

UNIVERSITY OF BIRMINGHAM

Research at Birmingham

Sequence-specific ^1H , ^{13}C and ^{15}N backbone resonance assignments of the plakin repeat domain of human envoplakin

Jeeves, Mark; Fogl, Claudia; Al-Jassar, Caesar; Chidgey, Martyn; Overduin, Michael

DOI:

[10.1007/s12104-015-9659-2](https://doi.org/10.1007/s12104-015-9659-2)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Jeeves, M, Fogl, C, Al-Jassar, C, Chidgey, M & Overduin, M 2016, 'Sequence-specific ^1H , ^{13}C and ^{15}N backbone resonance assignments of the plakin repeat domain of human envoplakin', *Biomolecular NMR Assignments*, vol. 10, no. 1, pp. 167-170. <https://doi.org/10.1007/s12104-015-9659-2>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Sequence-specific ^1H , ^{13}C and ^{15}N backbone resonance assignments of the plakin repeat domain of human envoplakin

Mark Jeeves¹ · Claudia Fogl¹ · Caezar Al-Jassar^{2,3} · Martyn Chidgey³ · Michael Overduin^{1,4}

Received: 11 September 2015 / Accepted: 31 October 2015 / Published online: 21 November 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract The plakin repeat domain is a distinctive hallmark of the plakin superfamily of proteins, which are found within all epithelial tissues. Plakin repeat domains mediate the interactions of these proteins with the cell cytoskeleton and are critical for the maintenance of tissue integrity. Despite their biological importance, no solution state resonance assignments are available for any homologue. Here we report the essentially complete ^1H , ^{13}C and ^{15}N backbone chemical shift assignments of the singular 22 kDa plakin repeat domain of human envoplakin, providing the means to investigate its interactions with ligands including intermediate filaments.

Keywords Envoplakin · Plakin repeat domain · Cornified envelope · Plakin · Cytoskeleton · Backbone resonance assignment

Biological context

Envoplakin is a member of the plakin family of cytolinkers. Plakin proteins connect elements of the cell cytoskeleton to each other and to junctional complexes at the cell membrane. The plakin proteins play a vital role in the maintenance of tissue integrity, particularly in tissues such as the heart and skin that are subjected to high levels of mechanical stress (Al-Jassar et al. 2013). The plakin superfamily consists of seven proteins in mammals, namely envoplakin, periplakin, desmoplakin, plectin, bullous pemphigoid antigen 1 (encoded by the dystonin gene), microtubule-actin cross-linking factor 1 and epiplakin. These proteins are widely expressed and have diverse modular structures but all are characterized by the presence of either a plakin domain, a plakin repeat domain (PRD), or both (Sonnenberg and Liem 2007; Bouameur et al. 2014).

Envoplakin is a 2033 amino acid protein which forms heterodimers with periplakin to initiate formation of the cornified envelope (DiColandrea et al. 2000, Candi et al. 2005). The cornified envelope forms beneath the plasma membrane of keratinocytes during the later stages of epidermal differentiation and is a core component of the epidermal permeability barrier in eukaryotes, preventing water loss and excluding foreign substances and organisms from the environment. In differentiating keratinocytes envoplakin is localized to the plasma membrane, forming contacts with desmosomes, the interdesmosomal membrane and the cell cytoskeleton (Ruhrberg et al. 1996). Envoplakin is targeted by autoantibodies in paraneoplastic pemphigus (Zimmermann et al. 2010), an autoimmune skin blistering condition that accompanies malignant and benign neoplasia, although the role of these antibodies in the aetiology of the disease remains to be determined.

✉ Michael Overduin
overduin@ualberta.ca

¹ Henry Wellcome Building for Biomolecular NMR Spectroscopy, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

² Present Address: MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK

³ School of Cancer Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

⁴ Present Address: Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta, 474 Medical Sciences Building, Edmonton, AB T6G 2H7, Canada

were grown in M9 media supplemented with $^{15}\text{NH}_4\text{Cl}$ and ^{13}C -glucose at 37 °C until an OD_{600} of 0.6 was reached. The temperature was then reduced to 18 °C and protein expression induced by the addition of 1 mM isopropyl- β -D-thiogalactopyranoside. Cells were grown for a further 16 h, harvested by centrifugation (7000 g for 15 min) and resuspended in phosphate buffered saline with complete EDTA-free protease inhibitors (Roche). The cells were lysed using an EmulsiFlex-C3 (Avestin) and the lysate cleared by centrifugation (75,000 g for 45 min). The envoplakin PRD was purified from the supernatant by nickel affinity chromatography using HisTrap HP columns (GE Life Sciences). The poly-His tag was cleaved using Tobacco Etch Virus protease, leaving 8 exogenous residues. The PRD was further purified by size exclusion chromatography using a Superdex-75 column (GE Life Sciences).

NMR experiments were performed at 298 K on Agilent NMR spectrometers equipped with cryogenic Z-axis pulse field gradient probes. Backbone assignments were made using BEST versions of the HNCA, HNCACB, HNCOCA, HNCO and HNCACO experiments (Schanda et al. 2006) and a standard CBCACONH pulse sequence (Grzesiek and Bax 1992). The HNCA, HNCACB, HNCO and HNCOCA experiments were performed on a 900 MHz spectrometer while HNCACO and CBCACONH experiments were collected on a 600 MHz spectrometer. Spectra were processed using NMRPipe (Delaglio et al. 1995) and analysed using CCPN software (Vranken et al. 2005).

