**Structural studies of chitinolytic enzymes from *Pyrococcus chitonophagus***

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**Summary**

This thesis presents structural and functional studies of enzymes involved in the chitin degradation pathway. Four proteins belonging to three different enzyme classes (chitinase, diacetylchitobiose deacetylase and *exo*-β-D-glucosaminidase) were selected for the study and purification protocols was established for them.

I obtained crystals for two of the enzymes (under several crystallization conditions) for which diffraction data were recorded. For diacetylchitobiose deacetylase, three data sets were recorded: for the unliganded protein, anomalous data at the zinc absorption wavelength, and for the enzyme-substrate complex. Thanks to additional research, I was able to confirm that the deacetylase belongs to the carbohydrate esterase (CE) family 14, as suggested by the literature, which includes zinc-dependent deacetylases. For *exo*-β-D-glucosaminidase, crystallographic data were obtained and the structure was solved of the unliganded enzyme.

In addition to structural research, which was the focus of my work, the scope of activities was extended through collaboration to complementary research by means of techniques such as SAXS, NMR, ITC, DSC.

The three enzymes that I investigated – chitinase, deacetylase and glucosaminidase – form a minimum set that should be able to degrade chitin all the way to glucosamine, provided that the latter two enzymes are not "too specific". Otherwise intermediate products will accumulate. The deacetylase in this study removes the acetyl group only from the non-reducing end of $(GlcNAc)_2$ and $(GlcNAc)_3$, but it can also deacetylate the monomeric GlcNAc. This means that it is sufficiently unspecific to be part of the chitinolytic trio, defined above. The specificity of glucosaminidase still remains to be determined and will be subject to further studies, but its structure has been solved and

early enzymatic studies indicate that it complements the activity of diacetylchitobiose deacetylase.

It is worth noting that both the hyperthormophilic enzymes, Dac-74 and GlmA-01, could be prepared at room temperature but required annealing before any enzymatic activity could be detected. The term “annealing” was defined in metallurgy and materials science, to describe heat treatment that alters physical or chemical properties of materials. It apparently applies also to at least some hyperthermophilic enzymes. The details of what actually takes place during the annealing process remain to be determined.