Microbial Cell Factories

Oral Presentation

Production, purification and structural analysis of a cation efflux membrane protein from *Thermus thermophilus*

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Background

A metal efflux protein CzrB, for **c**admium and **z**inc **r**esistance protein **B**, was isolated during phage display-based screening of a *Thermus thermophilus* genomic library in *Escherichia coli*. *E. coli* cells containing the *czrB* gene expressed from its native promoter exhibit increased efflux of, and resistance to, zinc and cadmium ions. Of biotechnological interest, however, *czrB*+ cells also display delayed cell lysis upon recombinant protein production [1].

We have undertaken the cloning, production and purification of CzrB in order to determine its structure. In addition, the 92-aa cytoplasmic tail of this 291-aa protein has been cloned and produced in *E. coli* for structural analysis.

Results

czrB has been cloned and expressed, with N-terminal, C-terminal and no hexahistidine tags, under the control of a number of promoters and in a variety of *E. coli* host strains to optimise production. Cellular fractionation and immunoblotting revealed from <5% to ~50% of the recombinant polypeptide to be associated with the cytoplasmic membrane, depending on production parameters. Purification of the protein has been carried out from cellular membrane fractions and cytoplasmic inclusion bodies to generate sufficient yields for crystallisation studies. In addition, co-production of rare tRNAs and engineering of *czrB* to improve its expression using the ribonuclease MazF approach [2] are also under investigation to increase yields.

Production and purification of the soluble, cytoplasmic tail of CzrB has been optimised in parallel with analysis of the full-length protein (Figure 1). Preliminary crystallisation conditions were obtained using a sparse matrix screen (Hampton Research, Crystal Screen I and II) with a protein concentration of 20 mg/ml. Polyethylene glycol and ammonium sulfate concentrations were optimised to produce crystals which diffracted to 2.8 Å.

Conclusion

We have produced and purified the cation efflux protein CzrB from *T. thermophilus* in an *E. coli* expression system. Expression has been optimised and crystallisation and structural analysis of the protein and its cytoplasmic tail are underway.

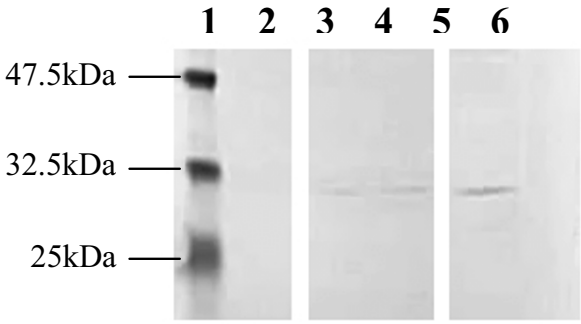

Figure 1
Production of CzrB-6his in *E. coli* BL21 (DE3) cells. Lane **1**. Molecular weight marker; lane **2**. *E. coli* control; lanes **3,4**. whole cell extracts after CzrB production at 25°C and 37°C; lanes **5,6**. membrane fractions (concentrated 3.5 × relative to whole extracts) after CzrB production at 25°C and 37°C.

References

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