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A Novel ‘Split-gene’ transketolase from the hyper-thermophilic bacterium Carboxydothermus hydrogenoformans: structure and biochemical characterisation

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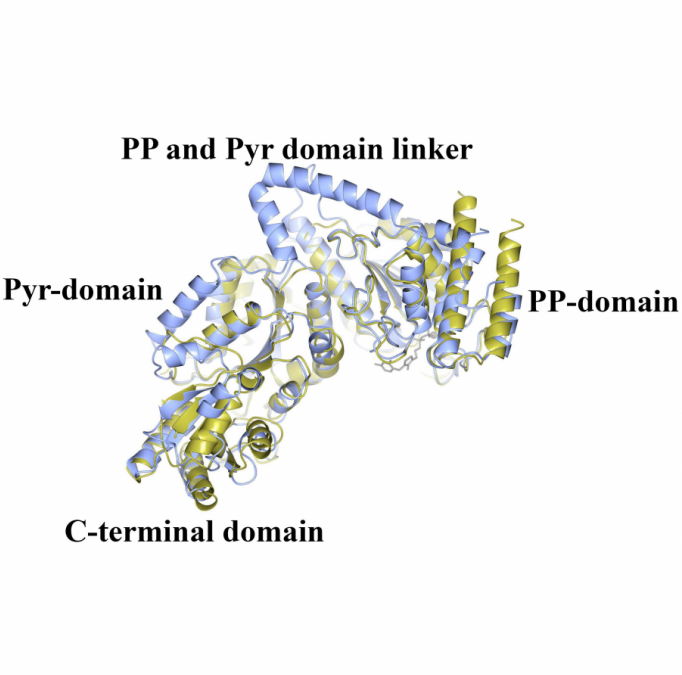
PURPOSE OF THE ABSTRACT

The genes encoding the two parts of a novel split transketolase were identified from the genome of the hyperthermophilic Carboxydothermus hydrogenoformans. These have been cloned and over-expressed in Escherichia coli before being reconstituted and purified by size exclusion chromatography. This novel enzyme is the first TK to be reconstituted and characterised both biochemically and structurally with the enzyme active using hydroxypyruvate as the ketol donor and different aldehyde acceptors. The reconstituted active alpha2beta2 tetrameric enzyme has been characterised biochemically and its structure determined to high resolution.

The activity of this enzyme has been monitored using the non-natural commercially interesting reaction using hydroxypyruvate as the ketol donor and a range of aldehydes including glycolaldehyde, butylaldehyde and cyclohexane aldehyde as the acceptor substrate. This reaction is used for the synthesis of a range of unusual sugars of interest for the pharmaceutical industries and proceeds to 100% completion due to the release of the product carbon dioxide This novel reconstituted transketolase is thermally stable with no loss of activity after incubation for 1 hour at 70 °C and is stable after 1-hour incubation with 50 % of the organic solvents methanol, ethanol, isopropanol, DMSO, acetonitrile and acetone. In the presence of the cofactor thiamine pyrophosphate and calcium, the crystals of the reconstituted alpha2beta2 tetrameric transketolase allowed the structure to be determined to 1.4 Å resolution. The ability of thiamine pyrophosphate (TPP) enzymes such as transketolase to form carbon-carbon bonds is a valuable feature used in the synthesis of high value chiral compounds and there is an interest to extend the range of substrates and products of these reactions. 1-Deoxy-D-xylulose 5-phosphate synthase (DXP synthase) is another TPP-dependent catalyst which transfers a two-carbon unit from pyruvate onto specific aldose D-glyceraldehyde 3-phosphate but is able to use the cheaper and more stable pyruvate as the ketol donor. A structural comparison of the split Carboxythermus transketolase, the Escherichia coli full length transketolase and the DXP synthase has been carried out in an attempt to rationalise the substrate specificity differences between the three enzymes. This reconstituted ‘split-gene' enzyme is the first example of a split transketolase to be fully characterised allowing its potential for industrial biocatalysis to be evaluated.

FIGURES



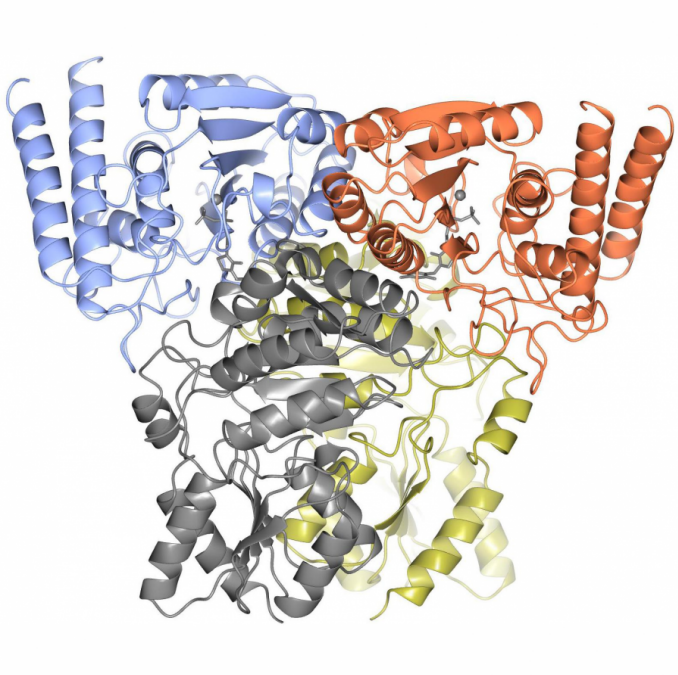


FIGURE 1

FIGURE 2

The overall structure of ChTK-F heterotetramer

The overall structure of ChTK-F heterotetramer is shown as a cartoon model with the ChTK-N monomers shown in blue and red and the ChTK-C monomers shown in gray and gold with the Ca2+ and TPP cofactor shown as stick models in gray.

KEYWORDS

'split-gene' transketolase | thermal stability | Industrial applications | hyperthermophilic

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