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Structural Analysis of Spider Silk Films

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Due to their outstanding mechanical properties, spider silks have fascinated men for a long time. Silk is composed of proteins which can not only be processed into fibers, as found in nature, but also be cast to form films in vitro. Starting with a protein solution in hexafluoroisopropanol, we were able to cast films with different properties deviated from the two spider silk proteins employed. All as-cast films revealed an α-helixrich structure and were water soluble. However, the secondary structure of One-protein films (silk films cast from one spider silk protein) could be converted from an α-helical rich to a β-sheet rich secondary structure by post-cast treatment with methanol or potassium phosphate. The structural conversion was accompanied by a higher stability as seen by water-insolubility. Depending on the employed proteins, silk films were stable in protein denaturants such as urea and guanidinium hydrochloride. Strikingly, Two-protein films (silk films cast from a mixture of both spider silk proteins) showed properties derived from both proteins, indicating that the process of film casting based on silk proteins is closely related to film casting of traditional chemical polymers. Our results reveal novel possibilities to generate protein films for applications that demand stable biocompatible polymer films.

Keywords: Araneus diadematus; Silk films; FTIR spectroscopy; Biopolymers

INTRODUCTION

Silks of insects and spiders are one of nature's best performing protein fibers. They have been optimized and adopted for different purposes over millions of years during evolution. Given the fact that spiders produce higher quality silks than the well-known silkworm *Bombyx mori*, spider silk is an interesting material for technical applications. Although fibers made from silk proteins are the prime object of material scientists, silks can also be assembled into other shapes. For instance starting from organic solutions such as hexafluoroisopropanol (HFIP), formic acid or trifluoro acetic acid, silk proteins can easily be cast into films [1–3]. Depending on the solvent, silk proteins adopt mainly α-helical or random coil conformation in solution, a conformation often maintained in as-cast films. This secondary structure of the proteins in films can be converted into a β-sheet rich one upon post-cast treatment with potassium chloride, ethanol or other alcohols, leading to a higher chemical stability of the films [1–4].

Here, we investigated films cast from HFIP solutions of recombinant spider silk proteins, whose sequence was adopted from dragline silk proteins of the garden spider *Araneus diadematus*. Dragline silk is composed of two related proteins, ADF-3 and ADF-4 (*Araneus diadematus fibroin*), which differ in their properties such as hydrophobicity and solubility [5–9]. Both proteins mainly exhibit repetitive amino acid sequences with characteristic polyalanine and glycine-rich motifs. Additionally highly conserved non-repetitive (NR) regions are located at the carboxylterminus of each protein [5,8,10–14]. While the repetitive part of the proteins adopted a random coil conformation in aqueous solution, the NR-regions of ADF-3 and ADF-4 revealed α-helical structure [15,16].

Here, we made use of the recombinant spider silk proteins $(AQ)_{12}$ and $(AQ)_{24}$NR3, based on the sequence of ADF-3, as well as C_{16} and C_{16}NR4, based on the sequence of ADF-4, to study structural and chemical properties of protein films thereof [15]. Films were cast from solutions of a single protein (One-protein films) or of two proteins (Two-protein

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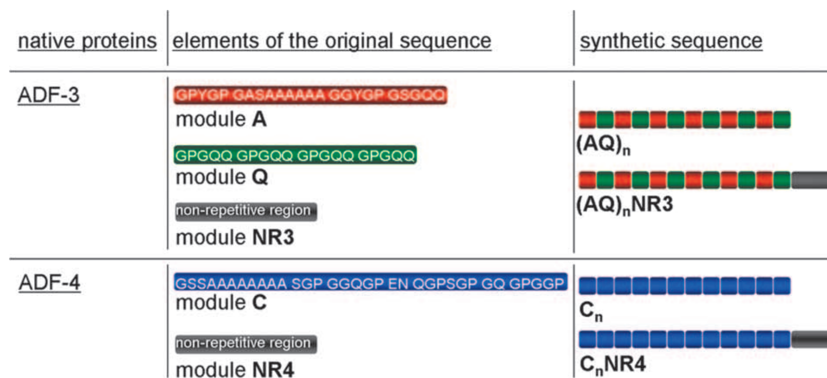

