FEBS Letters 587 (2013) 3273–3280

FEBS Letters

journal homepage: www.FEBSLetters.org

Nanoparticle self-assembly by a highly stable recombinant spider wrapping silk protein subunit

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ARTICLE INFO

Article history:
Received 12 August 2013
Accepted 20 August 2013
Available online 28 August 2013

Edited by Miguel De la Rosa

Keywords:
Biomaterial
Spider silk protein
Argiope trifasciata AcSp1
Nanoparticle

ABSTRACT

Artificial spider silk proteins may form fibers with exceptional strength and elasticity. Wrapping silk, or aciniform silk, is the toughest of the spider silks, and has a very different protein composition than other spider silks. Here, we present the characterization of an aciniform protein (AcSp1) subunit named W_1, consisting of one AcSp1 199 residue repeat unit from *Argiope trifasciata*. The structural integrity of recombinant W_1 is demonstrated in a variety of buffer conditions and time points. Furthermore, we show that W_1 has a high thermal stability with reversible denaturation at ~71 °C and forms self-assembled nanoparticle in near-physiological conditions. W_1 therefore represents a highly stable and structurally robust module for protein-based nanoparticle formation.

1. Introduction

Protein-based biomaterials have many potential applications, ranging from coating of surgical implants to tissue engineering scaffolds to drug delivery vehicles [1–4]. Spider silks are especially promising biomaterials because of their exceptionally high tensile strength, elasticity, toughness and biocompatibility. Notably, the mechanical properties of some types of spider silks surpass even the strongest of synthetic materials, including nylon, Kevlar, and steel [5]. Previous studies have described spider silk fibers as potential biomaterials for biocompatible artificial nerve conduits [6], enhancement of skin regeneration [7], and tissue engineering scaffolds [8]. Beyond fiber formation, a miniature spider dragline silk protein was shown to form nanoparticles with promise as drug delivery vehicles [9,10]. However, the particle formation process described involves salting out with potassium phosphate, where the high salt concentration required may make this system unsuitable for drug delivery under physiological conditions. Spiders produce at least six other types of silk fibers that differ significantly from dragline silk in physical properties and protein makeup. Therefore, alternative biomaterials with improved properties may be found through study of other spider silk proteins.

Spider wrapping silk, or aciniform silk, is distinctive from dragline silk in both physical properties and amino acid content. It is used to wrap and immobilize prey, to build the inner layer of egg sacs and for web decoration [11]. Wrapping silk is one of the toughest spider silks because of a combination of high tensile strength and high elasticity [11–13]. Unlike the dragline silk proteins MaSp1 and MaSp2, which have small sequence motifs like GGX and A_n repeated up to a hundred or more times [5], the wrapping silk protein AcSp1 contains a much longer repetitive sequence (Fig. 1) which lacks the small motifs of the dragline silk proteins [12].

The wrapping silk protein (AcSp1) repetitive domain has long (>150 residue) repeat units with complex amino acid compositions and little internal repetition. Similarity of AcSp1 across spider spe-

Abbreviations: λ_{em} and λ_{ex}, wavelengths of emission and excitation, respectively; λ_{max}, wavelength of maximum of emission intensity; AcSp1, *Argiope trifasciata* aciniform spidroin 1; ANS, 8-anilino-1-naphthalenesulfonic acid; CD, circular dichroism; DLS, dynamic light scattering; EM, electron microscopy; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy; TEM, transmission electron microscopy; T_m, thermal denaturation midpoint; UV, ultraviolet; W_1, subunit of the repetitive domain of AcSp1

