Precisely Patterned Nanofibers for
High Performance Bioseparations

BIOPRODUCTION OF MODIFIED SPIDROINS

Contents

Bioproduction of modified spidroins

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Abbreviations

Abbreviation / Acronym	
Aha	azidohomoalanine
DBCO-Cy3	dibenzocyclooctyne-cyanine3
eADF4	engineered *Araneus diadematus* fibroin 4
ncAA	non-canonical amino acid
SDS-PAGE	sodium dodecyl sulfate - polyacrylamide gel electrophoresis
UV	ultraviolet
Vis	visible
WP	work package

1. Introduction

Deliverable 2.6 includes at least 500 mg of the modified spidroins eADF4(C16[Aha]), eADF4(Ω16[Aha]) and eADF4(κ16[Aha]), respectively; with a production yield of over 200 mg/l of culture. These recombinantly produced spidroins contain a T7-Tag for immunodetection and 16 repetitions of the respective module, resulting in a molecular weight of ca. 48 kDa [1]. eADF4(C16) represents the negatively charged protein variant, while eADF4(Ω16) possesses a neutral and eADF4(κ16) a positive net charge [1–3] . For the functionalization of the three unmodified spidroin variants, the three Met residues in the T7-tag were replaced by the ncAA analog azidohomoalanine (Aha) in an *in vivo* residue-specific incorporation during the recombinant protein production. As a bacterial host, a Met auxotrophic *E. coli* strain was chosen, since it is suitable for the replacement of Met by a ncAA analog in proteins after Met depletion in the medium [4–6]. This report presents the analysis of the purified Aha-modified spidroin variants.

2. eADF4(C16[Aha])

The eADF4(C16[Aha]) batch described here was labeled 2020_61.01. The batch was purified from the insoluble fraction after cell lysis (inclusion bodies). The production yield was at 2556 mg/l of culture, or rather 45 mg/g cell wet weight.

eADF4(C16[Aha]) possessed a 260/280 nm absorption ratio of 0.47, as determined by UV/Vis spectroscopy, indicating the absence of nucleic acids (Figure 1).

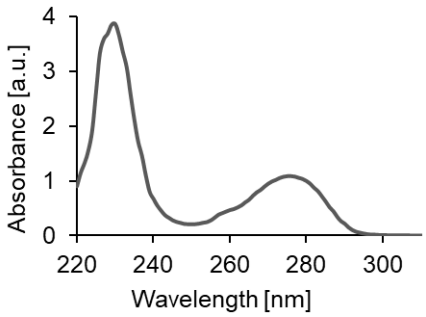

Figure 1: UV/Vis absorption spectrum of eADF4(C16[Aha]). Values were normalized to 1 at 280 nm.

The purified protein was analyzed using SDS-PAGE followed by a silver staining to reveal potential protein contaminations (Figure 2A). eADF4(C16[Aha]) appeared as a clear protein band, and no protein contamination was visible. The incorporation of Aha into the spidroins was demonstrated using strain-promoted azide-alkyne cycloaddition by adding DBCO-Cy3 to the protein sample prior to SDS-PAGE (Figure 2B). The spidroin showed strong binding to DBCO-Cy3 compared to the unmodified eADF4(C16), as determined by exposition to UV light. This indicated the presence of azido-groups.

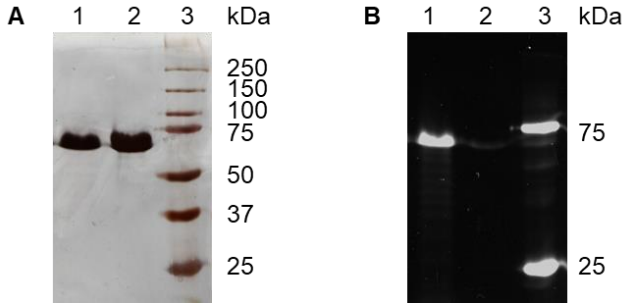

Figure 2: Characterization of eADF4(C16[Aha]) using SDS-PAGE. Spidroins were labeled with DBCO_Cy3. The gel was silver-stained (A) and exposed to UV light followed by fluorescence imaging (B). 1: eADF4(C16[Aha]); 2: eADF4(C16); 3: protein ladder.