FIGURE 1 Synthetic spider silk engineering approach. Synthetic genes optimized for bacterial expression were created after back translation of characteristic amino acid motifs of the sequence of ADF-3 and ADF-4. Modules A and Q (repetitive region) and NR3 (non-repetitive region) are based on the sequence of ADF-3, whereas modules C (repetitive region) and NR4 (non-repetitive region) are based on the sequence of ADF-4. The genes of these modules were combined to gain the synthetic spider silk genes and respective proteins $(AQ)_n$, $(AQ)_n$NR3, C_n and C_nNR4 as described in (15).

films). We investigated the secondary structure and the structural transition due to treatment with methanol or potassium phosphate of these films employing infrared spectroscopy. Furthermore we analyzed the chemical stabilities of the films as well as the influence of the NR-regions on film properties.

RESULTS

Recombinant Silk Proteins

We employed recombinantly produced proteins whose amino acid sequence was derived from that of ADF-3 and ADF-4 of the garden spider *Araneus diadematus* (Fig. 1) [5–9,15,18]. Repetitive elements from the sequence of ADF-3 revealed a polyalanine-rich motif which we termed A and a glutamine and glycine-rich motif which we termed Q. Based on the ADF-4 sequence a single conserved repetitive motif was determined which we named C. The non-repetitive carboxyltermini were referred to as NR3 (derived from ADF-3) and NR4 (derived from ADF-4). Our previously established engineering approach allowed combination and multimerization of the single motifs yielding in a huge variety of proteins of which we employed $(AQ)_{12}$ (with a molecular weight of 48 kDa), $(AQ)_{24}$NR3 (106 kDa), C_{16} (48 kDa) and C_{16}NR4 (58 kDa) in this study [15].

One-protein Films

As shown previously, the proteins $(AQ)_{24}$NR3 and C_{16} can be cast into several μm thick films starting from HFIP solutions (Fig. 2) [19]. The thickness of the films can be controlled by the concentration of the employed protein solution (data not shown). The films in this study had a thickness of approximately 1 μm. We used FTIR spectroscopy to investigate the secondary structure of as-cast films as well as structural changes of the protein films upon treatment with methanol or potassium phosphate.

Films of the employed recombinant spider silk proteins revealed typical protein FTIR spectra. The complete spectrum of C_{16}NR4 (Fig. 3) is shown representative for all other spectra. The three important protein bands are amide A (3250–3300 cm^{-1}), amide I (1600–1700 cm^{-1}) and amide II (1480–1575 cm^{-1}). To analyze the secondary structure

FIGURE 2 As-cast C_{16}-film fixed in a slide frame.

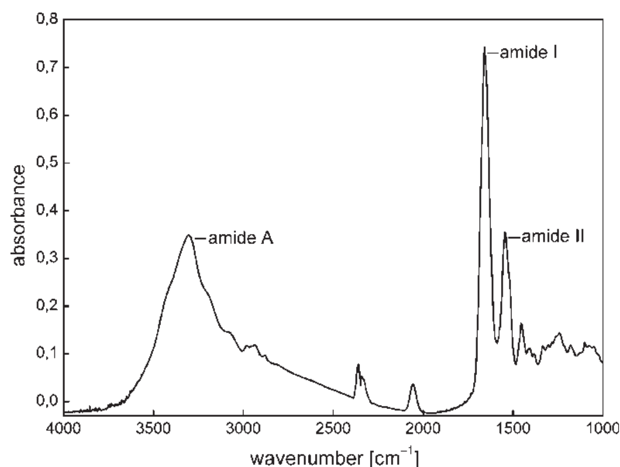

FIGURE 3 Complete FTIR spectrum of C_{16}NR4.

