Microbial Cell Factories

Poster Presentation

Heterologous overexpression of a halophilic α-amylase

Vanesa Bautista*, Julia Esclapez, Rosa Ma Martínez-Espinosa, Francisco Pérez-Pomares, Mónica Camacho and Ma José Bonete

Address: Department of Biochemistry and Molecular Biology, University of Alicante, 03080 Alicante, Spain
* Corresponding author

from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P13 doi:10.1186/1475-2859-5-S1-P13

Background

Extracellular hydrolytic enzymes such as α-amylases are widely used in diverse applications in different industrial areas. α-amylase (EC 3.2.1.1) is an important endo-type carbohydrase that hydrolyzes α-1,4 glycosidic linkages of D-glucose oligomers and polymers. This enzyme has been found in organisms of the three Domains, being a key enzyme of carbohydrate metabolism. *Haloferax mediterranei* is an extremely halophilic Archaea that requires high salt concentrations to grow. This microorganism is able to grow in a minimal medium with ammonium acetate as the only source of carbon and nitrogen.*H. mediterranei* shows α-amylase extracellular activity when grows in this minimal medium in the presence of starch. The main role of this enzyme is the starch metabolism in the extracellular medium, so a lot of microorganisms depend on amylases for survival [1].

Results

The extracellular halophilic *H. mediterranei* α-amylase has been purified to electrophoretic homogeneity [2]. The enzyme has been digested in the presence of trypsin to analyse the resulting peptides using the technique of nanoelectrospray LC/MS. From the obtained peptides and by sequence homology with other α-amylases, the *H. mediterranei* α-amylase gene has been isolated and sequenced, using a library of genomic DNA from *H. mediterranei* in bacteriophage lambda EMBL3. The molecular mass estimated from the deduced amino acid sequence was similar to the calculated by SDS-PAGE. This enzyme is rich in acidic amino acids with a 16% Asp and Glu content which is also the case in other halophilic enzymes [3].

The PCR product for α-amylase was ligated in the plasmid pSTBlue1 and cloned into *E. coli* NovaBlue cells. The insert was digested and subcloned in expression vector pET3a in *E. coli* NovaBlue cells, and introduced in the *E. coli* BL21(DE3) cells for expression. The recombinant protein was mainly obtained as inclusion bodies, although a small amount of protein was presented in the cytoplasmic soluble fraction (Figure 1). The protein obtained as inclusion bodies was refolded by solubilisation in 8 M urea followed by dilution into a high salt concentration buffer in the presence of calcium (Figure 2), and the protein of the soluble fraction was obtained in active state.

Furthermore, four genes have been sequenced by *primer walking*, which match up with four proteins belonging to ABC maltose transport system.

Conclusion

The α-amylase gene from *H. mediterranei* has been cloned, sequenced and overexpressed. The recombinant protein has been obtained in large amounts and refolded with a high efficiency.