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.08.024

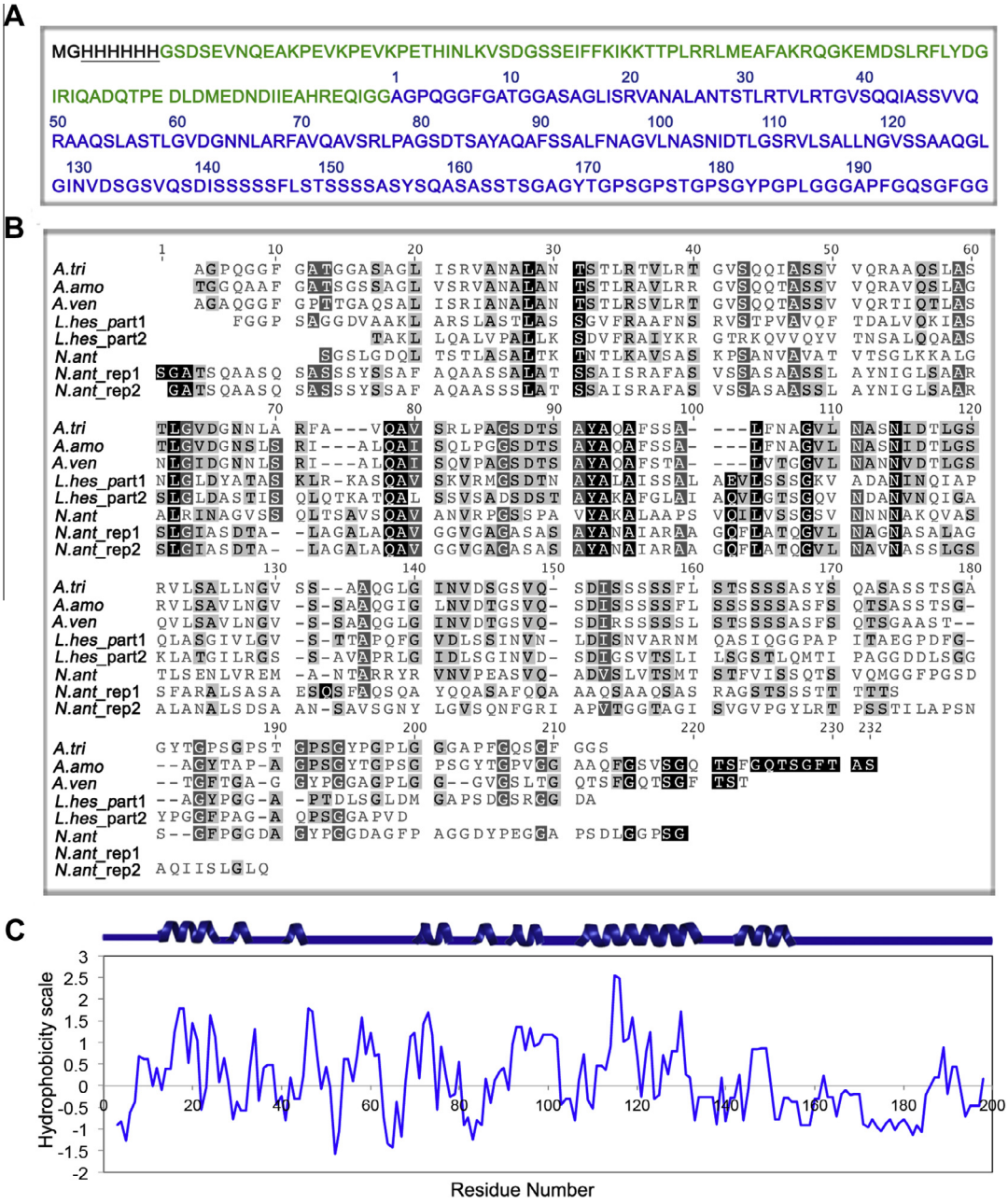

Fig. 1. Sequence and features of spidroin repetitive domains. (A) Sequence of His_6–SUMO–W_1 fusion protein. SUMO sequence is colored green and W_1 (*Argiope trifasciata* AcSp1 repeat unit) sequence blue. (B) Sequence alignment of aciniform silk proteins (AcSp1) from *Argiope trifasciata* (*A. tri*); *Argiope amoena* (*A. amo*); *Araneus ventricosus* (*A. ven*); *Latrodectus hesperus* (*L. hes*, divided into parts 1 and 2); *Nephila antipodiana* (*labeled N. ant*); and, tubuliform silk TuSp1 repetitive sequence types 1 and 2 from *N. antipodiana* (*labeled N. ant* rep1 and rep2). Grey background indicates sequence similarity, with darker indicating higher conservation. (C) Structural features [15 and manuscript in preparation] overlying Kyte-Doolittle hydrophobicity index [34] of W_1.

cies and with other spidroins consisting of long repeats such as tubuliform spidroin (TuSp1) tends to be low (Fig. 1). AcSp1 from *Argiope trifasciata* has been estimated to be at least 280 kDa in size, comprising a large, repetitive core domain of 200 residue subunits repeated at least 14 times in addition to a small C-terminal domain and a putative small N-terminal domain [13]. Beyond its sheer length, the 200 residue repeat is significantly different from MaSp1 and MaSp2 in amino acid content. For example, the combined Gly and Ala content in AcSp1 is ~30% vs. ~70% in dragline silk and a Ser content of ~21% in AcSp1 vs. <2% in dragline silk [14]. The relationship between primary structural differences and silk properties is unknown, but is suggestive of differing protein secondary and tertiary structure and self-assembly abilities. Both our nuclear magnetic resonance (NMR) spectroscopy-based chemical shift assignments [15] and our ongoing structural refinement (manuscript in preparation) of the AcSp1 repeat unit from *A. trifasciata* and the NMR structure of residues 1–160 of *Nephila antipodiana* AcSp1 from Yang and co-workers [16] show predominantly α-helical structures with generally good sequence alignment between helical segments. Interestingly, the AcSp1 fold is determined by Yang and co-workers is highly similar to that of the eggcase TuSp1 repeat units they previously solved [17] while our NMR structure implies a less helical globular core in *A. trafisciata* (Fig. 1C). The implications of these differences remain to be seen.

Here, we show that a 199 residue construct of AcSp1 from *A. trifasciata* self-assembles in near-physiological buffer into