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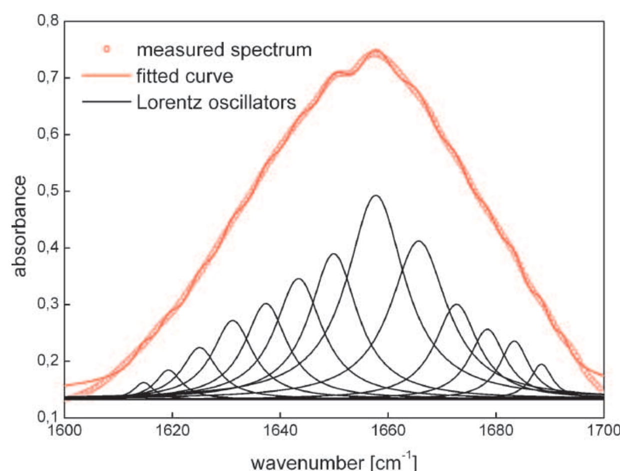

FIGURE 4 Deconvolution of the amide I band with Lorentzian oscillators shown for C_{16}NR4.

of the silk films, the amide I band, representing primarily the C═O stretching vibrations, was deconvoluted with Lorentzian oscillators. The number of oscillators and their initial band position in the fitting procedure have been determined by second derivatives of the absorbance spectra (Fig. 4). The influence of relative humidity (RH) on film structure was determined in dried (2% RH) and undried air (31% RH) for different samples. The spectra were reproducible (within the limits of 1.5%) and the results (Table II and IV) derived from the fit parameters could be determined within a margin of 10%. Since the deviations between samples in dried and undried air were well within the inherent uncertainties of the fitting procedure, the RH was neglected in further experiments. Another aspect in data analysis is the not well defined assignment for the different sub peaks to structural elements [20]. These uncertainties do not play a significant role since this study is focused on the determination of the contributions from α-helical and β-sheeted structural elements.

A general assignment for the different peaks of an FTIR spectrum concerning secondary structure elements is given in Table I. In Fig. 5 the amide I peak of individual spectra is shown from 1600 cm^{-1} to 1700 cm^{-1}. The sub peak at approximately 1660 cm^{-1} originated predominantly from an α-helical structure in the films, whereas no strong sub peak was found at wave numbers around 1630 cm^{-1}, which would be typically associated with β-sheet structures. The presence of NR-regions seemed not to influence film assembly or film structure, since no significant differences between the spectra of proteins with or without NR-regions neither in the case of (AQ)-proteins (Fig. 5A), nor in the case of C-proteins could be detected (Fig. 5B).

Post-cast Processing of One-protein Films Using Methanol or Potassium Phosphate

The treatment (= processing) of all silk protein films with 100% methanol or 1 M potassium phosphate (pH 8.0) yielded in a shift of the maximum of the IR-absorbance to lower wave numbers (~1630 cm^{-1}). This shift indicated the conformational conversion of the underlying proteins from α-helical to β-sheet rich structures (Fig. 6). The structural change upon processing has been additionally confirmed by circular dichroism spectroscopy (data not shown). In Table II the proportion of structural elements for different One-protein films is listed. Interestingly the random coil structure content found in as-cast films remained constant upon treatment, independent of the employed protein. Regarding the calculated secondary structure contents, treatment with methanol and potassium phosphate had slightly different influences on the silk films, especially in the case of $(AQ)_{12}$ (Fig. 6C). Here, the treatment with potassium phosphate resulted in a weaker sub peak at lower wave numbers in comparison to methanol, indicating a different β-sheet content.