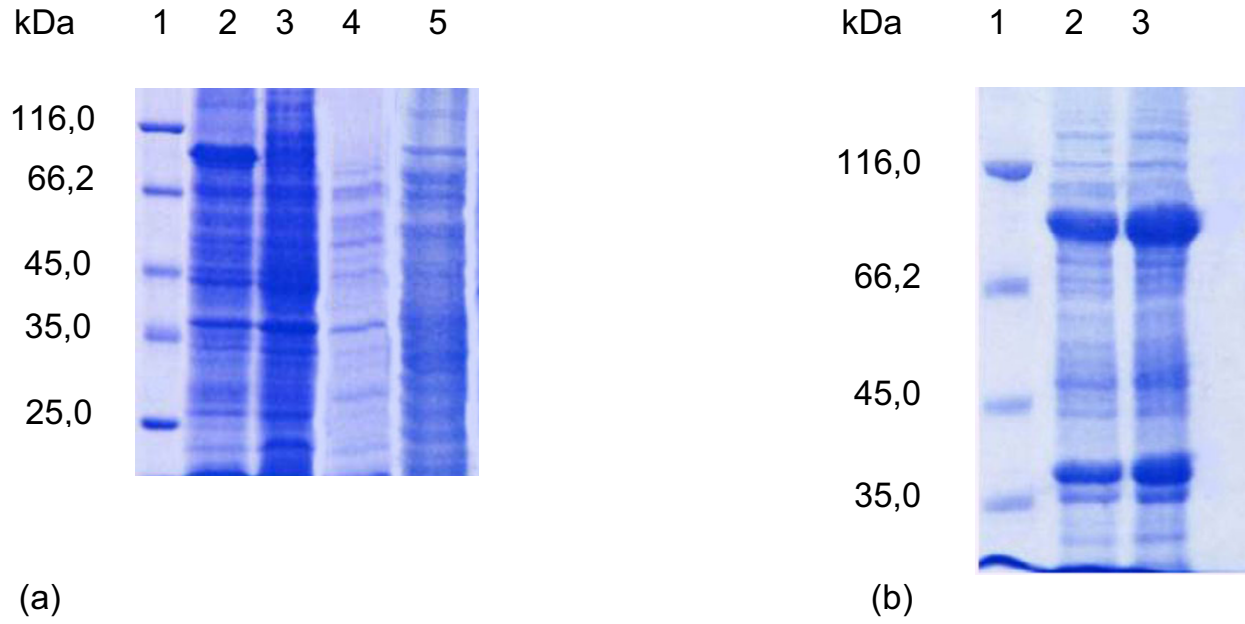

Figure 1
SDS-PAGE for expression cell fractions.**(a)** Lane 1: molecular weight standards; Lanes 3 and 4:*E. coli* BL21(DE3) containing pET3a; Lanes 2 and 5: *E. coli* BL21(DE3) containing pET3a-Amy; Lanes 2 and 3: total cell proteins; Lanes 4 and 5: soluble cytoplasmic fractions.**(b)** Lane 1: molecular weight standards; Lanes 2 and 3: inclusion bodies *E. coli* BL21(DE3) containing pET3a-Amy with different concentrations.

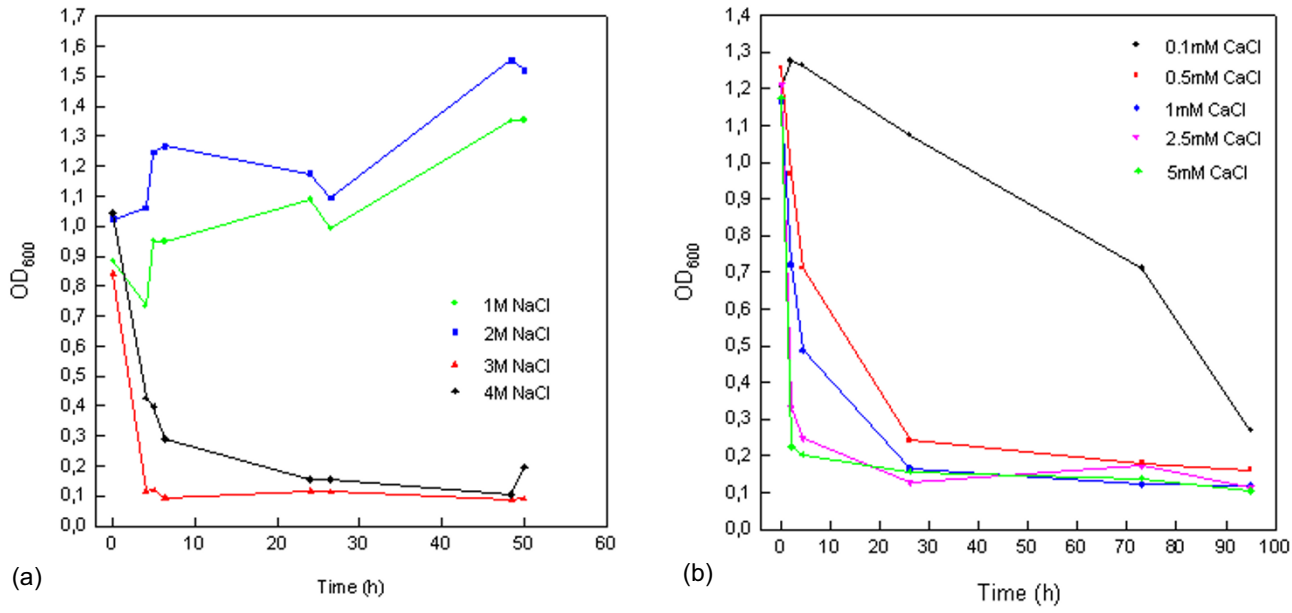

Figure 2
(a) Effect of NaCl concentration on the folding efficiency of α-amylase inclusion bodies dissolved in 8 M urea. Refolding buffer: 20 mM Tris-HCl pH 7.4 with salt. **(b)** Effect of calcium concentration on the folding efficiency of α-amylase inclusion bodies dissolved in 8 M urea. Refolding buffer: 20 mM Tris-HCl pH 7.4, 3 M NaCl and calcium.

References

1. Jones RA, Jermiin LS, Easteal S, Patel BKC, Beacham IR: **Amylase and 16S rRNA genes from a hyperthermophilic archaebacterium.** *J Appl Microbiol* 1999, **86:**93-107.
2. Pérez-Pomares F, Bautista V, Ferrer J, Pire C, Marhuenda-Egea FC, Bonete MJ: **Alpha-amylase activity from the halophilic archaeon *Haloferax mediterranei* .** *Extremophiles* 2003, **7(4):**299-306.
3. Pire C, Esclapez J, Ferrer J, Bonete MJ: **Heterologous overexpression of glucose dehydrogenase from the halophilic archaeon *Haloferax mediterranei*, an enzyme of the medium chain dehydrogenase family.** *FEMS Microbiol Lett* 2001, **200:**221-227.