WORKING HARD AND IN THE COLD: CHITINASE FROM *M. marina*

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A wide variety of microorganisms are present in cold environment, displaying a diverse range of adaptations. The major part of the marine biosphere is characterized by permanent low temperatures (-2 – 10°C) and therefore is a good source of cold-adapted marine bacteria, the so-called psychrophilic bacteria. Chitin is a very abundant insoluble biopolymer in the marine environment and is composed of linear chains of β-1,4-linked N-acetyl-D-glucosamine (NAG) residues that are highly cross-linked by hydrogen bonds. Chitin is abundant in nature, second after cellulose, as a crucial structural component of the cell walls of fungi and certain green algae, and as a major constituent of shells, cuticles and exoskeletons of worms, molluscs and arthropods, including crustaceans and insects. Chitin and its partially deacetylated derivative, chitosan, as well as other derivatives exhibit interesting properties and constitute a valuable raw material for biomedical, agricultural, cosmetics, and innovative biotechnological applications. In the aquatic biosphere, approximately 10^{11} tons of chitin are produced annually.

Chitinases (EC 3.2.1.14) hydrolyse the β-1,4-linkages in chitin. Chitinases produced by psychrophilic bacteria, responsible for degradation of the krill chitin, should have high catalytic activities under these low-temperature conditions and most often, if not always, exhibit high thermosensitivity. These properties can be very useful for various applications.

We report the crystal structure of a chitinase from the psychrophilic bacterium *Moritella marina*. The enzyme has been examined in complexes with the reaction intermediate, with the reaction product and in an unliganded form. The enzyme consists of 528 amino-acid residues arranged into four domains: a β/α-barrel that includes the substrate-binding and the active site, two elongated domains having an Ig-like fold, and a small chitin-binding domain (Figure 1). The enzyme is active in the crystal form. It has reduced a NAG_4 substrate, added to the cryo-protecting solution, to an oxazolinium reaction intermediate. In another experiment it reduced NAG_3 to NAG_2. The results of its activity are clear in the electron density and have been examined in order to determine the basis of substrate recognition and the enzymatic activity.

Comparisons with related enzymes have been used to identify features that are conserved, and therefore are presumed to be essential, and those that have been evolving and are therefore likely to be the enzyme's response to the specific environment.

Psychrophilic enzymes have been proposed to possess some "additional flexibility" that allows them to function efficiently at low temperature. We have examined the crystal structure of the psychrophilic chitinase looking for such features.

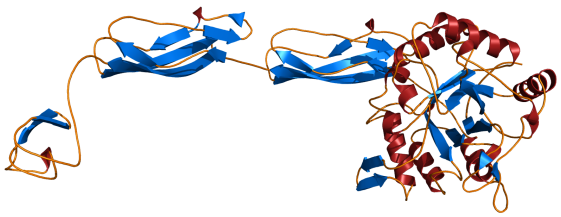

Figure 1: Ribbon diagram of chitinase from *M. marina*.

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References

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