Microbial Cell Factories

BioMed Central

Oral Presentation

An optimized method to produce halophilic proteins in Escherichia coli

Julia Esclapez*, Ma José Bonete, Mónica Camacho, Carmen Pire, Juan Ferrer, Vanesa Bautista, Rosa Ma Martínez-Espinosa, Basilio Zafrilla, Francisco Pérez-Pomares and Susana Díaz

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):S22 doi:10.1186/1475-2859-5-S1-S22

Background

The homologous and heterologous expression of genes is a prerequisite for most biochemical studies of protein function. Many systems have been carried out for protein production in members of the *Bacteria* and *Eukarya*, however members of the *Archaea* are less amenable to genetic manipulation. Only a few systems for high-level gene expression have been developed for halophilic microorganisms. Because of this, mesophilic hosts, in particular *Escherichia coli*, have been used to produce halophilic proteins for biochemical characterization and crystallographic studies. Expression in *E. coli* has the advantage to be faster and it will easily allow production on a commercial scale. In contrast, difficulties are encountered since enzymes from extreme halophiles require the presence of high salt concentration for activity and stability, and the overexpressed product will need either reactivation or refolding in a salt solution, and so the purification techniques should be compatible with the high salt concentration required.

For the last years, we have developed and refined a system to produce and purify large amounts of recombinants proteins from *Haloferax mediterranei* and *Haloferax volcanii* in the mesophilic host *E. coli*.

Results

Halophilic proteins have been overexpressed using the pET3a vector in *E. coli* BL21(DE3), for instance glucose dehydrogenase, glutamate dehydrogenase, nitrite reductase, extracellular α-amylase and isocitrate lyase from *H. mediterranei* and isocitrate dehydrogenase from *H. volcanii*. The recombinant proteins were always obtained as inclusion bodies (see Figure 1), which were solubilised in the presence of urea. In most cases, the proteins were refolded by rapid dilution in a high salt concentration buffers. The purification procedure of the recombinant proteins was based on the halophilic properties of this kind of enzymes. On the whole, the method consists of a precipitation using ammonium sulphate and a chromatography on DEAE-cellulose in the presence of that salt. The elution with sodium/potassium chloride yielded proteins in a pure and highly concentrated form (see Figure 2 and Table 1). Halophilic recombinant proteins have been characterized and shown the same biochemical characteristics as the enzymes isolated from *H. mediterranei* and *H. volcanii* [1-3]. The high protein concentrations obtained has allowed us to carry on crystallization assays. In particular, the glucose dehydrogenase from *H. mediterranei* has been crystallized by the hanging-drop method using sodium citrate as the precipitant [4,5].

Conclusion

The overexpression, refolding and purification method of halophilic proteins developed provides a fast, simple and efficient process that yields enzymes of high purity in large amounts.

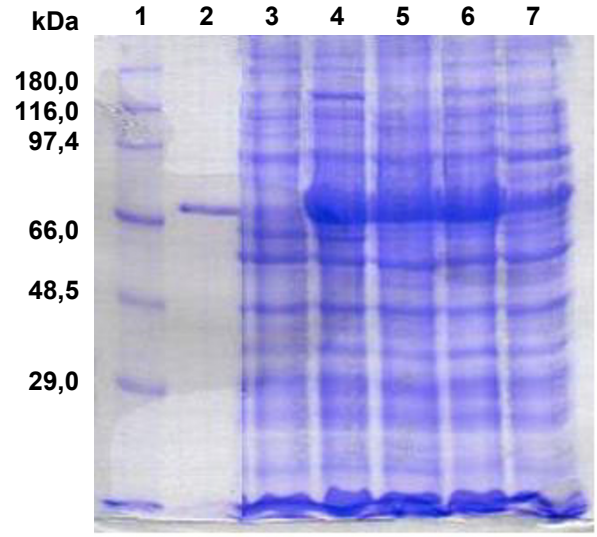

Figure 1
Expression of recombinant isocitrate dehydrogenase from *H. volcanii* under different temperatures. Lane 1. Molecular weight standards. Lane 2. Wild-type isocitrate dehydrogenase. Lanes 3. Uninduced insoluble fraction. Lanes 4 and 5. Induced insoluble fraction at 37°C. Lanes 6 and 7. Induced insoluble fraction at 25°C.

