$^1$H, $^{13}$C, $^{15}$N resonance assignment of the enzyme KdgF from Bacteroides eggerthii

Agnes Beenfeldt Petersen$^{1,2}$ · Idd Andrea Christensen$^2$ · Mette E. Rønne$^3$ · Emil G. P. Stender$^3$ · David Teze$^3$ · Birte Svensson$^3$ · Finn Lillelund Aachmann$^3$

Received: 15 August 2022 / Accepted: 17 August 2022 / Published online: 30 August 2022 © The Author(s) 2022

Abstract
To fully utilize carbohydrates from seaweed biomass, the degradation of the family of polysaccharides known as alginates must be understood. A step in the degradation of alginate is the conversion of 4,5-unsaturated monouronates to 4-deoxy-L-erythro-5-hexoseulose catalysed by the enzyme KdgF. In this study $Be$KdgF from Bacteroides eggerthii from the human gut microbiota has been produced isotopically labelled in Escherichia coli. Here the $^1$H, $^{13}$C, and $^{15}$N NMR chemical shift assignment for $Be$KdgF is reported.

Keywords KdgF · Alginate degradation · 4-deoxy-L-erythro-5-hexoseulose (DEH) · Human gut microbiota

Biological context
There is a growing demand for new biomass globally. A recent report by EIT Climate-KIC concluded that by 2050 the biomass usage in Europe is expected to increase 70−150%, which current biomass sources cannot sustain (Material Economics 2021). A currently underutilised biomass is seaweed. Seaweed is a fast-growing plant that does not require arable land, fresh water, or fertilizer (Enquist-Newman et al. 2014). Seaweed therefore has the potential to be a sustainable source of biomass e.g. for the production of chemical commodities, like biofuels. Alginate composes 30−60% of the polysaccharides found in seaweed. Other carbohydrates include mainly mannitol and glucan, which, unlike alginate, can readily be used in current industry (Enquist-Newman et al. 2014).

Methods and experiments
Construct design
Genomic DNA was isolated from a culture of Bacteroides eggerthii DSM 20,697 purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Germany) using Microbiome DNA Purification Kit (Invitrogen) according to manufacturer’s specifications. The gene encoding $Be$KdgF (UniProt: R5JNH6) was amplified using a modified Phusion High-Fidelity DNA polymerase with primers designed...
for USER cloning (Salomonsen et al. 2014). BeKdgF was cloned into the pET15b-USER vector by the restriction sites Ndel and BamHI, which extended BeKdgF with an N-terminal His-tag (MGSSHHHHHHGS) resulting in 126 amino acid residues in total, and the full-length construct was used for the resonance assignment. The resulting plasmid (pET15b-USER-BeKdgF) was verified by sequencing (GATC Biotech, Germany).

**Sample preparation**

For resonance assignment $^{15}$N–$^{13}$C-labelled BeKdgF from *B. eggerthii* was produced by recombinant expression in *Escherichia coli*.

*E. coli* T7 Express cell (New England bioLabs; BL21 (DE3)) transformed with the pET15b-USER-BeKdgF plasmid were incubated overnight (37°C) in a petri dish containing LB medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl), agar (1.5%) and ampicillin (100 μg/mL). One colony was transferred to LB medium (25 mL) containing ampicillin (100 μg/mL) and incubated overnight (30°C, 225 rpm). The preculture (1% v/v) was added to M9 medium (500 mL, 6 g/L Na$_2$HPO$_4$, 3 g/L KH$_2$PO$_4$, 0.5 g/L NaCl, 1.0 g/L (15NH$_4$)$_2$SO$_4$), along with ampicillin (100 μg/mL), 2 mL MgSO$_4$ (1 M), Trace Metal solution (0.1 g/L ZnSO$_4$, 0.8 g/L MnSO$_4$, 0.5 g/L FeSO$_4$, 0.1 g/L CuSO$_4$, 1 g/L CaCl$_2$), Gibco™ MEM vitamin solution (100x), 10 mL Bioexpress Cell Growth Media (15 N labelled) (Cambridge Isotope Laboratories), and 6.0 g/L 13C$_6$ D-glucose. All isotopes were purchased from Cambridge Isotope Laboratories, Tewksbury, USA. The culture was incubated (30°C, 225 rpm) until OD$_{600nm}$ was between 0.6 and 0.8, and the culture was then cooled on ice for 5 min. Expression was induced by adding IPTG (isopropyl β-D-1-thiogalactopyranoside) (final conc. 0.5 mM), hereafter the culture was incubated overnight (37°C, 225 rpm).

