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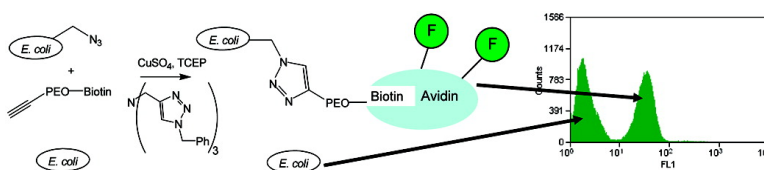
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Cell Surface Labeling of *Escherichia coli* via Copper(I)-Catalyzed [3+2] Cycloaddition

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The display of proteins and polypeptides on the surfaces of cells is an important technology which has been used extensively[1] for applications ranging from screening of antibody fragments[2,3] to the creation of whole cell bioremediation agents.[4,5] These approaches are powerful, but are limited by the fact that the proteins and peptides displayed on the cell surface are composed of just the canonical amino acids. Expansion of the range of chemical functionality available on the mammalian cell surface has been demonstrated elegantly by the Bertozzi group,[6–8] and a recent paper from the Schultz laboratory has shown the addition of the ketone functional group to the surface of bacteria.[9] In this report, we demonstrate the display and selective modification of the azido functional group on the surface of *Escherichia coli*. The use of azidohomoalanine (**1**) as a methionine surrogate as well as its selective modification via the Staudinger ligation has been reported previously.[10] Here, we show that **1** can be metabolically incorporated into outer membrane protein C (OmpC), one of the abundant outer membrane porins of *E. coli*,[11] and that selective modification of the azide functional group can be realized via copper-mediated [3+2] azide-alkyne cycloaddition (Scheme 1).[12] The utility of this chemistry in biological contexts has recently been established[13,14] and thus has inspired us to explore the prospects for labeling intact bacterial cells. The specificity of the modification was verified by Western blotting and flow cytometry.

Naturally occurring *E. coli* OmpC contains three methionine residues and hence only three potential sites for replacement with azidohomoalanine. According to a published model[4] of *E. coli* OmpC, only one of these methionine sites is exposed to the extracellular milieu. To increase the number of sites for functionalization on the surface of the cell, six additional methionine residues were engineered into OmpC by site-directed mutagenesis. The residues chosen are all exposed on the outside of the cell according to the published model. When possible, residues with similar size and hydrophobic character were replaced with meth-

Scheme 1. Structure of Azidohomoalanine **1** and Biotin-PEO Propargylamide **2**; Biotinylation Reaction of Whole *E. coli* via [3+2] Cu-Mediated Azide-Alkyne Cycloaddition
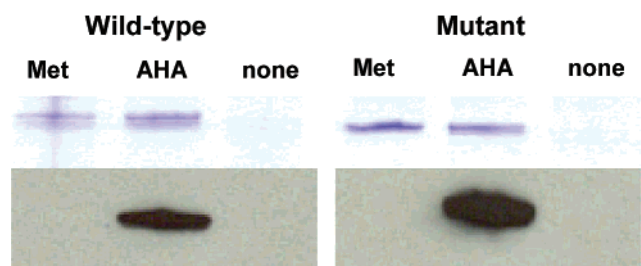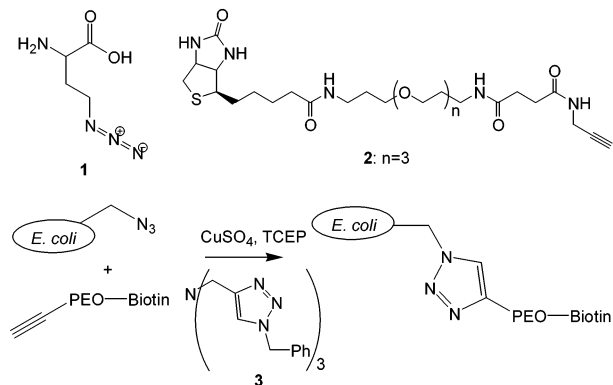

Figure 1. Coomassie blue stained gel (top) and Western blot (bottom) of outer membrane fractions after [3+2] biotinylation. Met, OmpC containing methionine; AHA, OmpC containing azidohomoalanine; none, OmpC produced when cells are induced in the presence of 19 amino acids (no methionine). The amount of protein expressed and targeted to the outer membrane is similar for cells expressing OmpC-met and OmpC-AHA. Only OmpC-AHA is detected by avidin-HRP for both the wild-type and the mutant proteins.

ionine.[15] When expressed in media supplemented with **1**, the mutant protein was produced at a level comparable to that of the wild-type (Figure 1). Furthermore, the mutant OmpC is targeted to the outer membrane of the cell, even when expressed in media supplemented with **1**.

Cells expressing either wild-type OmpC or mutant OmpC containing azidohomoalanine (OmpC-AHA) were prepared by using a medium shift procedure as previously described.[16] The cells were then washed and subjected to the [3+2] reaction with the biotinylated alkyne reagent **2** (Scheme 1). The concentrations of $CuSO_4$, tris(carboxyethyl)phosphine (TCEP), and triazole ligand **3** were 10-fold more dilute than in previous reports[13,14] because of the low solubility of **3** in aqueous media. Cells expressing OmpC containing methionine (OmpC-met) and cells which were induced in media supplemented with only 19 amino acids (no methionine) were prepared and subjected to similar reaction conditions as controls. As demonstrated in Figure 1, functionalization is specific toward both wild-type OmpC-AHA and mutant OmpC-AHA; we cannot detect background staining of either recombinant OmpC-met or naturally expressed OmpC in the Western blot. Furthermore, OmpC-AHA is the only band detected in the Western blot; although incorporation of **1** into other cellular proteins must occur, labeling of such proteins is not detected.

