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Neffe, A.T.; Federico, S.; Lendlein, A.:

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Secondary Structure of Decorin-Derived Peptides in Solution

Axel T. Neffe[1,2], Stefania Federico[1,2], and Andreas Lendlein[1,2]
[1] Institute of Biomaterial Science and Berlin-Brandenburg Centre for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstrasse 55, 14513 Teltow, Germany
[2] Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

ABSTRACT

Decorin is a small leucine-rich repeat proteoglycan supporting collagen fibril formation by controlling the rate of collagen fibrillogenesis and fibril dimensions. Peptides derived from the inner surface of decorin have been shown to bind to collagen, while peptides derived from the outer surface do not display such binding affinity. As typical secondary structural elements such as β-sheets and α-helical regions were found in the decorin X-ray crystal structure, here it was investigated by Circular Dichroism (CD) spectroscopy in solution, whether the same structural elements can be found in the derived peptides. Here it is shown that the peptide derived from decorin's outer surface has the propensity to adopt helical conformation, as it was found in the crystal structure. The results were more pronounced in 80 vol% TFE solution, which led to an increase in the number as well as the length of helices. In contrast, peptides derived from the inner surface had a higher tendency to adopt β-sheet conformation, also in TFE, which corresponds to the conformation of the original sequence in the crystal structure of decorin. This suggests that the peptides derived from decorin adopt the structures present in the native protein.

INTRODUCTION

Decorin is a small leucine-rich repeat proteoglycan, which is involved in collagen fibrillogenesis in the extracellular matrix [1]. The protein part of decorin has a horseshoe-like shape with two distinct sides, the concave inner surface and the convex outer surface, which are formed by highly repetitive peptide sequences [2]. We recently developed peptides derived from these two distinct sides and could demonstrate by surface plasmon resonance experiments that only peptides derived from the inner surface of decorin bind to collagen [3], while the peptides from the outer surface did not show binding affinity. This provided experimental evidence to identify the binding epitope of decorin to collagen, which is present on the inner surface.

In decorin, the two distinct surfaces not only differ in amino acid sequence, but show different and distinct secondary structures in the X-Ray analysis. While the inner surface sequences adopt beta sheet conformation, the outer surface sequences form helical structures. The interaction of peptides with target structures not only depends on their amino acid sequence, but also on their conformation [4]. For example, it is known that RGD-sequences ideally form a small loop to increase their binding affinity to integrins [5-6], or the misfolding of prion proteins induces their aggregation [7]. It was therefore of interest to study whether the peptides derived from decorin can also adopt the secondary structure known from the full protein, as otherwise missing binding affinity could potentially be attributed to a false secondary structure.

In solution, the secondary structure of proteins and peptides can be studied by circular dichroism spectroscopy [8]. The received curves can be postulated to be represented by the sum of all-α-helical, all-β-sheet, and unordered sequences and allows the calculation of the percent (%) secondary structural element. It should be noted that the stability of secondary structures generally increases with the length of the peptide and changes with the hydrophilicity of the environment [9]. It is well established that addition of 2,2,2-trifluoroethanol (TFE) to the solution emulates the amphiphilic environment putatively present in membrane proteins as well as in protein-protein interactions. It therefore is often employed, such as here, to study peptide conformation under conditions supporting stable secondary structures [10].

EXPERIMENTAL DETAILS

Materials

N-Methylpyrrolidone (NMP, peptide reagent grade), piperidine, acetonitrile (ACN) (HPLC grade), dichloromethane (CH_2Cl_2), and trifluoroacetic acid (TFA) were purchased from IRIS biotech (Marktredwitz, Germany). *N,N*-diisopropylethylamine (DIPEA) and acetic anhydride were purchased from Sigma-Aldrich (Munich, Germany). All 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids, Fmoc-Asn(Trt)-polystyrene resin with a Wang linker, and benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) were purchased from Novabiochem (Darmstadt, Germany). *Tert.*-butylmethylether (TBME) and triisopropylsilane (TIPS) were purchased from Merck (Darmstadt, Germany). When not specified differently, all solvents were in analytical grade and were used without further purification.

