Expression and Properties of the Highly Alkalophilic Phenylalanine Ammonia-Lyase of Thermophilic *Rubrobacter xylanophilus*

Klaudia Kovacs\(^2\), Gergely Banoci\(^1\), Andrea Varga\(^3\), Izabella Szabo\(^3\), Andras Holczinger\(^4\), Gabor Hornyanszky\(^1\), Imre Zagyva\(^2\), Csaba Paizs\(^3\), Beata G. Vertessy\(^2,4\), Laszlo Poppe\(^1\)

\(^1\) Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budapest, Hungary
\(^2\) Institute of Enzymology, Research Centre for Natural Sciences of Hungarian Academy of Sciences, Budapest, Hungary
\(^3\) Biocatalysis Research Group, Babes-Bolyai University of Cluj-Napoca, Cluj-Napoca, Romania
\(^4\) Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary

Abstract

The sequence of a phenylalanine ammonia-lyase (PAL; EC: 4.3.1.24) of the thermophilic and radiotolerant bacterium *Rubrobacter xylanophilus* (RxPAL) was identified by screening the genomes of bacteria for members of the phenylalanine ammonia-lyase family.

A synthetic gene encoding the RxPAL protein was cloned and overexpressed in *Escherichia coli* TOP 10 in a soluble form with an N-terminal His\(^6\)-tag and the recombinant RxPAL protein was purified by Ni-NTA affinity chromatography.

The activity assay of RxPAL with L-phenylalanine at various pH values exhibited a local maximum at pH 8.5 and a global maximum at pH 11.5.

Circular dichroism (CD) studies showed that RxPAL is associated with an extensive helical character (far UV CD) and two distinctive near-UV CD peaks.

These structural characteristics were well preserved up to pH 11.0. The extremely high pH optimum of RxPAL can be rationalized by a three-dimensional homology model indicating possible disulfide bridges, extensive salt-bridge formation and an excess of negative electrostatic potential on the surface. Due to these properties, RxPAL may be a candidate as biocatalyst in synthetic biotransformations leading to unnatural L- or D-amino acids or as therapeutic enzyme in treatment of phenylketonuria or leukemia.