



STRUCTURAL
BIOLOGY

Volume 73 (2017)

Supporting information for article:

Learning from oligosaccharide soaks of crystals of an AA13 lytic polysaccharide monooxygenase: crystal packing, ligand binding and active-site disorder

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Table S1 Macromolecule production information

| | |
|--|--|
| Source organism | <i>Aspergillus oryzae</i> RIB40 |
| DNA source | Genomic DNA |
| Forward primer | 5'-CGAGGATCCAACATAATGAAGGTCTTCG-3' |
| Reverse primer | 5'-AGCAAGCTTCAGTGACGAAATGCCAT-3' |
| Cloning vector | pMStr57 |
| Expression vector | pMStr80 |
| Expression host | <i>Aspergillus oryzae</i> MSTR212 |
| Complete amino acid sequence of the construct produced | HGYMYIPSSRTRLGHEAGIDSCPECAILEPVSSWPDLDAAPVG RSGPCGYNARDSIDYNQPTTNWGS DAVQSYSPGEEIEVQWCV DHNGDHGGMFTYRICDQSI VDKFLDPSYLP TNDKQAAEDC FDAGLLPCTDVSGQECGYSADCTEGEACWRNDWFTCN GFEA SDRPKCQGV DNAELNSCYTSIAGGYTVTKKVKLPEYTSNHTLI SFKWNSFQTGGQIYLS CADIAIQ |

Table S2 Zn sites in the AoAA13 structures

| | Zn-AoAA13 (unliganded) | | AoAA13- His91flip | | AoAA13- G2(α1,4) | | AoAA13- G3(α1,4)G1(α1,6) | |
|--------------------------------|-----------------------------------|-------|------------------------------|-------|---|-------|---|-------|
| Soaking time | - | | 48 hours | | 3 hours | | 18 hours | |
| Zinc sites | Occ | B | Occ | B | Occ | B | Occ | B |
| meH1-Zn-H91 [§] | 1.00 | 15.28 | 0.20 | 17.13 | 0.80 | 5.24 | 0.70 | 12.87 |
| | - | - | - | - | 0.20 | 5.40 | - | - |
| H15-Zn-E29* | 0.50 | 14.37 | - | - | 0.70 | 9.71 | 0.50 | 16.95 |
| | - | - | - | - | 0.20 | 14.20 | - | - |
| H87-Zn- E142* | 0.80 | 35.09 | - | - | 0.20 | 12.91 | - | - |
| D36-Zn-D38 | 0.75 | 14.08 | 0.30 | 10.77 | 0.70 | 7.50 | 0.75 | 12.16 |
| | - | - | - | - | 0.20 | 15.09 | - | - |
| (E203*) | - | - | - | - | 0.10 | 11.97 | - | - |
| D148-Zn-D119* | 0.50 | 19.17 | - | - | - | - | - | - |
| D102-Zn-S104 | 0.20 | 18.48 | 0.50 | 13.04 | 0.80 | 11.60 | 0.60 | 13.94 |
| E125-Zn-D129 | 0.50 | 28.27 | - | - | - | - | - | - |
| E153-Zn | 0.50 | 45.25 | - | - | 0.35 | 7.14 | - | - |
| Q233-Zn-S74 | 0.50 | 22.56 | - | - | 0.20 | 8.39 | - | - |
| (Q219)-H ₂ O-Zn | - | - | - | - | 0.20 | 11.42 | - | - |
| (G66/L110)-H ₂ O-Zn | - | - | 0.10 | 13.85 | - | - | - | - |

[§] Active site.

*Symmetry related molecule.