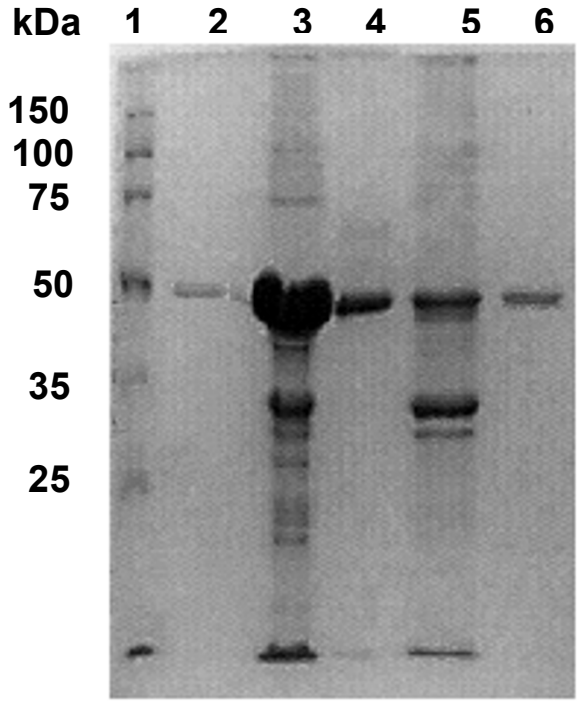

Figure 2
Purification of recombinant glucose dehydrogenase from *H. mediterranei*. Lane 1: Molecular weight standards. Lane 2: Wild type glucose dehydrogenase. Lane 3: Inclusion body fraction. Lane 4: $(NH_4)_2SO_4$ precipitation supernatant. Lane 5: $(NH_4)_2SO_4$ precipitation pellet. Lane 6: Active fractions from DEAE-cellulose.

Table 1: Purification of recombinant glucose dehydrogenase from *H. mediterranei*

Enzyme fraction		Vol. (ml)	U_T	mg_T	Specific activity (U/mg)	Enrichment (-fold)	Yield (%)
Inclusion bodies refolding in 20 mM Tris-HCl pH 7.4, 2 M NaCl, 1 mM EDTA		500	2210	20.9	106	1.0	100
$(NH_4)_2SO_4$ Precipitation	**Supernatant**	500	2180	18.7	117	1.1	99
	Pellet	20	27	1.7	16	----	----
DEAE-cellulose		18	1666	8.9	187	1.8	75

References

1. 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.
2. Camacho M, Rodríguez-Arnedo A, Bonete MJ: **NADP-dependent isocitrate dehydrogenase from the halophilic archaeon *Haloferax volcanii* : cloning, sequence determination and overexpression in *Escherichia coli*.** *FEMS Microbiol Lett* 2002, **209:**155-160.
3. Díaz S, Pérez-Pomares F, Pire C, Ferrer J, Bonete MJ: **Gene cloning, heterologous overexpression and optimized refolding of the NAD-glutamate dehydrogenase from *Haloferax mediterranei*.** *Extremophiles* 2006, **10:**105-115.
4. Ferrer J, Fisher M, Burke J, Sedelnikova SE, Baker PJ, Gilmour DJ, Bonete MJ, Pire C, Esclapez J, Rice DW: **Crystallization and preliminary X-ray analysis of glucose dehydrogenase from *Haloferax mediterranei* .** *Acta Crystallogr D Biol Crystallogr* 2001, **57:**1887-1889.
5. Esclapez J, Britton KL, Baker PJ, Fisher M, Pire C, Ferrer J, Bonete MJ, Rice DW: **Crystallization and preliminary X-ray analysis of binary and ternary complexes of *Haloferax mediterranei* glucose dehydrogenase.** *Acta Crystallograph Sect F Struct Biol Cryst Commun* 2005, **61:**743-746.