Influence of the NR-regions on the Stability of One-protein Films

Next, we investigated the influence of the presence of NR-regions on the stability of the protein films. The chemical resistance of as-cast, as well as methanol processed films of $(AQ)_{12}$, $(AQ)_{24}$NR3, C_{16} and C_{16}NR4 were tested against water, 8 M urea, 6 M GdmCl and 6 M GdmSCN (Table III). The denaturants have different abilities to break hydrogen bonds and therefore lead to a denaturation of proteins accompanied by unfolding. All of

TABLE I Assignment of secondary structure elements of proteins to characteristic wave numbers ν as measured by FTIR-Spectroscopy [22].

secondary structure assignment	wave number $\tilde{\nu}$ [cm^{-1}] amide A	amide I	amide II
α-helix	3290–3300	1648–1660	1540–1550
β-sheet	3280–3300	1625–1640 (strong) 1690 (weak)	1520–1530
β-turn		1660–1685	
random coil	3250	1652–1660	1520–1545
3_{10}-helix		1660–1670	
aggregate		1610–1628	

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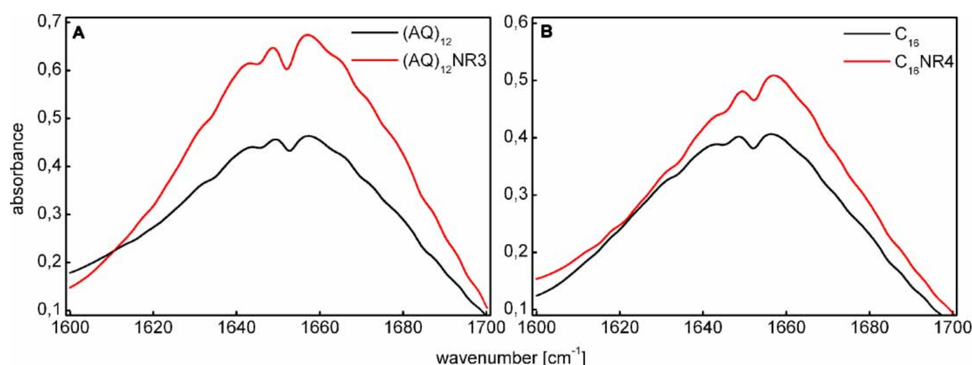

FIGURE 5 Amide I bands (1600 cm^{-1} to 1700 cm^{-1}) of the synthetic spider silk proteins $(AQ)_{12}$, $(AQ)_{24}NR3$ (A), C_{16} and $C_{16}NR4$ (B). As-cast films gave raise to maxima at about 1659 cm^{-1}, which is related to α-helical content.

the as-cast films were soluble in all tested solvents. Processed films, however, became water-insoluble, which was likely based on the structural change of the involved proteins. Processed films of C_{16} or $C_{16}NR4$ were additionally resistant to treatment with solutions of 8 M urea and 6 M GdmCl. Importantly, all films, even after methanol processing, were soluble in solutions of 6 M GdmSCN, which is the strongest of the tested solvents. In summary, the NR-regions did not influence the film's chemical stability under the conditions employed.

Two-protein Films

In nature, the interaction between the two protein components of the dragline, ADF-3 and ADF-4, is essential for dragline assembly, structure, and stability. The NR-regions, which contain a single cysteine residue, form intermolecular disulphide bridges, which could play a major role in the assembly process of the silk proteins [15,17,21].

Therefore, we analyzed whether films cast from solutions containing two protein components (Two-protein films) revealed a different structure or stability in comparison to One-protein films. $(AQ)_{12}$ and C_{16} or $(AQ)_{24}NR3$ and $C_{16}NR4$ were dissolved in HFIP (1% w/v each, 2% w/v in total). Two-protein films were cast from $(AQ)_{12}/C_{16}$ (molar ratio 1:1) or $(AQ)_{24}NR3/C_{16}NR4$ (molar ratio 1:1.8) mixtures.