Occupancy (Occ); B-factor (B) in Å²

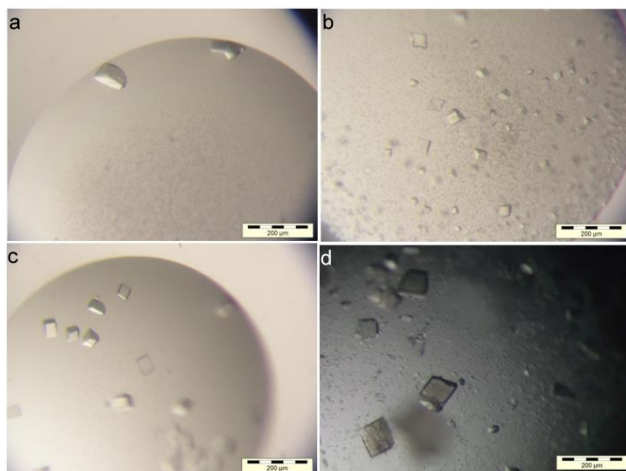


Figure S1 Crystals of *AoAA13* obtained in MRC (a-c) and VDX plates (d). **a**) Crystals grown in 10% (v/v) PEG 3000, 0.2 M Zn-acetate, 0.1 M Na-acetate pH 4.5. **b**) Crystals grown in 20% (v/v) PEG 3000, 0.2 M Zn-acetate, 0.1 M imidazole pH 8.0. **c**) Crystals grown in 20% (v/v) PEG 3000, 0.2 M Zn-acetate, 0.1 M buffer system II pH 5.0. **d**) Crystal after 18 hours soak with 125 mM glucosyl-maltotriose

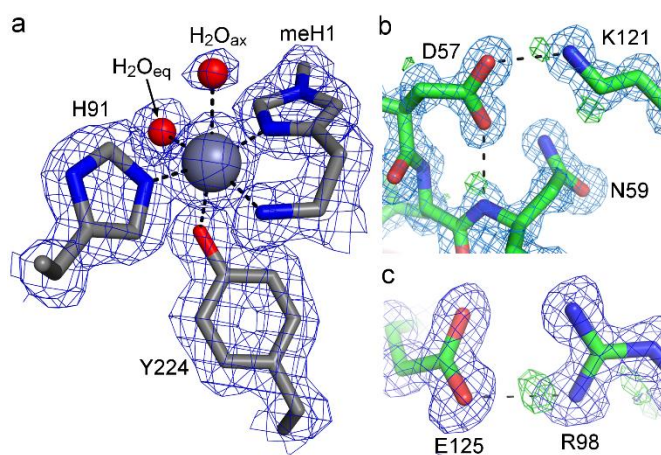


Figure S2 a) Active site of Zn-AoAA13. The active site residues are shown in grey sticks, and active site Zn and water ligands are shown as zinc sphere and red spheres, respectively. **b-c)** Well-ordered regions in the AoAA13-G2(α 1,4) structure (2Fo-Fc in blue) with hydrogens clearly visible in the Fo-Fc map (green). In all panels the 2Fo-Fc and Fo-Fc maps are contoured at 1σ and 3σ , respectively.

