**s8a.m1.p19 Snapshots of Enzyme Activation**. 
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Keywords: molecular interactions, hydrogen bonds.

Crystals of B. lentus subtilisin, when cryo-cooled diffract to 0.85 Å resolution or better, which has been sufficient to resolve a special hydrogen bond within the catalytic triad at pH 5.9 (1) We have collected data from crystals at pH 5.0 and 8.6 where the enzyme is inactive or fully active. These data sets extend to a resolution of 0.85 and 0.76 Å, respectively. Both data sets in the outermost shell; are > 94% complete and have $I/\sigma(I) \geq 1.9$. The aim of this work is to determine what changes may occur within the catalytic triad under conditions were the enzyme is inactive, partially and fully active. Preliminary difference electron density maps suggest that some subtle rearrangements do occur and refinement is underway.

[1] Kuhn *et al.* (1998) Biochemisrtry 37, 13446-13452.

**s8a.m1.p20 How to degrade sulfated galactans : the structure of κ- and ι-carrageenases.** 
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Keywords : carrageenases, β-sandwich, β-helix.

Carrageenans are sulfated polysaccharides found in the cell walls of marine red algae. They consist of a backbone of D-galactoses linked by alternating α(1, 3) and β(1, 4) linkages. According to the number of sulfates per repeating unit, one distincts κ-, ι- and λ-carrageenan. They form gel and are used as texturing agents in industries. The degradation of these galactans is achieved by hydrolases which cleave the internal β(1, 4) linkage with a strict substrate specificity. κ-carrageenases (κ-ases) belong to family 16 of glycoside hydrolases, whereas ι-carrageenases (ι-ases) constitute a new structural family. Oligosaccharides produced by the hydrolysis of carrageenans can be used to stimulate the resistance of plants against phytopathogens.

To understand the mechanisms of hydrolysis and recognition of carrageenans, the κ-ase of *P. carrageenovora* (33kDa) and the ι-ase of *A. fortis* (53 kDa) have been expressed in *E. coli* with a His-tag, in native form or with seleno-methionine, and purified to electrophoretic homogeneity. These proteins have been crystallized with PEG. κ-ase crystals belong to the space group $P2_12_12_1$ (a=56.4 Å, b=61.1 Å, c=75.5 Å), whereas ι-ase crystals belong to the space group $P2_1$ (a=56.8 Å, b=91.0 Å, c=124.9 Å, β=93.8°) with two molecules in asymmetric unit. Phases were determined by MAD method. Data were collected at the ESRF at three wavelengths at the selenium K edge at 1.75 Å and 2.3 Å resolution for κ- and ι-ase, respectively. After phase extension using higher resolution data, the first models were built with wARP, which assigned about 80% of the main chain. The missing parts were built manually using O. The models were refined using CNS (κ-ase : R=17.8%, $R_{free}$=19.3% at 1.54 Å ; ι-ase : R=20.7%, $R_{free}$=22.3% at 1.60 Å).

κ-ase displays a β-sandwich fold, similar to lichenase fold, only known structure in family 16. However, three additive β-sheets closed the active site, whereas a open cleft was found in lichenase. This tunnel topology, only observed in cellobiohydrolases, suggested an endo-processive mechanism. ι-ase comprises a right handed β-helix core with two inserted domains in C-terminal region. The structure revealed one chloride, three sodium and three calcium binding sites. Two calcium binding sites have a hairpin topology and could play a structural or functional role. The long cleft formed by the β-helix should be the binding area of the double-helix of ι-carrageenan. The flexibility of the inserted domains suggested they could change conformation to hold ι-carrageenan during the processive hydrolyzing mechanism.