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Insight into interactions of the von-Willebrand-factor-A-like domain 2 with the FNIII-like domain 9 of collagen VII by NMR and SPR

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ABSTRACT

Type VII collagen as component of anchoring fibrils plays an important role in skin architecture, however, no detailed structural information is available. Here, we describe the recombinant expression, isotope labeling, and ^{1}H, ^{15}N, ^{13}C chemical shift assignment of a subdomain of the murine type VII collagen – the von-Willebrand-factor-A-like domain 2 (mvWFA2). vWFA2 interacts with type I collagen and plays a central role in certain skin blistering diseases. Based on these assignments a secondary structure prediction was performed showing a properly folded protein. An interaction of mvWFA2 with its neighboring domain mFNIII-9 was characterized with NMR spectroscopy and SPR.

Structured summary of protein interactions:
mFNIII-9 and **mvWFA2** bind by nuclear magnetic resonance (View interaction)
mvWFA2 binds to **mFNIII-9** by surface plasmon resonance (View interaction)

1. Introduction

Type VII collagen (Col7) is the main component of the anchoring fibrils, connecting the basal lamina with the tissue beneath by interacting with other collagen types and parts of the extracellular matrix [1,2].

Col7 is a homotrimer consisting of one central collagenous domain interrupted by a short non-collagenous region. The central domain is flanked by two non-collagenous domains. The C-terminal non-collagenous domain 2 (NC-2) contains conserved cysteine residues which accompany an antiparallel assembly of these collagen molecules. In contrast the N-terminal non-collagenous domain 1 (NC-1) consists of several subdomains with high homologies to adhesion proteins. The C-terminal subdomain of NC-1 reveals high homology to von-Willebrand-factor A and is called von-Willebrand-factor-A like domain 2 (vWFA2) [3]. A schematic representation of a single Col7 α-chain forming a homotrimer is shown in Fig. 1.

Investigations have shown that vWFA2 plays a crucial role for binding to type I collagen but also for interactions with other components of the extracellular matrix [2,4]. It is assumed that these connections contribute to skin stability. However, detailed knowledge of the skin architecture and the role of Col7 are still sparse and no high resolution structural information at atomic level is available.

Col7 is involved in different skin blistering diseases. A heritable form is caused by mutations in type VII collagen, which are also located within vWFA2 [5]. Loss of tolerances to Col7 leads to the autoimmune skin blistering disease epidermolysis bullosa acquisita (EBA) characterized by autoantibodies against Col7. During the course of disease the dermis separates from the epidermis resulting in blisters and lesions [6]. Former investigations have shown that antigenic epitopes can be found also in vWFA2 [7,8]. So far only little is known about the exact contribution of this domain in the architecture of the extracellular matrix and its role in developing EBA.

Therefore, structural investigations of the murine von-Willebrand-factor-A-like domain 2 (mvWFA2) should help to gain more insight into the connection of the skin layers and the role of this domain in skin blistering diseases.

Nuclear magnetic resonance (NMR) spectroscopy is advantageous for structural investigations due to its possibility of investigating dynamic systems as interactions with possible ligands. Based on NMR resonance assignment secondary structure elements have

Abbreviations: Col7, type VII collagen; EBA, epidermolysis bullosa acquisita; mFNIII-9, murine fibronectin-III-like domain 9; NC-1, non-collagenous domain 1; NMR, nuclear magnetic resonance; SPR, surface plasmon resonance; TALOS, torsion angle likelihood obtained from shift and sequence similarity; mvWFA2, murine von-Willebrand-factor-A-like domain 2; TSP-d_4, 3-(trimethylsilyl)propionic acid-d_4 sodium salt

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Fig. 1. Scheme of an α-chain of type VII collagen. Three identical α-chains build up Col7 forming anchoring fibrils. Each chain consists of CMP: cartilage-matrix-protein-like domain; FNIII-(1–9): fibronectin like domains; vWFA2: von-Willebrand-factor-A-like domain 2.

been obtained with the program torsion angle likelihood obtained from shift and sequence similarity+ (TALOS)+ [9]. Additional information has been achieved by ^{3}J-H(N)–H(α)-coupling constants and hydrogen–deuterium exchange. The experimental data are in good agreement with a homology model. Interestingly, an interaction with its neighboring domain the murine fibronectin-III-like domain 9 (mFNIII-9) has been shown the first time and was characterized by surface plasmon resonance (SPR) and NMR spectroscopy.

2. Methods

2.1. Protein expression and purification

A codon optimized sequence of mvWFA2 and of mFNIII-9 of Col7 was commercially synthesized (Mr. Gene, Regensburg, Germany) and cloned in pTWIN1 (NEB, Frankfurt, Germany). Cloning of the genes differs in the position of their intein affinity-tags. Whereas mvWFA2 has an N-terminal affinity-tag the affinity-tag of mFNIII-9 is C-terminal. Protein expression in *Escherichia coli* ER2566 and purification of both proteins followed the IMPACT™-TWIN protocol (NEB). mvWFA2 has been labeled uniformly with ^{15}N and ^{13}C according to Marley et al. [10]. Selective labeling for the amino acids A, R, and V was done according to Griffey et al. [11]. Cloning, expression and purification of mFNIII-9 was performed analog to mvWFA2.

