CLONING AND PURIFICATION OF A TETRAMERIC OXIDOREDUCTASE FROM ARTHROBACTER NICOTINOVORANS pAO1

Marius Mihașan1, Vlad Artenie1*, Roderich Brandsch2

1. Alexandru Ioan Cuza University, Faculty of Biology, Department of Molecular and Experimental Biology, Iasi, Romania
2. Institute for Biochemistry and Molecular Biology, Center for Biochemistry and Molecular Cell Research, Albert Ludwigs University, Freiburg, Germany

* marius.mihasan@uaic.ro

Introduction

The soil bacterium Arthrobacter nicotinovorans carries the pAO1 catabolic megaplasmid which enables it to grow on nicotine (Igles and Brandsch, 2003). Besides the well-characterized pathway for nicotine degradation (Brandsch, 2006), PAO1 carries a gene cluster of a hypothetical pathway for carbohydrate utilization. This cluster consists of ORF of a transcriptional regulator, of a sugar ABC-transporter, and of several putative dehydrogenases and oxidoreductases. Previously, we established that the pAO1 gene encodes an aldehyde-dehydrogenase (Mihașan, 2010) and ORF4 encodes a sugar dehydrogenase. Here we focus on further characterization the ORF40 protein and elucidation of its possible role in the cell. By cloning the gene in the plasmid vector pHI6EX3, we were able to express it as a recombinant His-tagged protein and to easily purify it to homogeneity.

Table 1. Oligo-nucleotides used for the isolation and cloning of orf40

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orf40f</td>
<td>5'-C TCT GAG G4A GTC TTG ACT AAA ACA GC-3'</td>
</tr>
<tr>
<td>Orf40r</td>
<td>5'-G GAA GTA GGC TCG AGG TCA TTA GAG C-3'</td>
</tr>
</tbody>
</table>

Figure 1. General organisation of the putative carbohydrate utilisation gene cluster from pAO1

Figure 2. Orf40 encoded protein was purified to homogeneity. M – Molecular Weight Marker Sigma Wide Range 1,2,3 – Purified protein 5, 10, 15 microglobally

Figure 3. Determination of native molecular mass of ORF40 protein. 1.6 mg purified ORF40 protein was injected on a HiLoad 16/60 Superdex 200 previously calibrated using Blue-Dextran, Ferrite (440 kDa), Catalase (232 Kda), Aldolase (158 KDa), Albumine (67 Kda), Ovalbumine (43 Kda), Ribonuclease (17.4 KDa).

Table 2. Zn content in the analyzed samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purification buffer Hepes 40 mM, NaCl 500 mM</td>
<td>1.7</td>
</tr>
<tr>
<td>Flow-through</td>
<td>27.2</td>
</tr>
<tr>
<td>ORF40 protein (15 mg/ml)</td>
<td>43.2</td>
</tr>
</tbody>
</table>

Method

Isolation and cloning of orf40. The orf40 was isolated by PCR using the primers in table 1 and a suspension of Arthrobacter nicotinovorans cells as template. Directional cloning (Sambrook, J., Frisch, EF, Maniatis, T, 1989) of the fragment containing the orf40 in the pHi6EX3 vector was achieved by using HindIII and XbaI (NEB, UK) enzymes and Rapid DNA ligation Kit, Roche. Transformed E. coli XL1 Blue competent cells were selected on plates containing ampicilin (50 microg/ml) and the recombinant plasmid was checked for the presence of insert by restriction enzyme digestion. Protein expression was achieved using auto-inducible medium as described elsewhere. (Mihașan, Ungureanu & Artenie, 2007) Protein purification was done using standard IMAC techniques (Auseb M, Frederick et al., 2002) on Fast-Flow Ni-chelating Sepharose (Amersham Biosciences, Sweden). Native molecular weight determination was done using gel permeation chromatography on an HiLoad 16/60 Superdex 200 column connected to an AKTA Basic FPLC system. Protein concentration was assayed using the dye-binding method of Bradford (Bradford, 1976). SDS-PAGE was performed using the discontinuous system of Laemmli following the procedure described by Sambrook, 1989.

Conclusions

The ORF40 was cloned, expressed and purified to homogeneity. It consists of a novel sugar-oxidoreductase of 47 kDa, which is a tetramer as assayed by gel-permeation chromatography. The brown color of the purified enzyme preparations indicated that it contains an co-factor. Mass spectroscopy indicated that the enzyme contains 2 atoms of Zn per molecule of monomer, classifying the enzyme as a alcohol dehydrogenase. In order to fully establish the biotechnological potential of this enzyme, serious efforts are undertaken to develop a suitable method for assaying the enzyme activity and to further characterise the enzyme (heat stability, pH stability, Km, Kcat).

Bibliography


Acknowledgements. This work was supported by CNCSIS-UEFISCDU, project number PN II- RUS 337/2010.

www.bio.unic.ro/cercetare/contract/P1317-Mihasan/pd137.html