Surface Display of Poly(His)$_n$ on the *Escherichia coli* using OmpC as Surface Anchoring Motif

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Abstract

A novel cell surface display system was developed by employing the *Escherichia coli* outer membrane protein C (OmpC) as an anchoring motif. Poly-histidine (poly-His) peptides of 19, 32, 45, 84, and 162 amino acids (aa) could be successfully displayed by inserting them into the seventh exposed loop (L7) of OmpC. Recombinant cells displaying poly-His of 19, 32, 45, and 84 aa could absorb 18.9, 23.9, 26.1, and 32.0 μmol of Cd^{2+} per gram cell dry weight, respectively and therefore, would be useful as the biosorbents of heavy metals.

Introduction

Cell surface display aims to display proteins or peptides on the surface of prokaryotic or eukaryotic cells, especially of bacterial and yeast cells. Its possible applications include: (i) live vaccine development[1] (ii) antibody production[2] (iii) peptide libraries screening[3] (iv) environmental bioadsorbents development[4] (v) whole cell catalysts construction[5] and (vi) biosensor development to anchor enzymes, receptors, or other signal-sensitive components on cell surface to develop novel biosensors for diagnostic, industrial or environmental purpose.

In this study, we aimed to develop a novel cell surface display system using the *E. coli* OmpC, one of the most abundant (may up to 10^5 molecules per cell) OMPs in *E. coli* cells. we used one of these external loops as the point of inserting foreign peptides for cell surface display. Poly-His peptides (several hexa-histidine linkers) were used as model inserts for two main reasons: (i) they are good chelators for divalent metal ions, such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+} or

Pb^{2+}, and therefore may be used as biosorbents for heavy metal removal, and (ii) the permissive size limit of polypeptide to be fused to the external loops of OmpC can be easily examined by inserting varying number of copies of 6His linkers.

Materials and methods

E. coli K-12 strain MC4100 (*F- araD139 (argF-lac)U169 rpsL150 (Str^r) relA1 flbB5301 deoC1 ptsF25 rbsR*; ATCC35695) was used as a host strain. All recombinant strains were cultivated in LB medium supplemented with 50 μg/ml of ampicillin. Recombinant cells were induced at the OD_{600} of 0.6 by adding isopropyl-β-D-1-thiogalactopyranoside (IPTG) to the final concentration of 10 μM. Strains harboring pTCdP, pTCHP1, pTCHP2, pTCHP3, or pTCHP6 were cultivated at 30℃, and were grown for 2 more hours after induction; and strains harboring pTCHP12 or pTCHP18 were cultivated at 25℃, and were grown for 4 more hours after induction. Adhesion of recombinant *E. coli* cells to Nickel-nitrilotriacetic acid (Ni-NTA)-agarose (Qiagen GmbH, Hilden, Germany) beads was examined. Adsorption of Cd^{2+} was analyzed by Atomic Analysis System (Perkin-Elmer 3100, Norwalk, CT) using an air-acetylene flame and a hollow cathode lamp. Outer membrane proteins were prepared and analyzed as described by Puente *et al.*, except that 0.5% (w/v) Sarkosyl was used instead of Triton X-100 and samples were incubated on ice. Protein samples were analyzed by electrophoresis on a 12% (w/v) sodium dodecyl sulfate polyacrylamide gel eletrophoresis (SDS-PAGE) as described by Laemmli.

Results and discussion

In this study we examined for the first time the possibility of employing *E.coli* OmpC as a surface display motif. In particular, we developed a sandwich display system employing one of the exposed loop of OmpC. The entire *ompC* gene including the signal sequence was inserted into the *Nco*I-*Bam*HI site of pTrc99A, right downstream of the *trc* promoter, to make pTrcC. Plasmids

pTCHP$_n$ series bearing different *ompC-(6His)$_n$* fusion genes were constructed: pTCHP1, pTCHP2, pTCHP3, pTCHP6, which contain one, two, three, and six sets of *6His* linkers, respectively.

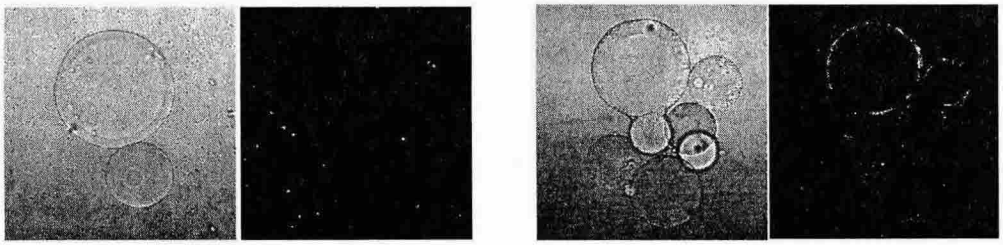
Poly-His peptides up to 84 aa could be targeted efficiently to the *E. coli* outer membrane by OmpC protein. The expression level of OmpC-(6His)n decreased as the number of inserted 6His linker increased: 34.7%, 33.1%, 32.8%, and 26.7% of total outer membrane proteins (OMPs) for OmpC-(6His)$_1$, OmpC-(6His)$_2$, OmpC-(6His)$_3$, and OmpC-(6His)$_6$, respectively. This finally turned out to be true because the amount of fusion proteins displayed could reach over 30% of the total outer membrane proteins.

Fig. 1 shows the recombinant *E. coli* cells expressing OmpC-(6His)n fusion proteins bound to the Ni-NTA-agarose beads. MC4100(pTCdP) did not bind to the beads, and therefore, the beads were invisible under fluorescent field. For MC4100(pTCHPn) strains, the outlines of the beads were clearly visible due to the attached recombinant *E. coli* cells. these micrographs indicate that OmpC-(6His)n fusion proteins were successfully exposed outside the recombinant cells.

Another goal we tried to achieve from this study was to extend the heavy metal removal potential of *E. coli* cells by surface engineering techniques. Recombinant *E. coli* cells displaying OmpC-(6His)n fusion proteins up to six copies of 6His were examined for their abilities to adsorb Cd^{2+}. Cells harboring pTCHP1, pTCHP2, pTCHP3 and pTCHP6 could absorb 18.9, 23.9, 26.1 and 32.0 mol of Cd^{2+} per gram of cell dry weight, respectively. The biosorption capacity increased along with the increasing number of 6His units.

In conclusion, we have developed a novel cell surface displaying system using the *E. coli* OmpC as an anchoring motif. A large poly-His peptide of up to 162 aa could be successfully displayed at the *E. coli* outer membrane, which disproves the size limit of peptides to be inserted previously suggested for sandwich fusion motifs. Furthermore, the recombinant strains developed in this study could adsorb large amounts of Cd^{2+}, suggesting their usefulness as

bioadsorbents.



A B

Figure 1. Adhesion of recombinant strain to Ni-NTA-agarose beads. Transmission micrographs(left) and matching fluorescence micrographs(right) A: MC4100(pTCdP) B: MC4100(pTCHP6)

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