| | | |
|-------------------|-----|---|
| Q2U8Y3_AoAA13 | 1 | HG-----YMYIPS-----SRTRLGHE-----AGI |
| Q7SCE9_NcAA13 | 1 | HG-----YLTIPF-----SRTRLGAE-----AGL |
| Q5B1W7_AnAA13 | 1 | HG-----YLTVPA-----SRTRLGFE-----AGI |
| B6H504_P.chrysog. | 1 | HG-----YLTIPS-----SRTRLGFE-----AGI |
| B2AD24_P.anserina | 1 | HGGHSGSRPWLPDHPVQSDTSWLRGKSKLRVWNLSTDTWAQQ-----AGL |
| E5A5Y5_L.maculans | 1 | HG-----YLSSPM-----SRTGLNAQDLDRNLSNSKCSQAGA |
| Q2KEQ8_M.grisea | 1 | HG-----YLTFPM-----SRTGLNAQ-----AGP |
| | ** | * * * |
| Q2U8Y3_AoAA13 | 20 | DSCPECAILEPVSSWPDLDAAAPVGRSGPCGYNARDSIDYNQPTTN-WGSDAVQSYSPGEE |
| Q7SCE9_NcAA13 | 20 | DTCPECSILEPVTAWPNVTEAKVGRSGPCGYNARVSIDYNQPATN-WGNSPVVYTAGDT |
| Q5B1W7_AnAA13 | 20 | DTCPECSILEPVSAWPDLTAAQVGRSGPCGYNARVSDYNQPGDY-WGNEPVVSYTAGDV |
| B6H504_P.chrysog. | 20 | DTCPECSILEPVSAWPDLEEAQVGRSGPCGYNARVGVVDYNQPAAH-WGNSVVATYTNQI |
| B2AD24_P.anserina | 48 | DSCPECSILEPVSAWPDLDVAPVGRSGPCGYNARVSIDYNQPRAGLWGNSPVARYSPGQT |
| E5A5Y5_L.maculans | 33 | DTCPECTILEPVPAPWPDLDAAAVGRSGPCGFNARVSDYNQPGPR-WGSQPVITYKGET |
| Q2KEQ8_M.grisea | 20 | DTCPECTILEPVTAWPDLDQAQVGRSGPCGYNARVSDYNQPGPR-WGSAPVVITYKGGDV |
| | * | **** * *** ** * * * ** ** * * * * |
| Q2U8Y3_AoAA13 | 79 | IEVQWCVDHNGDHGGMFTYRICQDQSIVDKFLDPSYLPNTDEKQAAEDCFDAGLLPCTDV |
| Q7SCE9_NcAA13 | 79 | VDVQWCVDHNGDHGGMFSYRICQDQELVNKFLTPGYLPTAEAKQAAEDCFEKGTLPCTDV |
| Q5B1W7_AnAA13 | 79 | VEVQWCVDHNGDHGGMFTYRICQNTLVDLFLTPGYLPTNEEKQAAEDCFLEGELSLCHV |
| B6H504_P.chrysog. | 79 | VEVEWCVDNNGDHGGMFTYRICQNTLVDLFLDPNYLPTNAEKQAAEDCFLEGELKCTDV |
| B2AD24_P.anserina | 108 | IDVQWCVDNNGDHGGMFAYRICQDHALVKKFLTPGYLPTDEEKQAAEDCFERGTLPCTDV |
| E5A5Y5_L.maculans | 92 | VDVQWCVDNNGDHGGMFSYRICQDQSIVDKFLITPGYLPTEAEKAAAEDCFNKGLLKCTDV |
| Q2KEQ8_M.grisea | 79 | ADVQWCVDNNGDHGGMFTYRICQDQALVDKLLTPGYLPSEAEKQAAENCFRAGTLPCTDV |
| | * | * |
| Q2U8Y3_AoAA13 | 139 | SGQECGYSADCTEAGEACWRNDWFTCNQGFASDRPKCQGVDNAELNSCYTSIAGGYTVTKK |
| Q7SCE9_NcAA13 | 139 | NGQSCDFSPDCQQGQACWRNDWFTCNQAFQADSRRCQGVDNAALGSCFTIAGGYTVTKK |
| Q5B1W7_AnAA13 | 139 | PGQTCNYPDCSAGEPCYQNDWFTCNQAFQADNNRACQGVGAAALNSCMTIAGGYTVTKK |
| B6H504_P.chrysog. | 139 | PGQTCGYSPDCTSDQPCWRNDWFTCNQAFNAGSRRGCQGVGAPLNSCKTIAGGYTITKK |
| B2AD24_P.anserina | 168 | SGQNCGFSPDCSPGQPCWRNDWFTCNQAFNAGDRRACQGVDDAPRGSCYTSIAGGFVPTKK |
| E5A5Y5_L.maculans | 152 | AGQSCGFNPDCRQGPCWRNDWFTCKGFQDG--PRCKGVNAPINSCTYSIAGGYTVSSK |
| Q2KEQ8_M.grisea | 139 | NGQSCGYSPDCSPGQACWRNDWFTCKGFQD---TKCRGVNAPLNSCYTSIAGGYTTP-- |
| | * | * * ** * * * * * * * * * * * * * * * * |
| Q2U8Y3_AoAA13 | 199 | VKLPEYTSNHTLLISFKWNSFQTGQIYLSCADIAIQ |
| Q7SCE9_NcAA13 | 199 | IKIPNYISGHTLLSFRWNSFQTAQVYLSCADIAIV |
| Q5B1W7_AnAA13 | 199 | IKIPDYSSHTLLRFRWNSFQTAQVYLHCADIAIV |
| B6H504_P.chrysog. | 199 | IKIPDYSAHTLLRFRWNSFQTAQVYLGADISIV |
| B2AD24_P.anserina | 228 | IKLPNINVGHTLLSFKWNSFQTGQIYLSCADIAIG |
| E5A5Y5_L.maculans | 210 | IKIPNYVSNHTLLSLKWNAFETPQIYSTCADIKIT |
| Q2KEQ8_M.grisea | 194 | -QIYLTCADIKITAPDSQSPPTTTTSTPAS---- |
| | * | * * |

Figure S3 Multiple sequence alignment of selected members of the AA13 family with Uniprot entries in the CAZy database. Uniprot accession numbers are given followed by either the protein- or organism name. The residues, R58 and S188, involved in interactions with maltose in AoAA13-G2(α 1,4) are highlighted in green, while the residues, Q82, Q141 and T194, interacting with glucosyl-maltotriose in AoAA13-G3(α 1,4)G1(α 1,6) are highlighted in yellow. Fully conserved residues for the complete AA13 family (as found in (Lo Leggio *et al.*, 2015)) are indicated by asterisks.