Solid phase peptide synthesis

The peptides were synthesized on a LibertyTM Automated Microwave Peptide Synthesizer (CEM Corporation, Kamp-Lintfort, Germany) according to an Fmoc protocol. The deprotection steps were performed with 20% (v/v) piperidine in NMP solution. The coupling reactions were performed using 5 eq of PyBOP in NMP (0.5 M), 5 eq of amino acids in NMP (0.2 M), and 10 eq of DIPEA in NMP solution (2 M). Each deprotection and coupling reaction was performed with microwave energy (at a temperature of 75 ºC) and nitrogen bubbling. Each coupling was followed by a capping step using 10 vol% acetic anhydride in NMP. The peptide was then cleaved from the resin by treatment with a solution of 95 vol% trifluoroacetic acid (TFA), 2.5 vol% water, and 2.5 vol% TIPS for 90 min. The peptide was precipitated with TBME and collected by centrifugation. The final residual was dissolved in water, and then lyophilized. The purification of the peptide was performed on a Prostar Model 701 HPLC (Varian, Darmstadt, Germany) by using a Polystyrene / Divinylbenzene (PS/DVB) reversed-phase semipreparative column (PLRP-S, pore size: 100 Å, 8 μm; 300 mm × 25 mm) with acetonitrile/water as liquid phase.

Circular dichroism spectra

CD measurements were performed on a CD spectrophotometer (J-815, Jasco, Gross-Umstadt, Germany). Spectra were recorded in the 175-260 or 190-250 nm wave length with 1 nm

increments and 4 s integration time. Measurements were carried out at 25 ºC using a 10 mm path length quartz cuvette with peptide samples at a concentration ranging from 0.25 to 2 mg/mL in 10 mM sodium phosphate buffer, pH 7.4 with/without TFE, at a concentration 40 vol%, 60 vol%, and 80 vol%. The CD spectra were recorded in millidegrees of ellipticity as a function of wavelength. Single experiments were performed and are reported. Analysis was performed with the support of dichroweb [11], which is a web based server offering different deconvolution algorithms for quantifying the structural element contents of a sample from the CD spectra. In short, the spectra are uploaded, an algorithm is chosen, and the data output stating % structural elements is accompanied by a measure of exactitude such as the normalized root-mean-square deviation (NRMSD). Different algorithms were tested for the spectra and were chosen as specified in the results section. It should be highlighted, that the different algorithms may over- or underrepresent certain structural elements in the analysis. For the SELCON 3 method, Pearson's correlation coefficents of about 0.94 for evaluation of α-helices and about 0.64 for evaluation of β-sheets have been calculated [12], i.e., a higher probability to correctly evaluate helical than strand parts can be stated. This method requires spectra to be acquired from 180-260 nm. For the k2D networks, Pearson's correlation coefficents are given to be about 0.93 for evaluation of α-helices and about 0.97 for evaluation of β-sheets [13], but did not give interpretable results for the spectrum of KITKVEAASLKGLNN, for reasons unknown.

RESULTS AND DISCUSSION

Secondary structure of a peptide derived from the outer decorin surface

The peptide KITKVEAASLKGLNN derived from the outer surface of decorin [3] could be isolated after synthesis and HPLC purification in 2% yield in >95% purity. The identity of the peptide could be shown by MALDI-ToF mass spectrometry and 2D NMR spectra (see ref. 3 for full characterization). Typical CD spectra are reported in Fig.1. Determined by the SELCON 3 method [12], in aqueous solution this peptide showed primarily unordered conformation (62%), with low amounts of 3/10 helices (5%), β-strands (8%), and turns (25%). In 80 vol% TFE solutions, predominantly helical conformations were adopted (59% α-helical and 23% 3/10 helical), while β-strand (0%), turns (6%) and unordered parts (15%) were less present. At the same time, the calculated number (1.1 vs. 5.6 per 100 residues) and average length of the helices (3.2 vs. 14.4 residues) increased in the TFE solution in comparison to aqueous solutions. The investigations were performed on solutions containing 1 or 2 $mg \cdot mL^{-1}$, and were not concentration dependent. Altogether, the investigations showed that the peptide derived from the outer surface of decorin is prone to adopt helical conformation.