2.2. NMR spectroscopy and structure prediction

NMR experiments have been performed on a Bruker Avance DRX 500 spectrometer equipped with a TCI cryoprobe at 298 K in 10 mM sodium phosphate buffer, pH 7.4 and 10% D_2O. 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP-d_4) was added as internal chemical shift reference. ^{13}C and ^{15}N chemical shifts have been referenced using the absolute frequency ratios [12]. A set of three dimensional triple resonance experiments namely CBCACONH, HNCA, HCCCONH, HBHACONH, HNCOCA, HNCO, HNHA and NOESY-HSQC have been performed. ^{1}H,^{15}N-HSQC spectra of ^{15}N–^{13}C mvWFA2 in 100% D_2O have been recorded additionally to identify slow exchanging amide protons. ^{1}H,^{15}N-HSQC spectra have been recorded immediately after ^{1}H–^{2}H exchange and 3 days later.

Spectra processing was done with Bruker Topspin 2.0. For data analysis and resonance assignment Sparky has been used. Scalar coupling constants have been calculated for information of flexibility of mvWFA2 from an HNHA experiment according to Vuister and Bax [13]. Secondary structure determination has been performed with TALOS+ on the eNMR web portal [9,14].

The interaction site of mvWFA2 with mFNIII-9 was determined via chemical shift mapping as described by Farmer et al. $\Delta\delta = [(\delta^b_{HN} - \delta^f_{HN})^2 + (0.17(\delta^b_N - \delta^f_N))^2]^{0.5}$ with b and f representing bound and free state [15]. The molar ratio of mvWFA2 to mFNIII-9 was approximately 1:2 with a concentration of 100 μM labeled mvWFA2.

A three dimensional homology model has been obtained from Swiss Model [16]. The homology model was built on the basis of an alignment of the amino-acid sequence of mvWFA2 with a similar protein of known structure. This template protein is the crystal structure of the von-Willebrand-factor-A3 domain in complex with a Fab fragment of IgG RU5 (PDB: 1FE8C) and the overall sequence identity is 20.1%.

2.3. SPR binding assay

A BIAcore 3000 has been used for SPR measurements. mvWFA2 was dissolved in 10 mM sodium acetate buffer pH 4 and immobilized on a CM5 sensor chip resulting in 6000 resonance units. This would correspond to a theoretical RU_{max} of 1156 for mFNIII-9. Binding experiments were performed in triplicate at room temperature in 10 mM sodium phosphate buffer pH 7.4. Dilutions of mFNIII-9 ranged from 100 μM to 2 mM. The K_D was determined via steady-state curve fitting analysis of BIAevaluation software assuming an 1:1 binding stoichiometry.

3. Results and discussion

3.1. Chemical shift assignment

Expression and purification of ^{15}N and ^{15}N, ^{13}C-labeled mvWFA2 yielded a single band in the SDS–PAGE (Supplementary data) with a typical overall yield of 19 mg protein per liter minimal medium. mvWFA2 shows a well-resolved ^{1}H,^{15}N-HSQC spectrum indicating that the recombinant mvWFA2 is properly folded (Fig. 2). The sample was stable over several months as identical spectrum patterns attest. Backbone assignments were obtained using standard triple resonance experiments. The assignment of the backbone chemical shifts is 96% complete. The N-terminal residues belonging to the affinity-tag could not be assigned due to flexibility. Solely from the chemical shifts a first structural character a disulfide bridge was identified. SDS–gel electrophoresis under non-reducing conditions revealed those to be intramolecular (data not shown). These chemical shift assignments present the initial point for structure determination of mvWFA2 and for protein–protein interaction studies.

3.2. Secondary structure prediction

Based on chemical shift assignments a secondary structure prediction of mvWFA2 employing TALOS+ via the eNMR web-portal was performed [9,14]. This program predicts torsion angles from C_α, C_β, N, H and H_α NMR backbone chemical shifts (see Supplementary data). The resulting prediction for mvWFA2 is consistent with idealized secondary structure elements for the majority of the residues. Only a short region in the middle part (residues 78–87) is less well defined making a clear conclusion difficult. Overall a β-strand is predicted for this region.

A comparison of the derived prediction with the homologous von-Willebrand-factor-A3 domain (PDB: 1AO3) matches well. It shows a similar distribution of α-helical and β-sheet sequences but they differ in length of secondary structure elements (Fig. 3c).

3.3. Coupling constants

With the help of an HNHA spectrum scalar coupling constants have been determined. These permit also to draw conclusions