The cells were harvested by centrifugation (10 min, 4°C, 5000×g), the supernatant was discarded, and the pellet resuspended in ice cold lysis buffer (50 mM HEPES, 300 mM NaCl, pH 7.7) along with one cOmplete™ EDTA-free protease inhibitor tablet (Roche). The resuspended cells were lysed using sonication for 10 min. using a Branson Sonifier equipped with a microtip. The cell lysate was centrifuged (30 min, 4°C, 16000×g) and the supernatant sterile filtered (0.22 μm filter).

BeKdgF was purified with affinity chromatography using Ni$_2^+$-resin. A column was filled with 3 mL cOmplete His-tag Purification Resin (Roche), and the resin was first rinsed with ethanol (20%, 20 mL) and ultrapure water (20 mL), before being equilibrated with 20 mL lysis buffer. The lysate was loaded onto the column and the column was washed with lysis buffer (3 × 7.5 mL) and washing buffer (50 mM HEPES, 300 mM NaCl, 20 mM imidazole, pH 7.7, 8 × 7.5 mL). The protein was eluded using elution buffer (50 mM HEPES, 300 mM NaCl, 300 mM imidazole, pH 7.7) and stored at 4°C. The enzyme molecular size and purity were assessed using SDS-PAGE.

The buffer was exchanged to the buffer used for NMR data collection (25 mM Na$_2$HPO$_4$, 50 mM NaCl, pH 7.2) using a VivaSpin column (5 kDa cut-off, Sartorius) and the sample was then concentrated to a volume of ≈ 130 μL on an Amicon Ultra centrifugal filter (3 kDa cut-off). The concentrated protein sample was transferred to a 3 mm NMR tube and 99.9% D$_2$O (10 v/v%) (Sigma Aldrich) was added. The BeKdgF concentration in the NMR sample was calculated to 430 μM using an extinction coefficient of 10,095 M$^{-1}$ cm$^{-1}$ (Gasteiger et al. 2005) and measuring absorbance at 280 nm (NanoDrop One Microvolume UV–Vis spectrophotometer, Thermo Fisher Scientific). The N-terminal His-tag did not interfere negatively the NMR work thus removal of the His-tag was not attempted.

**NMR experiments**

All NMR spectra were recorded at 20°C on a Bruker Avance III HD 800 MHz spectrometer using a 5 mm Z-gradient CP-TCI (H/C/N) cryogenic probe at the NV-NMR-Center/ Norwegian NMR Platform (NNP) at the Norwegian University of Science and Technology (NTNU). $^1$H signals were internally referenced to the water signal, and $^{13}$C and $^{15}$N signals were indirectly referenced to the water signal based on absolute frequency ratios (Zhang et al. 2003).

Backbone and side-chain resonance assignment were accomplished using $^1$H–$^{15}$N HSQC, $^1$H–$^{13}$C HSQC, HNCO, HNCA, HNCA(CB), HN(C)CA, HNCOHA, HNHA, HcCH-TOCSY, and $^{15}$N-NOESY-HSQC with 80 ms mixing time. All spectra were processed using TopSpin version 3.6.1.

Spectra were analysed using CARA (Computer Aided Resonance Assignment) version 1.8.4.2 (Keller 2004). A dihedral angle analysis based on the measured backbone and side-chain chemical shifts was made using TAŁOS-N (Shen and Bax 2013).

**Assignment and data deposition**

Here we report the backbone and side-chain assignment of BeKdgF from *B. eggerthii*. The $^{13}$N-HSQC spectrum of BeKdgF with the assigned resonances is shown in Fig. 1. The backbone assignment is almost complete (H$^N$, H$^C$, C$^\alpha$, N, and C$'$ > 92%). The five unassigned residues (H52, F53, P86, D87, and V88) define short two sections in BeKdgF, which may be ascribed to multiple conformations in intermediate exchange, enhance relaxation or fast exchange regime. The side-chain assignment is partially complete (side-chain H and C $\approx$ 59.3%). The unassigned...
smaller peaks in the $^1$H-$^{15}$N HSQC are due to impurities of the sample. The amino acid residue R108 has an unusual chemical shift as the $^1$H, $^1$C, and $^13$C chemical shifts are significantly lower than expected (Ulrich et al. 2008). The chemical shifts have been deposited in the Biological Magnetic Resonance Data Bank (BMRB) under the accession number 51288.