The amide I band, as seen in FTIR spectra of as-cast Two-protein films, revealed maxima at about

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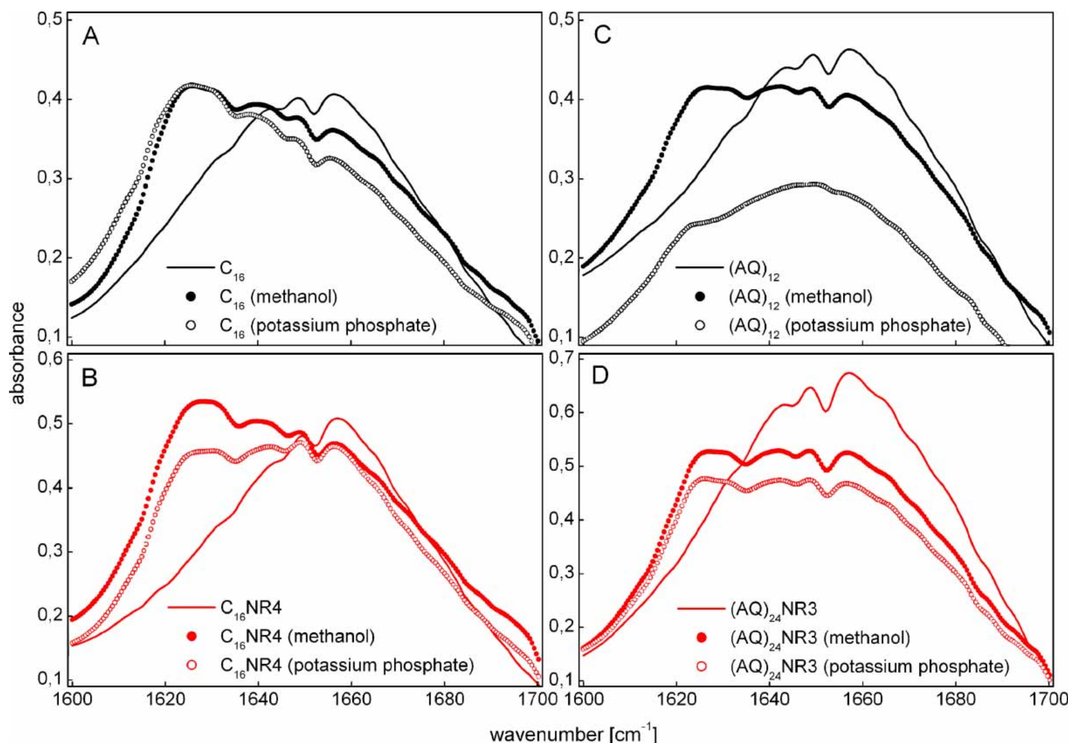

FIGURE 6 The amide I bands of the synthetic spider silk proteins C_{16} (A), $C_{16}NR4$ (B), $(AQ)_{12}$ (C) and $(AQ)_{24}NR3$ (D) are shown before and after treatment with methanol or potassium phosphate. The maxima of the amide I bands at about 1659 cm^{-1}, detected in as-cast films, shifted to lower wave numbers with maxima at about 1630 cm^{-1}, indicating a transformation from α-helical to β-sheet rich structures.

TABLE II Distribution of secondary structure elements of all investigated One-protein films after deconvoluting the amide I bands. As-cast films have a higher amount of α-helical structure than processed films, which show a higher amount of β-sheet structure.