Extent of assignments and data deposition

The ^1H , ^{15}N HSQC of the envoplakin PRD is shown (Fig. 1). Backbone assignments have been completed for 96 % of amide groups, 95 % of C' , 94 % of $\text{C}\alpha$ and 93 % of $\text{C}\beta$ non-proline residues. The C' , $\text{C}\alpha$ and $\text{C}\beta$ have been determined for all of the proline residues. Assignments for residues 1–3, 7 and 8 of the artefactual remnants of the N-terminal His-tag are missing, and those peaks which are assigned for this element exhibited sharp NMR signals, indicative of disorder. The resonance assignments of Asp1823, Phe1825, Thr 1854, Gln1900, Val1910, Ile1955, Asn1890, Thr1893 and Gln1894 are incomplete. Analysis using TALOS+ (Shen et al. 2009) indicated that Asp1823, Asn1890, Val1910 and Phe1925 are in unstructured elements, Thr1893, Gln1894 and Gln1900 are found in the fourth helix of the protein, and Ile1955 is located in the eighth helix of the domain. Although the latter four residues are in a structured region of the PRD, according to TALOS+ and structure comparison with the known desmoplakin PRD structures (ILM5 and ILM7, Choi et al. 2002) and the crystal structure of the envoplakin PRD (4QMD), the repetitive nature of the 4.5 plakin repeat

motifs that define the PRD fold creates difficulties in resolving the highly overlapped chemical shifts of these particular residues (Fig. 1). The chemical shift values for the ^1H , ^{13}C and ^{15}N resonances of envoplakin PRD have been deposited at the BioMagResBank (<http://www.bmrwisc.edu>) under accession number 26642.

Acknowledgments We thank Sara Whittaker, Christian Ludwig and other staff of the Henry Wellcome Building for Biomolecular NMR Spectroscopy for support. This research was funded by the Leukaemia and Lymphoma Research, Medical Research Council, and the Wellcome Trust which also funds HWB-NMR as a national biomedical resource.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Alcalai R, Metzger S, Rosenheck S, Meiner V, Chajek-Shaul T (2003) A recessive mutation in desmoplakin causes arrhythmogenic right ventricular dysplasia, skin disorder, and woolly hair. *J Am Coll Cardiol* 42:319–327. doi:10.1016/S0735-1097(03)00628-4
- Al-Jassar C, Bikker H, Overduin M, Chidgey M (2013) Mechanistic basis of desmosome-targeted diseases. *J Mol Biol* 425:4006–4022. doi:10.1016/j.jmb.2013.07.035
- Bouameur JE, Favre B, Borradori L (2014) Plakins, a versatile family of cytolinkers: roles in skin integrity and in human diseases. *J Invest Dermatol* 134:885–894. doi:10.1038/jid.2013.498
- Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 6:328–340. doi:10.1038/nrm1619
- Choi HJ, Park-Snyder S, Pascoe LT, Green KJ, Weis WI (2002) Structures of two intermediate filament-binding fragments of desmoplakin reveal a unique repeat motif structure. *Nat Struct Biol* 9:612–620. doi:10.1038/nsb818
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR* 6:277–293. doi:10.1007/BF00197809
- DiColandrea T, Karashima T, Määttä A, Watt FM (2000) Subcellular distribution of envoplakin and periplakin: insights into their role as precursors of the epidermal cornified envelope. *J Cell Biol* 151:573–586. doi:10.1083/jcb
- Grzesiek S, Bax A (1992) Correlating backbone amide and side chain resonances in larger proteins by multiple relayed triple resonance NMR. *J Am Chem Soc* 114:6291–6293. doi:10.1021/ja00042a003
- Jonkman MF, Pasmooij AM, Pasmans SG, van den Berg MP, Ter Horst HJ, Timmer A, Pas HH (2005) Loss of desmoplakin tail causes lethal acantholytic epidermolysis bullosa. *Am J Hum Genet* 77:653–660. doi:10.1086/496901
- Ruhrberg C, Hajibagheri MA, Simon M, Dooley TP, Watt FM (1996) Envoplakin, a novel precursor of the cornified envelope that has homology to desmoplakin. *J Cell Biol* 134:715–729. doi:10.1083/jcb.134.3.715
- Schanda P, Van Melckebeke H, Brutscher B (2006) Speeding up three-dimensional protein NMR experiments to a few minutes. *J Am Chem Soc* 128:9042–9043. doi:10.1021/ja062025p

- Shen Y, Delaglio F, Cornilescu G, Bax A (2009) TALOS + : a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. *J Biomol NMR* 44:213–223. doi:[10.1007/s10858-009-9333-z](https://doi.org/10.1007/s10858-009-9333-z)
- Sonnenberg R, Liem RK (2007) Plakins in development and disease. *Exp Cell Res* 313:2189–2203. doi:[10.1016/j.yexcr.2007.03.039](https://doi.org/10.1016/j.yexcr.2007.03.039)
- Vranken WF, Boucher W, Stevens TJ et al (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins* 59:687–696. doi:[10.1002/prot.20449](https://doi.org/10.1002/prot.20449)
- Yu CC, Yu CH, Hsueh CH, Yang CT, Juang JM, Hwang JJ, Lin JL, Lai LP (2008) Arrhythmogenic right ventricular dysplasia: clinical characteristics and identification of novel desmosome gene mutations. *J Formos Med Assoc* 107:548–558. doi:[10.1016/S0929-6646\(08\)60168-0](https://doi.org/10.1016/S0929-6646(08)60168-0)
- Zimmermann J, Bahmer F, Rose C, Zilikens D, Schmidt E (2010) Clinical and immunopathological spectrum of paraneoplastic pemphigus. *J Dtsch Dermatol Ges* 8:598–606. doi:[10.1111/j.1610-0387.2010.07380.x](https://doi.org/10.1111/j.1610-0387.2010.07380.x)