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New features of the biocatalyst transketolase from Geobacillus stearothermophilus emerging from biochemical and structural analyses

AUTHORS

Camilla LEOGRANDE / GEORG AUGUST UNIVERSITÄT GÖTTINGEN, JULIA-LERMONTOWA-WEG 3, GÖTTINGEN
Fabian RABE VON PAPPENHEIM / GEORG AUGUST UNIVERSITÄT GÖTTINGEN, JULIA-LERMONTOWA-WEG 3, GÖTTINGEN
Laurence HECQUET / UNIVERSITÉ CLERMONT AUVERGNE, AVENUE BLAISE PASCAL 24, AUBIERE
Wolf-Dieter FESSNER / TECHNISCHE UNIVERSITÄT DARMSTADT, ALARICH-WEISS-STRASSE 4, DARMSTADT
Kai TITTMANN / GEORG AUGUST UNIVERSITÄT GÖTTINGEN, JULIA-LERMONTOWA-WEG 3, GÖTTINGEN

PURPOSE OF THE ABSTRACT

Transketolases are enzymes with a high biocatalytic potential: they are able to transfer a chemical moiety from a ketose donor to an aldose acceptor thereby creating a new carbon-carbon bond. The transketolase from the thermophilic organism Geobacillus stearothermophilus (gstTK) has unique stability properties that make it particularly suitable for industrial applications [1]. Therefore, it has been elected to undergo extensive directed-evolution efforts for accepting non-natural substrates.

Here, we report on the structural analysis of gstTK. We present the first experimental structure of gstTK solved by X-ray crystallography (PDB code 8CIP). The gstTK crystal was assigned to space group P1 with two homodimers in the asymmetric unit, a feature only shared with the sole other thermophilic TK released to date (ttTK from Thermus Thermophilus, PDB code 7WRR) and, interestingly, with the TK from Mycobacterium tubercolosis (mtTK, PDB code 3 RIM). While in vitro assays on mtTK relegated the tetramerization to a mere crystallization artifact, contrary evidence exists for ttTK. It was postulated that a higher oligomerization state could be a strategy adopted by the enzyme to cope with higher temperatures [2]. We here prove the existence of a gstTK tetramer in solution with several independent biochemical assays, using the well-studied mesophilic orthologue from Escherichia coli (ecTK) as comparison. Our support to the above-mentioned hypothesis could enable the engineering of new thermostable catalysts in the future.

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FIGURES

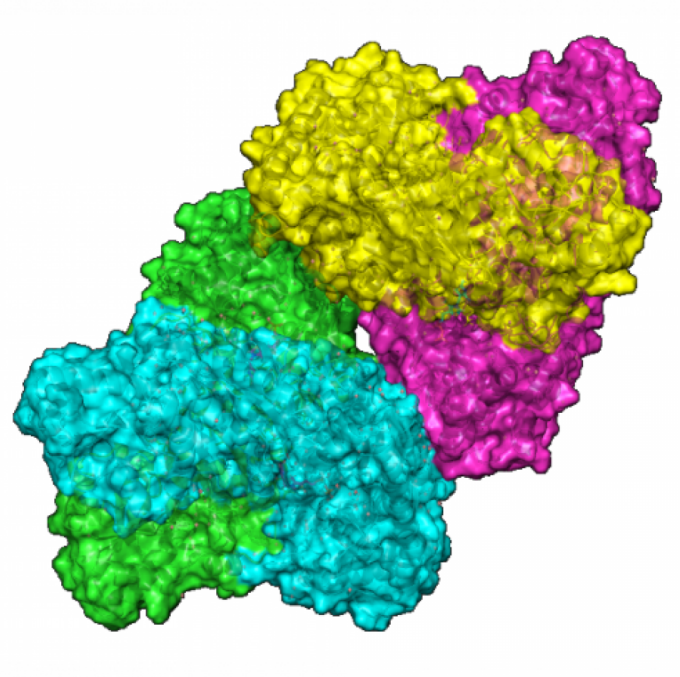

FIGURE 1

PDB 8CIP

The gstTK tetramer as appears in the crystallographic asymmetric unit.

FIGURE 2

KEYWORDS

transketolase | thermostability | tetramerization | X-ray crystallography

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