	α-helix	β-sheet	β-turn	random coil
$(AQ)_{12}$ as-cast	23.7	27.7	30.1	18.5
$(AQ)_{12}$ methanol	18.4	34.8	29.2	17.6
$(AQ)_{12}$ potassium phosphate	9.9	36.5	32.0	21.6
$(AQ)_{24}$ NR3 as-cast	19.5	28.3	39.4	12.8
$(AQ)_{24}$ NR3 methanol	18.5	41.1	28.6	12.1
$(AQ)_{24}$ NR3 potassium phosphate	11.5	38.1	35.8	13.8
C_{16} as-cast	19.0	35.0	32.2	13.8
C_{16} methanol	12.9	45.2	23.8	18.0
C_{16} potassium phosphate	13.2	52.1	25.5	9.2
C_{16} NR4 as-cast	25.6	21.1	32.6	20.7
C_{16} NR4 methanol	17.1	40.5	29.6	12.8
C_{16} NR4 potassium phophate	18.7	36.8	30.1	14.4

1657 cm^{-1} indicating α-helical structure (Fig. 7). In this respect, the Two-protein films showed no significant difference in secondary structure to the previously investigated One-protein films under the employed conditions. Processing Two-protein films with 100% methanol shifted the maximum of the amide I band to lower wave numbers (~1627 cm^{-1}), indicating the formation of β-sheet structures. In Table IV, the apportionment of secondary structure is shown, gained after deconvoluting the spectra. Based on the deconvolution, the content of α-helix decreased (by 12%) and the content of β-sheet structure increased (by 25%) in the processed Two-protein films.

Remarkably, Two-protein films showed a combination of the properties of the films cast from the single silk proteins. As-cast Two-protein films made of $(AQ)_{12}/C_{16}$ have been soluble in all tested reagents. After processing with methanol, these films became insoluble in water and urea, but soluble in solutions of GdmCl and GdmSCN, reflecting a chemical stability between that of plain C_{16} or plain $(AQ)_{12}$ films.

As-cast Two-protein films of $(AQ)_{24}NR3/C_{16}NR4$ could not be completely dissolved in water. After water treatment, amorphous protein aggregates were remaining. The formation of intermolecular disulphide bridges between the NR-regions could be excluded to cause amorphous aggregation, since the behaviour of the films did not change in the presence of a reducing agent such as β-mercaptoethanol (5% (v/v)). Processing of the $(AQ)_{24}NR3/C_{16}NR4$ films with methanol led to chemical stability in water and urea. Regarding the stability in solutions of GdmCl, the films could be partially dissolved, but again amorphous aggregates were visible.

DISCUSSION

Previously, it had been shown that the presence of NR-regions critically influenced the aggregation behaviour of recombinantly produced spider silk proteins in aqueous solutions [15]. Our study revealed, that in the case of organic solvents such as HFIP, no significant influence of the NR-regions on solubility and aggregation behaviour of single proteins could be observed. Additionally after casting, One-protein films revealed no differences in chemical stability, regardless of the presence of

TABLE III Solubility assay of different recombinant spider silk films in water, 8 M urea, 6 M GdmCl and 6 M GdmSCN. The films were incubated at room temperature over night. As-cast films are shown in (A), methanol processed films in (B). + resolubilized, − remaining as a film, +/− aggregates.

	water	8 M urea	6 M GdmCl	6 M GdmSCN
A				
$(AQ)_{12}$	+	+	+	+
$(AQ)_{24}$ NR3	+	+	+	+
C_{16}	+	+	+	+
C_{16} NR4	+	+	+	+
$(AQ)_{24}/C_{16}$	+	+	+	+
$(AQ)_{24}$ NR3/C_{16} NR4	+/−	+	+	+
B				
$(AQ)_{12}$	−	+	+	+
$(AQ)_{24}$NR3	−	+	+	+
C_{16}	−	−	−	+
C_{16}NR4	−	−	−	+
$(AQ)_{12}/C_{16}$	−	−	+	+
$(AQ)_{24}NR3/C_{16}NR4$	−	−	+/−	+