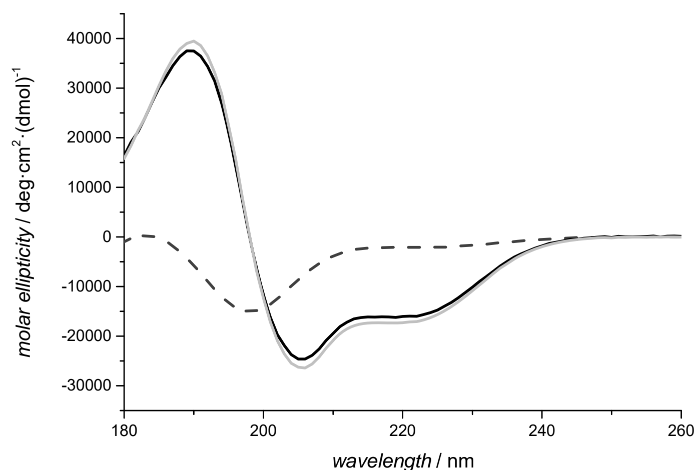

Figure 1. Circular dichroism spectrum of the peptide KITKVEAASLKGLNN from the outer surface of decorin: in water, dark grey dashed line (2 mg·mL^{-1}), in 80 vol% of TFE, black line (1 mg·mL^{-1}) and grey line (2 mg·mL^{-1}).

Secondary structure of a peptides derived from the inner decorin surface

The nonapeptides derived from the inner surface of decorin were synthesized in 13-60 mol% yield and ≥95% purity after HPLC purification. The identity of the peptide was demonstrated by MALDI-ToF mass spectrometry and 2D NMR spectra (see ref. 3 for full characterization). The percent secondary structure adopted was quantified from the CD-spectra by analysis with the neural network K2D [13]. These shorter peptides, in contrast to the longer peptide described above, did not display stable conformations in aqueous solution. In 80 vol.% TFE solution, the peptides derived from the inner surface of decorin adopted more stable secondary structures (spectra see Fig. 2, values see Table 1). While three peptides mostly adopted helical conformations, two peptides displayed a noticeable amount of β-sheet conformation. While the peptide concentration had only a minor effect on the adopted conformation (Fig. 2c/d), an increase in TFE concentration led to more pronounced secondary structure adoption (Fig. 2e).

There was no correlation found between the β-sheet content of the peptides and their binding affinity. One potential explanation is that short peptides generally do not adopt very stable conformations in aqueous solutions, but undergo a conformational rearrangement upon binding to the binding partner, in this case collagen. This binding would then lead to an induced fit, which enables a strong binding, with the dissociation constant possibly suffering an entropy penalty because of the necessary loss of conformational freedom. Furthermore, while TFE as solvent generally increases the hydrophobicity of the solvent in comparison to water and thereby promotes stabilizing hydrogen bonds, generally a stabilization of helical structures, which display internal hydrogen bonds, are easier to be promoted through TFE than β-strands, which can only be stabilized through hydrogen bonds with a second peptide sequence. The display of β-sheet conformation in several of the peptides is suggesting that such a conformation can principally be adopted.

Table 1: Secondary structural elements of peptides derived from the inner surface of decorin in 80 vol% TFE solution, and their binding affinity to collagen.

Peptide	% α-helix/ 3/10-helix[a]	% β-sheet[a]	K_D[b]
LTELRLSNN	44	12	n.b.o.
LSELRLHEN	29	30	$3.4\cdot10^{-5}$
LTELHLDNN	89	3	$1.6\cdot10^{-4}$
LSELRLHNN	10	35	$2.2\cdot10^{-4}$
LSELRLHAN	83	0	$1.9\cdot10^{-5}$

a: determined in 80 vol% TFE and analysis with the neural network K2D [13] b: to collagen, determined with surface plasmon resonance, data from ref. [3]

