Thermus thermophilus amylomaltase: a new, closed conformation of the enzyme is observed

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Amylomaltases transfer glycosyl groups to and from amylose chains. Despite strong homology to amylases, they are incapable of catalyzing amylose hydrolysis. Like the amylases, the amylomaltases employ a reaction mechanism based on a covalent intermediate. Using amylomaltase, starch can be converted to a number of interesting biotechnological products like cycloamyloses and thermoreversible starch gels.

We study the amylomaltase from *Thermus thermophilus*, which is easily prepared in large amounts of high purity, with a view to understanding the mode of action of this enzyme. In particular, we wish to understand the reasons for the low hydrolysis rate to guide a directed evolution effort aimed at producing more hydrolytic variants of amylomaltase.

Other researchers have reported the crystal structure of the virtually identical (99.8%) amylomaltase from *Thermus aquaticus*, both native [1] and in complex with the α-amylase inhibitor acarbose [2]. This latter study was performed at pH 9.0.

We have succeeded in growing crystals of the *Thermus thermophilus* enzyme at pH 5.6, close to the pH optimum of 5.5. These crystals diffract to better than 2.0 Å resolution. A dataset was collected at the BW7A beamline of the EMBL outstation at the DESY synchrotron in Hamburg, Germany. The data were phased using MR, using the structure of the *Thermus aquaticus* enzyme as a search model.

A comparison of the structure in the new crystal form with the *Thermus aquaticus* amylomaltase structure reveals a striking difference in conformation: in the new crystal form, a more closed conformation is observed (figure 1). This new conformation may be seen as arising from a hinging motion of the protein structure, around an axis going through one of the catalytic residues.

We are currently analyzing the possible role of this new conformation in the catalytic cycle by comparing the conformations observed in different complexes of amylomaltase. In the future, we hope to have a detailed understanding of the reaction cycle of these enzymes, to use as a guide in protein engineering.

References

[1] I. Przylas, Y. Terada, K. Fujii, T. Takaha, W. Saenger, N. Strater, Eur. J. Biochem. 267, 6903-6913 (2000)

[2] I. Przylas, K. Tomoo, Y. Terada, T. Takaha, K. Fujii, W. Saenger, N. Strater, J. Mol. Biol. 296, 873-886 (2000)

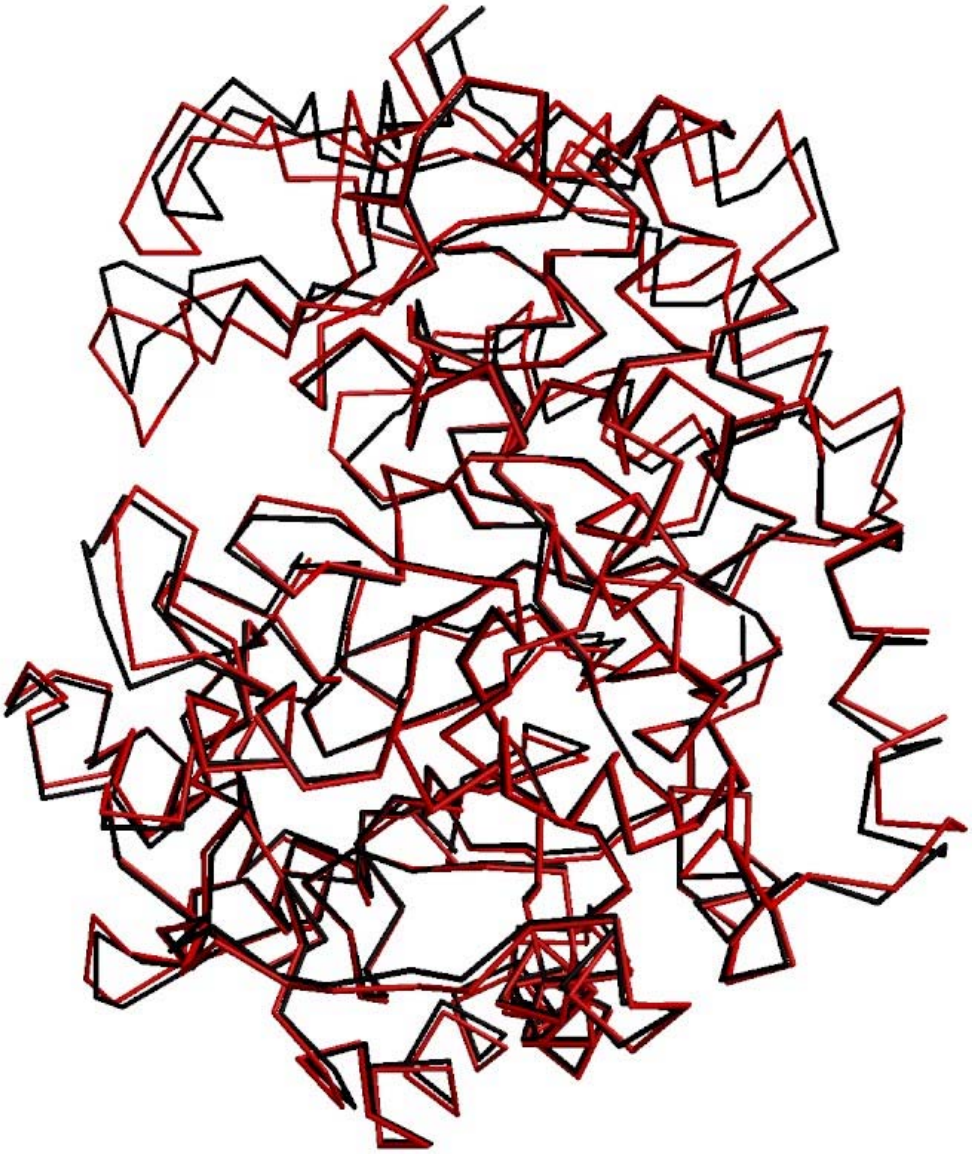

Figure 1: Open (black) and closed (red) conformation of *Thermus* amylomaltase