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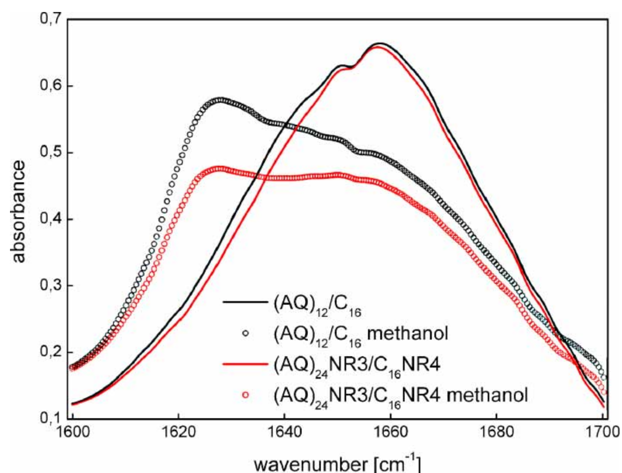

FIGURE 7 Amide I bands of the Two-protein films ($(AQ)_{12}/C_{16}$ and $(AQ)_{24}NR3/C_{16}NR4$). These films revealed a maximum at $1657\,cm^{-1}$, which is assigned to α-helical structure, while processing with methanol shifted the maxima to lower wave numbers ($1627\,cm^{-1}$), indicating the formation of β-sheet structures.

NR-regions. Treatment with methanol or potassium phosphate changed the content of secondary structure of the underlying proteins from an α-helical rich to a more β-sheet rich state in all investigated One-protein silk films. Importantly, processed films always had a higher chemical stability than non-processed ones, based on a structural change of the employed protein.

In the case of Two-protein films, the treatment with methanol led to a chemical stability influenced by both proteins. Compared to conventional synthetic polymers, where blending usually causes mixed properties, such finding is astonishing for proteins. In general, the structure and interaction of proteins is complex and depends on many factors. When mixed, two proteins, that do not interact, usually remain their chemical stability found in the absence of the second protein. In case they do interact, usually both proteins show a higher chemical stability. In our case, both proteins seemed to interact, but the gained chemical stability was between that of the single compounds. As-cast Two-protein films containing proteins with NR-regions could not be completely dissolved in water, leaving amorphous aggregates. Therefore, the NR-regions are influencing the solubility of spider silk protein films [15]. Furthermore, dissolving methanol processed Two-protein NR-films in solutions of GdmCl also led to the formation of amorphous aggregates. These findings indicated that the NR-regions influence protein–protein interactions, although no formation of disulphide bridges could be observed. Probably, non-covalent interactions, as found in native dragline silks between ADF-3 and ADF-4, contribute to the chemical stability of these films and the aggregation behaviour upon film dissolution [18].

Based on the presented findings, our setup is a promising tool to create stable, biocompatible protein films with adjustable properties depending on the employed protein components.

MATERIALS AND METHODS

Proteins Production

Protein synthesis and purification have been performed as described previously [15].

Sample Preparation

All recombinant spider silk proteins were dissolved in 100% HFIP at room temperature at 2% (w/v) prior to film casting. Solutions were cast on a polystyrene surface and the solvent was evaporated. As-cast films were transparent, soluble in water and could be easily removed from the surface after casting. In order to render films water-insoluble, films were treated with 100% methanol or 1 M potassium phosphate (pH 8.0) [19].

FT-IR

Polarized absorbance spectra of films were recorded in the spectral range between 700 and $6000\,cm^{-1}$ with a resolution of $4\,cm^{-1}$. An accumulation of 32 spectra ensured a high signal to noise ratio. Unless differently quoted, the measurements were carried out at 26°C and 30% relative humidity. The FTIR spectrometer (Bio-Rad FTS 6000) was coupled to an IR microscope (UMA 500 with 15x lenses) in order to additionally monitor the film surface. The measurement spot at the sample had a size of $250 \times 250\,\mu m$.

TABLE IV Proportion of the secondary structure of the as-cast and processed (treatment with methanol) Two-protein films ($(AQ)_{12}/C_{16}$ and $(AQ)_{24}NR3/C16NR4$) calculated by deconvoluting the amide I band. Processing led to a decreased amount of α-helical and an increased amount of β-sheet structures.