Secondary structural propensity was evaluated by investigating secondary chemical shift values of $^{13}$C-$^{15}$N labelled $BeKdgF$. The chemical shift deviations of $C_\alpha$ and $C_\beta$ from the random-coil values for each residue (Wishart et al. 1995) were calculated, and the results can be seen in Fig. 2. Generally, the $C_\alpha$ chemical shifts of $BeKdgF$ are lower than the predicted random-coil values, whereas the $C_\beta$ values are higher. This indicates that $BeKdgF$ generally consists of $\beta$-sheets.

The probability of secondary structural elements was calculated based on the dihedral angle analysis using TALOS-N (Shen and Bax, 2013). The results can be seen in Fig. 3. The dihedral angle analysis shows that 50% of the amino acid residues in $BeKdgF$ form $\beta$-strands and the rest random coils with a possibility of a three amino acid residue $\alpha$-helix present. The $\beta$-sheets content is consistent with the three previously reported structures of KdgF from the organisms Yersinia enterocolitica and Halomonas sp (Hobbs et al. 2016).

The $^1$H, $^{13}$C, $^{15}$N resonance assignment of $BeKdgF$ has been presented. The assignment gives the possibility of...
Further investigation of \textit{BeKdgF} with NMR spectroscopy. Future functional studies will include metal ion interactions, pH titration, and protein dynamics. Understanding the biological role of KdgF can aid the industrial use of alginates extracted from seaweed as biomass.

**Acknowledgements** We are grateful to Christian Dybdahl Andersen for identifying the \textit{BeKdgF} gene and Kari F. P. Sollerud for optimizing the isotope protein production and NMR conditions. Support from Innovation Fund Denmark grant 1308-00011B (StrucSat) and PhD fellowships from Technical University of Denmark (to EGPS and MER) is gratefully acknowledged.

**Author contributions** ABP has designed experiments, performed research and analyzed data, and wrote the first draft of the manuscript. IAC, MER, EGPS, DT has performed research, analyzed data, and reviewed the manuscript. BS and F.L.A. initiated the research, conceptualization, designed experiments, analyzed data, edited the manuscript, carried out supervision, and acquired funding.
Funding Open access funding provided by NTNU Norwegian University of Science and Technology (incl St. Olavs Hospital - Trondheim University Hospital). This work was financed by the Norwegian Research Council (NFR) Grants 315385 (AlgModE) and 226244 (Norwegian NMR Platform) and Innovationsfonden (Grant No: 1308-00011B)

Data availability The assigned chemical shifts have been deposited in the Biological Magnetic Resonance Data Bank (BMRB) under the accession number 51288.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments conducted do not violate any ethical principles.

Consent for publication All authors declare that they consent to the publication of this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Enquist-Newman M et al (2014) Efficient ethanol production from brown macroalgal sugars by a synthetic yeast platform. Nature 505(7482):239–243. https://doi.org/10.1038/NATURE12771
Gasteiger E et al (2005) The proteomics protocols handbook. Humana Press, Totowa. https://doi.org/10.1385/1592598900
Gorin PAJ, Spencer JFT (1966) Exocellular alginic acid from Azotobacter Vinelandii. Can J Chem 44(9):993–998. https://doi.org/10.1139/v66-147
Haug A, Larsen B, Smidsrød O (1967) Studies on the sequence of uronic acid residues in Algicin acid. Acta Chem Scand 21:691–704
Hobbs JK et al (2016) KdgF, the missing link in the microbial metabolism of uronate sugars from pectin and alginate. Proc Natl Acad Sci USA 113(22):6188–6193. https://doi.org/10.1073/pnas.1524214113
Keller R (2004) The computer aided resonance assignment. Goldau, Switzerland C Verlag [Preprint]
Kim HS, Lee C-G, Lee EY (2011) alginate lyase: structure, property, and application. Biotechnol Bioprocess Eng 16:843–851. https://doi.org/10.1007/s12257-011-0352-8

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.