Furthermore, fluorescence spectroscopy was employed for detection of bacterial protein contaminations in the purified protein fractions. This is based on the fact that the spidroins contain Tyr but no Trp residues, which are both present in *E. coli* proteins. The absence of a Trp emission signal confirmed that there was no bacterial protein contamination (Figure 3).

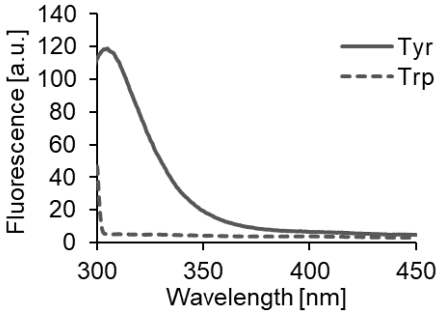

Figure 3: Fluorescence emission spectrum of eADF4(C16[Aha]). Tyr was excited at 278 nm and Trp at 295 nm.

3. eADF4(κ16[Aha])

The eADF4(κ16[Aha]) batch described here was labeled 2021_65. The batch was purified from the soluble fraction after cell lysis. The production yield was at 302 mg/l of culture, or rather 5 mg/g cell wet weight.

The UV/Vis spectrum of purified spidroin is shown in Figure 4. The 260/280 nm absorption ratio was at 0.71, indicating a slight contamination by nucleic acids.

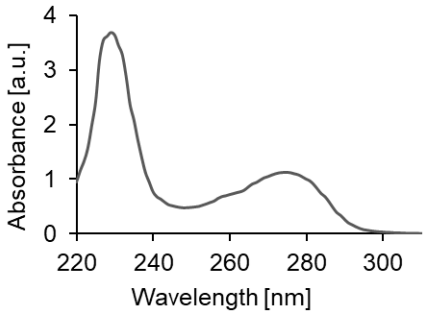

Figure 4: UV/Vis absorption spectrum of eADF4(κ16[Aha]). Values were normalized to 1 at 280 nm.

In SDS-PAGE analysis, eADF4(κ16[Aha]) appeared as a strong protein band at ca. 50 kDa (Figure 5A). Two less intense bands with a lower molecular weight were visible in the eADF4(κ16[Aha]) fraction. One of them might be a degradation product of the target protein, since it was visible in the fluorescence-detected gel as well. Labeling with DBCO-Cy3 specifically revealed the presence of Aha in the target protein (Figure 5B).

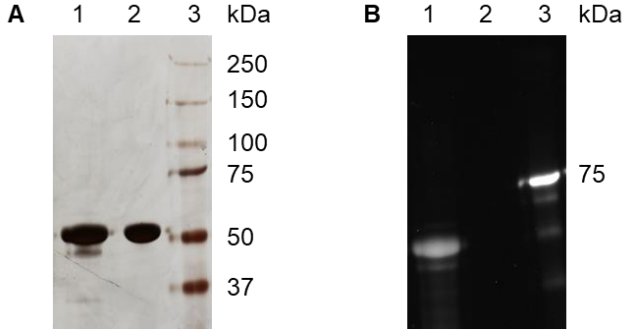

Figure 5: Characterization of eADF4(κ16[Aha]) using SDS-PAGE. Spidroins were labeled with DBCO_Cy3. The gel was silver-stained (A) and analyzed using a fluorescence imager (B). 1: eADF4(κ16[Aha]); 2: eADF4(κ16); 3: protein ladder.

Fluorescence spectroscopy of eADF4(κ16[Aha]) indicated a very weak Trp signal at ca. 350 nm, demonstrating a negligible protein contamination (Figure 6).