	α-helix	β-sheet	β-turn	random coil
$(AQ)_{12}/C_{16}$ as-cast	20.5	20.5	35.7	23.3
$(AQ)_{12}/C_{16}$ methanol	8.2	46.4	19.9	25.5
$(AQ)_{24}NR3/C_{16}NR4$ as-cast	22.5	17.7	33.7	26.1
$(AQ)_{24}NR3/C_{16}NR4$ methanol	9.9	38.8	30.3	21.0

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Chemical Stability

To investigate the chemical stability of the silk protein films, they were exposed to aqueous solutions of 8 M urea, 6 M guanidinium hydrochloride (GdmCl) and 6 M guanidinium thiocyanate (GdmSCN) and incubated over night at room temperature. The films were regarded chemically stable, when they remained completely intact after such treatment.

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References

[1] Zhao, C.; Yao, J.; Msuda, H.; Kishore, R.; Asakura, T. *Biopolymers* **2003**, *69*, 253.
[2] Um, I. C.; Kweion, H. Y.; Park, Y. H.; Hudson, S. *Int. J. Biol. Macromol.* **2001**, *29*, 91.
[3] Ha, S. W.; Tonelli, A. E.; Hudson, S. M. *Biomacromolecules* **2005**, *6*, 1722.
[4] Teramoto, H.; Miyazawa, M. *Biomacromolecules* **2005**, *6*, 2049.
[5] Guerette, P. A.; Ginzinger, D. G.; Weber, B. H.; Gosline, J. *Science* **1996**, *272*, 112.
[6] Gatesy, J.; Hayashi, C.; Motriuk, D.; Woods, J.; Lewis, R. *Science* **2001**, *291*, 2603.
[7] Hinman, M. B.; Lewis, R. V. *J. Biol. Chem.* **1992**, *272*, 19320.
[8] Hayashi, C. Y.; Shipley, N. H.; Lewis, R. V. *Int. J. Biol. Macromol.* **1999**, *24*, 271.
[9] Winkler, S.; Kaplan, D. L. *J. Biotechnol.* **2000**, *74*, 85.
[10] Colgin, M. A.; Lewis, R. V. *Protein Sci.* **1998**, *7*, 667.
[11] Craig, C. L.; Riekel, C. *Comp. Biochem. Physiol. Biochem. Mol. Biol.* **2002**, *133*, 493.
[12] Sponner, A.; Unger, W.; Grosse, F.; Weisshart, K. *Biomacromolecules* **2004**, *5*, 840.
[13] Xu, M.; Lewis, R. V. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 7120.
[14] Beckwitt, R.; Arcidiancono, S. *J. Biol. Chem.* **1994**, *269*, 6661.
[15] Huemmerich, D.; Helsen, C. W.; Quedzuweit, S.; Oschmann, J.; Rudolph, R.; Scheibel, T. *Biochemistry* **2004**, *43*, 13604.
[16] Dicko, C.; Knight, D.; Kenney, J. M.; Vollrath, F. *Biomacromolecules* **2004**, *5*, 758.
[17] Sponner, A.; Unger, E.; Grosse, F.; Weisshart, K. *Biomacromolecules* **2004**, *5*, 840.
[18] Huemmerich, D.; Scheibel, T.; Vollrath, F.; Cohen, S.; Gat, U.; Ittah, S. *Current Biol.* **2004**, *14*, 2070.
[19] Huemmerich, D.; Slotta, U.; Scheibel, T. *Appl. Phys. A* **2006**, *82*, 219.
[20] Surewicz, W. K.; Mantsch, H. H.; Chapman, D. *Biochemistry* **1993**, *32*, 38.
[21] Jin, H. J.; Kaplan, D. L. *Nature* **2003**, *242*, 1057.
[22] Tamm, L. K.; Tatulian, S. A. *Q Rev. Biophys.* **1997**, *4*, 365.

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