BIOTRANS
LA ROCHELLE 2023

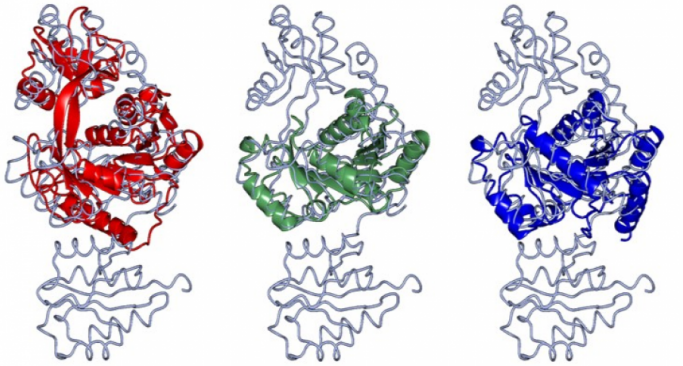
N°952 / PC
TOPIC(s) : Biocatalytic cascade reactions / Enzyme discovery and engineering

A Unique Cyclic Diphosphoglycerate Synthase enzyme for Extremolyte Production in Thermus thermophilus

AUTHORS
Jennifer LITTLECHILD / UNIVERSITY OF EXETER, HENRY WELLCOME BUILDING FOR BIOCATALYS, EXETER

PURPOSE OF THE ABSTRACT
The primative microorganism Methanothermus. fervidus was first isolated in 1981 by Setter and has a genome coding for only 1311 proteins and 50 RNA genes. It is smallest sequenced genome observed for a free-living organism. This anaerobic hyperthermophilic archaeon produces energy through the reduction of carbon dioxide with hydrogen to produce methane. It accumulates an unusual cyclic form of 2,3 phosphoglycerate for protein stabilisation at temperatures up to 97 degrees C.
The thermophilic bacteria Thermus thermophilus has been used as a host for expression for the two enzyme cascade for synthesis of the so called small molecule extremolyte cDPG which has healthcare and cosmetic applications [1]. The enzymes used in this synthetic pathway, 2-phosphoglycerate kinase (2PGK) and cyclic diphosphoglycerate synthetase (cDPGS) have been over-expressed in Escherchia coli to study them both biochemically and structurally. Both enzymes have low sequence identity to other known enzymes available in the Protein Data Base. The cDPGS structure has been solved to high resolution using seleno-methionine due to no molecular replacement models being available. It has also been solved in complex with 2,3-diphosphoglycerate and ADP Mg2+ showing the conformational changes that occur on substrate binding [2]. The structure appears unique with only one domain showing homology to other known proteins that have unrelated function. The structure of the other enzyme, 2PGK is currently being solved and is remote regarding homologues currently known.
This leads the way for other thermophilic proteins to be used for whole cell synthesis of related small molecules with industrial applications.

FIGURES



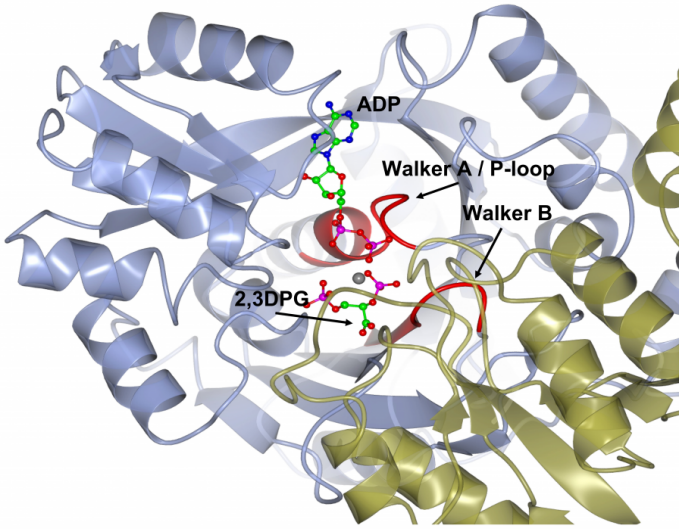


FIGURE 1

Figure 1

FIGURE 2

Figure 2

Cartoon illustration of the ADP and 2,3 DPG bound to cDPGS. The two monomeric subunits are shown in cyan and green. ADP and 2,3 DPG are shown in ball-and-stick representation (carbon, green; oxygen, red; nitrogen, blue; phosphorus, pink). The Mg2+ atom i

KEYWORDS

Extremolyte | Cyclic diphosphoglycerate synthase | Synthetic Pathway | Thermophilic host

BIBLIOGRAPHY

[1] De Rose, S. A., Finnigan, W., Harmer, N. J., Littlechild, J. A. (2021). Production of the Extremolyte Cyclic 2,3-Diphosphoglycerate Using Thermus thermophilus as a Whole-Cell Factory . Front. Catal. 1:80341

[2] De Rose, S. A., Isupov, M., Worthy, H., Harmer, N. J., Littlechild, J. A. Structural characterisation of cyclic 2,3-diphosphoglycerate synthetase from Methanothermus fervidus.in preparation