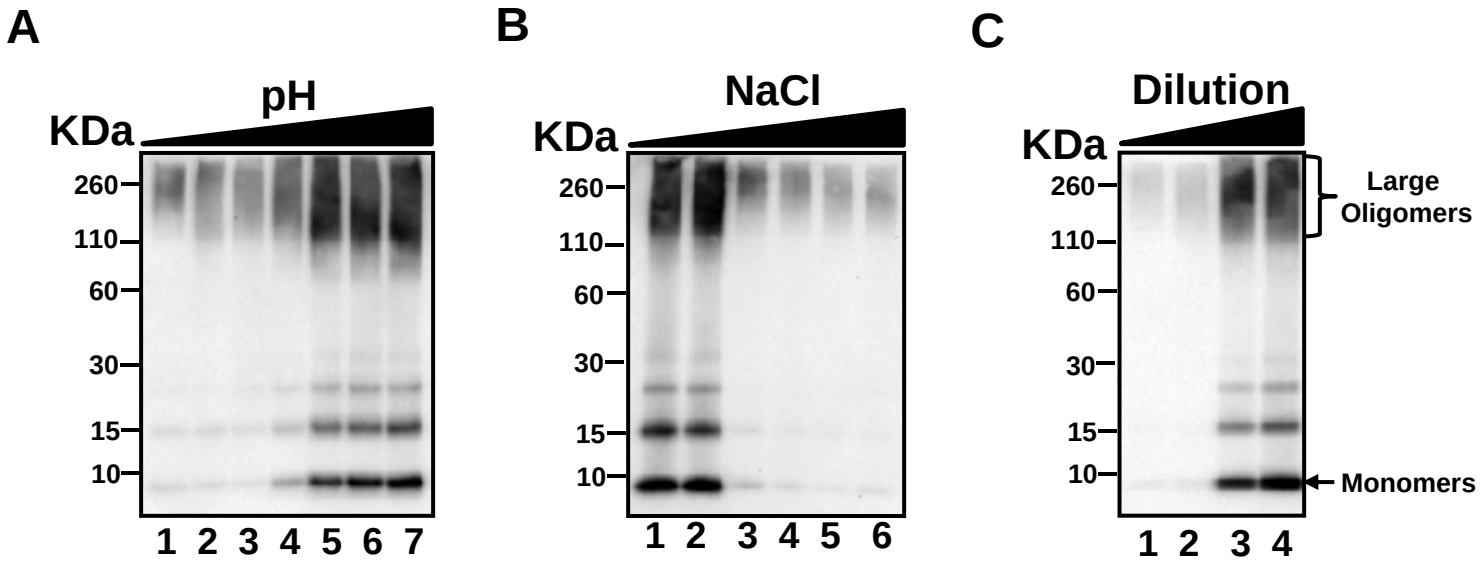
Calculation of monomers to oligomers ratios- To calculate monomers to oligomers ratios, Mcc fibrils were treated at varying pH (6.5 to 10.5) for 2 h at 25 ºC. After that samples were centrifuged at 16,500 x g for 10 min and resultant supernatants were fractionated by SEC. The elution profiles were detected by absorbance at 280 nm. The ratios of monomers to oligomers were calculated by measuring the area of peak 1, ~670 kDa (considered as oligomers) and Peak 3, ~15.7 kDa (considered as monomers although they co-exist with dimers) of the chromatograms. Because of being too small and short lived, peak 2, was excluded of this study. Detection of peak 2 requires comparatively larger and concentrated samples.

Supplemental Figure Legends

Supplemental Figure S1. SDS-PAGE and immunoblot analysis of Mcc fibrils disaggregation. Mcc fibrils were prepared *in vitro* by incubating soluble Mcc at 400 µg/ml in 50 mM PIPES-NaOH, pH 6.5, 500 mM NaCl at 37 °C with agitation for 24 h, and thereafter fibrils were treated, at varying pH (**A**), *lanes* 1-7 (2.5, 5.5, 6.5, 7.5, 8.5, 9.5 and 10.5); at varying NaCl concentrations (**B**), *lanes* 1-6 (0, 50, 150, 500, 750 and 1000 mM); and at varying dilutions (**C**), *lanes* 1-4 (1:1, 1:2, 1:5 and 1:10). Treatment was done for 2 h at 25 °C. Small aliquots were removed, centrifuged at 16,500 x g for 10 min, and supernatants were resolved by SDS-PAGE and immunoblotted with Mcc specific antibody.

Supplemental Figure S2. **Mcc fibrils disaggregation at basic pH promotes formation of increased levels of monomers and/or dimers**. **A**: Mcc fibrils were prepared *in vitro* by incubating soluble Mcc at 400 µg/ml in 50 mM PIPES-NaOH, pH 6.5, 500 mM NaCl at 37 °C with agitation for 24 h, and treated at different pH (6.5-10.5), as indicated for 2 h at 25 °C. Aliquots were removed, centrifuged at 16,500 x g for 10 min, and supernatants were fractionated by SEC. The elution profiles were detected by the absorbance at 280 nm. **B**: The ratios of monomers to oligomers were calculated by measuring the area of peak 1 (as oligomers) and peak 3 (as monomers) of chromatograms shown in *panel A*. Note: Even though peak fraction 3 contains small amount of small oligomers (dimers) as shown by SDS-PAGE analysis (Fig. 5E), to simplify calculation it was considered as monomers.

Supplemental Figure S1



Supplemental Figure S2