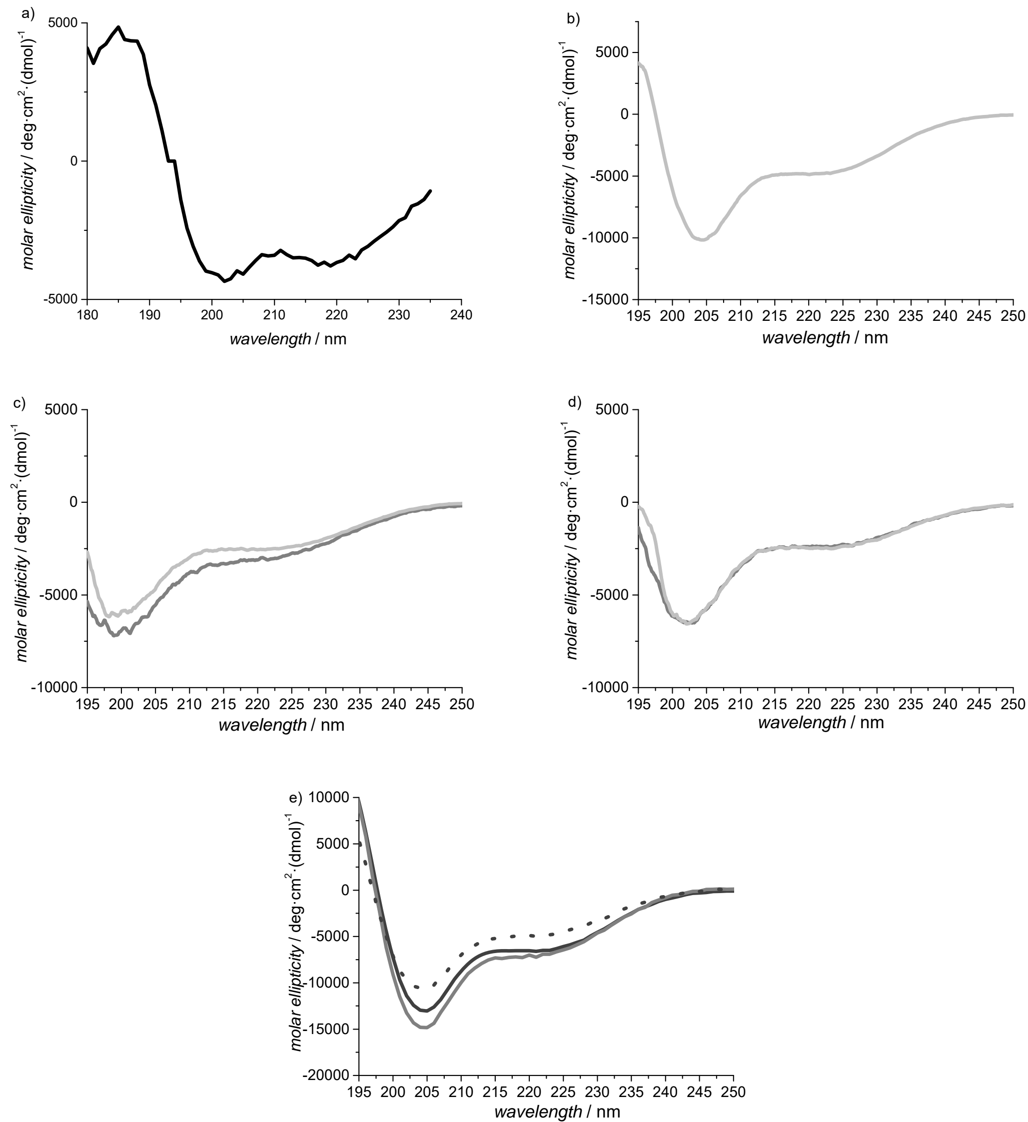

Figure 2: Circular dichroism spectra of the peptides LTELRLSNN (a), LSELRLHEN (b), LTELHLDNN (c), LSELRLHNN (d), LSELRLHAN (e) derived from the inner surface of decorin: in 80 vol% TFE in concentrations of 0.25 $mg\cdot mL^{-1}$ (light grey), 0.5 $mg\cdot mL^{-1}$ (grey), or 1 $mg\cdot mL^{-1}$ (black); in 50% TFE in a concentration of 1 $mg\cdot mL^{-1}$ (black dotted line)

CONCLUSIONS

The results shown here support the hypothesis that the investigated peptide derived from the outer surface of decorin indeed shows a propensity to adopt a helical conformation. The missing binding affinity to collagen can therefore unlikely be attributed to a misfolding, as this conformation is also present in the protein structure. Therefore, the CD studies support that decorin binds to collagen via its inner surface.

While the peptides derived from the inner surface of decorin have a much stronger propensity to adopt β-sheet structure than the longer peptide derived from the outer surface of decorin, the data do not offer a simple relation, which connects higher β-sheet content in TFE solution with higher binding affinity to collagen. It is likely that the exact peptide sequence is important for the interaction to collagen, e.g. through enabling hydrogen bonds or hydrophobic interactions, which may result in an induced fit of the two binding partners.

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