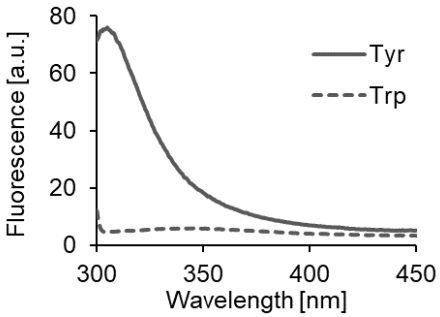

Figure 6: Fluorescence emission spectrum of eADF4(κ16[Aha]). Tyr was excited at 278 nm and Trp at 295 nm.

4. eADF4(Ω16[Aha])

The eADF4(Ω16[Aha]) batch described here was labeled 2022_6. The batch was purified from the insoluble fraction after cell lysis (inclusion bodies). The production yield was at 4327 mg/l of culture, or rather 68 mg/g cell wet weight.

The UV/Vis spectrum of purified spidroin is shown in Figure 7. The 260/280 nm absorption ratio was at 0.57, indicating a negligible presence of nucleic acids.

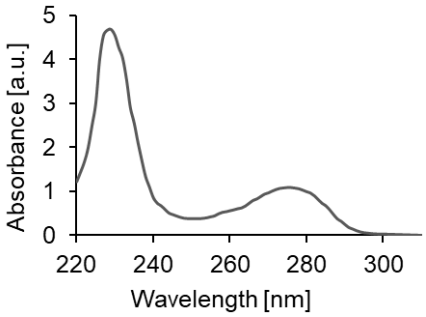

Figure 7: UV/Vis absorption spectrum of eADF4(Ω16[Aha]). Values were normalized to 1 at 280 nm.

In SDS-PAGE analysis, there was a strong protein band appearing at ca. 50 kDa representing eADF4(Ω16[Aha]) (Figure 8A). A weakly stained higher molecular weight protein was visible. The presence of Aha in the spidroin was verified upon labeling with DBCO-Cy3 (Figure 8B).

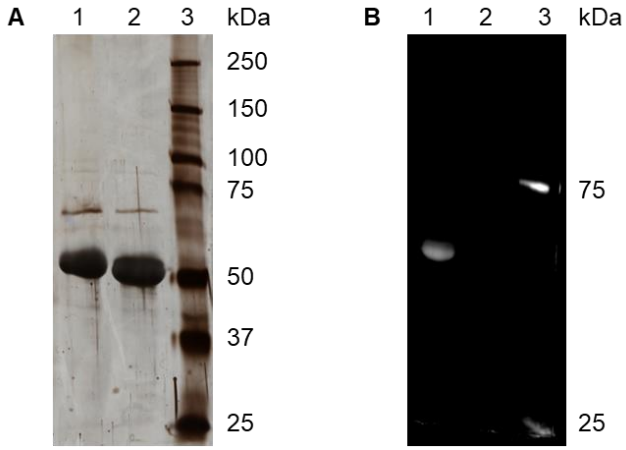

Figure 8: Characterization of eADF4(Ω16[Aha]) using SDS-PAGE. Spidroins were labeled with DBCO_Cy3. One gel was silver-stained (A) and another gel was analyzed using a fluorescence imager (B). 1: eADF4(Ω16[Aha]); 2: eADF4(Ω16); 3: protein ladder.

The absence of a Trp emission signal in fluorescence spectroscopy confirmed that there was no significant bacterial protein contamination (Figure 9).

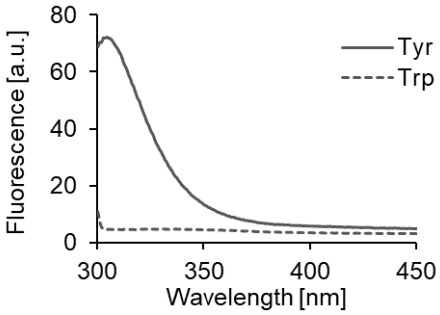

Figure 9: Fluorescence emission spectrum of eADF4(Ω16[Aha]). Tyr was excited at 278 nm and Trp at 295 nm.

5. Summary

The protein analyses demonstrated a sufficient purity of all three Aha-modified spidroin variants. The production yield was over 200 mg/l culture for every batch, and a total amount of more than 500 mg of each spidroin is available.

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