MS10 Protein-carbohydrate interactions

MS10-05

Polyuronic acid degradation by polysaccharide lyase family 7

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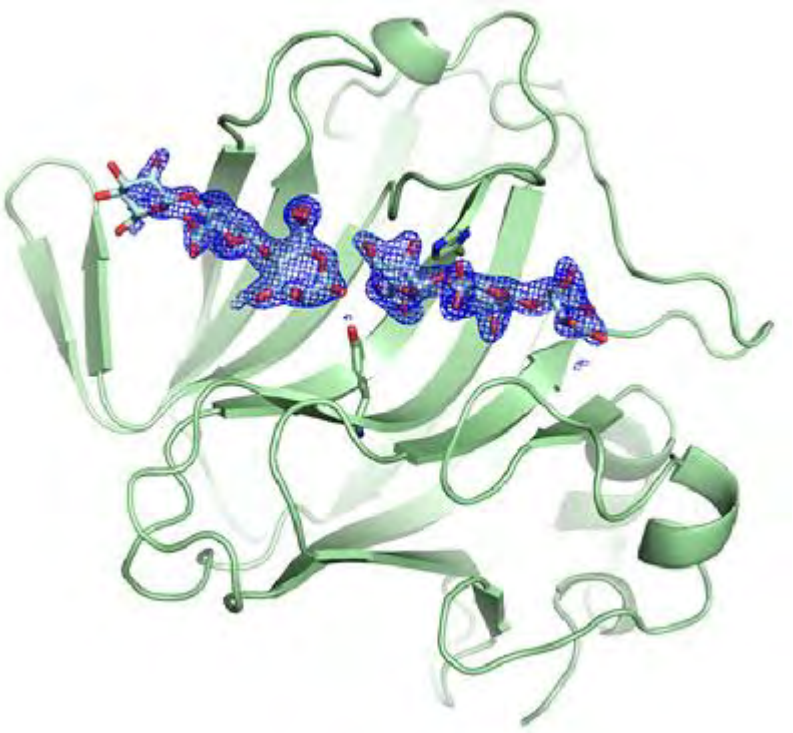
Abstract

Polysaccharide lyases (PLs) are in general efficient enzymes that generate short oligosaccharides not longer oligosaccharides that from an industrial perspective are far more promising. Unfortunately, the enzymatic mechanisms of PLs is not clearly understood preventing protein engineering leading to longer oligosaccharides. PLs are hypothesized to catalyze glycosidic linkage clevage *via* a 3-step beta-elimination mechanism generating an unsaturated uronic acid residue and a new reducing end. First, the carboxylate group of an uronic acid moiety is neutralized by an amino acid residue or a Ca^{2+} ion, which will lower the pK_a of the C5 proton allowing its abstraction by the catalytic base in step 2. In the 3rd step, an amino acid or water acts as a catalytic acid and donates a proton to the glycosidic linkage. This cleaves the glycosidic bond and creates the unsaturated bond between C4 and C5. Further, PLs catalyze bond cleavage by *syn*- or *anti*β-elimination. In *syn*, the C5-proton to be abstracted and protonation of the glycosidic oxygen at the new reducing end are on the same side of the sugar ring, while in *anti* on opposite sides. In theory, in the *syn* mechanism, the same residues can abstract the proton and donate it to the glyosidic bond, whereas in *anti* two catalytic groups are needed, however, this remains to be shown.

We have discovered PL7 members acting on β-glucuronan and alginate that are excellent model systems for studying PL7 mechanisms. Previously, it was suggested that a histidine acts as the general base and a tyrosine the general acid. We identified aβ-glucuronan lyase with a k_{cat} of 3119 s^{-1} lacking the histidine. The high k_{cat} enabled a mutational analysis based on the crystal structure (1.45 Å) in which residual activity could be detected for all mutated residues except the tyrosine, which suggests this enzyme acts *via* a *syn* mechanism. Crystal structures (>0.82Å) of two alginate lyases in complex with alginate oligosaccharides – both Michaelis-Menten-like complex' and products after catalysis, were solved indicating the histidine's role is to distort the sugar moiety at subsite +1, which positions the scissile glyosidic linkage within reach of the tyrosine that can then act as both the general acid and base. A neutron crystal structure (2.33 Å) shows that the tyrosine is deprotonated, enabling it to abstract the proton. QM/MM is currently on-going to determine if the tyrosine can donate the proton to the glyosidic linkage.

PsAlg7A in complex with products



PsAlg7A Michaelis-Menten-like complex