Biochemical and structural characterization of two new families of Transaldolases

Berichterstattung

Projektinformationen

NOVEL TRANSALDOLASES

ID Finanzhilfevereinbarung: 6317

Projekt abgeschlossen

Startdatum
1 November 2004

Enddatum
31 Oktober 2005

Finanziert unter
Human resources and Mobility in the specific programme for research, technological development and demonstration "Structuring the European Research Area" under the Sixth Framework Programme 2002-2006

Gesamtkosten
€ 40 000,00

EU-Beitrag
€ 40 000,00

Koordiniert durch
UNIVERSITAET STUTTGART
Germany

Final Activity Report Summary - NOVEL TRANSALDOLASES (Biochemical and Structural Characterization of Two New Families of Transaldolases)

Transaldolases transfer an activated dihydroxyacetone moiety from a ketose donor, e.g. fructose 6-

phosphate, to an aldose acceptor, e.g. erythrose 4-phosphate, reversibly. In this reaction transaldolases form, a new C-C bond and two new asymmetric C-atoms are generated with a 3S, 4R configuration. Because of their high stereo specificity for this asymmetric synthesis, transaldolases are desired tools in biocatalysis. Nevertheless, their use is limited by their substrate specificity. Therefore, numerous approaches were utilised to understand and modify the stereo and substrate specificity of transaldolases.

In order to identify transaldolases with new properties, we used the increasing number of available sequences and discovered three new families of larger transaldolases. One family consisted of cyanobacterial sequences, the other of plant sequences and the third was more heterogeneous and contained bacterial as well as plant sequences.

Representatives of all three families were cloned and the proteins were expressed heterologously in escherichia coli. These included the transaldolase of the bacteria synechocystis sp. PCC 6803 and corynebacterium glutamicum, as well as two transaldolases of the plant arabidopsis thaliana (AtTal1 and AtTal2). A transaldolase activity was proven for all genes that were cloned by the time of the project completion.

The transaldolase of synechocystis was investigated in further detail. It was demonstrated that the two EF-hand motifs at the C-terminus bound two Ca2+ ions. The combination of a Ca2+ binding site via EF-hands and a catalytic activity was very rarely observed in the past. A function for the bound Ca2+ could not yet be assigned. Size exclusion chromatography showed that the native protein formed a dimer, whereas the His-tagged protein was a monomer. Hence, the native protein should be used for further investigations and a purification protocol for the native protein was established. Further studies would compare the different transaldolases with respect to their substrate and stereo specificity to TalB from Escherichia coli. Their structure would be determined using X-ray crystallography.

In addition, an Escherichia coli strain which was deficient in both transaldolases was constructed. This strain did not show any difference in growth compared to wild type when grown on various carbon sources. However, it was a useful tool for the expression of new transaldolases avoiding a contamination with endogenous Escherichia coli transaldolases.

Letzte Aktualisierung: 23 November 2011

Permalink: https://cordis.europa.eu/project/id/6317/reporting/de