A series of optimization experiments was performed on the mutant to achieve balance between a high level of functionalization and maintenance of intact cells. Western blotting was performed to examine modulation of protein expression conditions and [3+2] reaction conditions. Among the factors tested were the concentration of the inducer, IPTG, concentration of the copper catalyst, reaction time, and addition of the triazole ligand **3**. Vigorous induction of OmpC-AHA (i.e., addition of 1 mM IPTG) was found to be necessary for extensive cell-surface labeling, although biotinylation was still detectable when cells were induced at 0.1 mM IPTG.
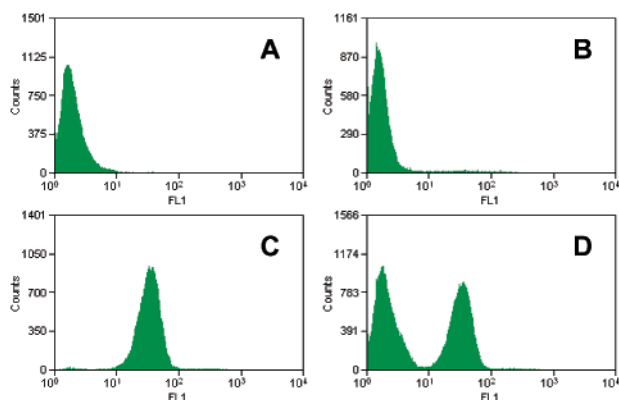
Figure 2. Flow cytometry data from (A) mutant cells bearing OmpC-met, (B) wild-type cells bearing OmpC-AHA, (C) mutant cells bearing OmpC-AHA, and (D) a mixed population of mutant cells bearing OmpC-AHA and cells induced in medium supplemented with only 19 amino acids.

Decreasing the concentration of $CuSO_4$ to 1 μM or 10 μM resulted in a complete loss of biotinylation, while the reaction proceeded well with 100 μM or 1 mM $CuSO_4$. After 1 h of reaction with cells induced at 1 mM IPTG and functionalized in 100 μM $CuSO_4$, essentially no biotinylation was observed by Western blotting. A small amount of biotinylation was observed after 2 or 4 h, but extensive modification required overnight reaction (16 h). A recent paper[14] reported that similar [3+2] reactions proceed to high yield without the triazole ligand **3**. However, when **3** was omitted from the whole cell reaction, essentially no biotinylation was detected by Western blotting. Through these experiments, we converged on optimal conditions for OmpC expression and functionalization. The conditions are as follows: induction at 1 mM IPTG and reaction with 100 μM $CuSO_4$ for 16 h at 4 °C (see Supporting Information for details). These conditions were used to generate functionalized cells both in the Western blot experiments shown in Figure 1 and in the flow cytometry experiments described below.

Cells expressing either wild-type or mutant versions of OmpC-met or OmpC-AHA, and cells that were induced in the presence of all amino acids except methionine, were biotinylated and subsequently stained with an avidin-Alexa Fluor 488 conjugate and subjected to flow cytometric analysis. Unlabeled cells were analyzed initially, and this population was placed in the first decade of the fluorescence channel by adjusting the photomultiplier tube voltage. Cells expressing OmpC-met and cells induced in the presence of 19 amino acids have the same mean fluorescence as the unlabeled cells, indicating that there is essentially no nonspecific staining of the cells by the avidin-Alexa Fluor conjugate. Given the Western blot data above, it was surprising that cells expressing wild-type OmpC-AHA yield only background fluorescence intensity (Figure 2). This result can be attributed to the fact that the naturally occurring methionine in OmpC is sterically encumbered according to the published model.[4] While it may be possible for azidohomoalanine at that position to be biotinylated, the residues surrounding the azidohomoalanine hinder binding of the bulky avidin molecule. In contrast to the wild-type OmpC, cells expressing the mutant OmpC-AHA display an approximately 10-fold increase

in mean fluorescence over background (Figure 2). When cells expressing mutant OmpC-AHA are mixed with cells induced in the presence of 19 amino acids, cytometry reveals two populations with essentially baseline separation (Figure 2). Thus, the mutant OmpC is a useful tool for detection of unnatural amino acid incorporation on an individual cell basis.[17]

The results reported here demonstrate that recombinant *E. coli* OmpC can be functionalized with azidohomoalanine and targeted to the outer membrane of the cell. We further illustrate that a cell surface that displays azide functionality can be chemoselectively modified with a biotin-bearing reagent by means of a copper-mediated azide-alkyne cycloaddition. Furthermore, after being stained with fluorescent avidin, cells incorporating the unnatural amino acid can be readily distinguished from cells lacking the unnatural amino acid. Such a flow cytometric assay may be used in a variety of applications, including screening for the in vivo incorporation of new, reactive unnatural amino acids. Such studies are currently underway in our laboratory.

Supporting Information Available: Experimental protocols and details about flow cytometry (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chen, W.; Georgiou, G. *Biotechnol. Bioeng.* **2002**, *79*, 496.
(2) Georgiou, G.; Stathopoulos, C.; Daugherty, P. S.; Nayak, A. R.; Iverson, B. L.; Curtiss, R. *Nat. Biotechnol.* **1997**, *15*, 29.
(3) Daugherty, P. S.; Chen, G.; Olsen, M. J.; Iverson, B. L.; Georgiou, G. *Protein Eng.* **1998**, *11*, 825.
(4) Xu, Z.; Lee, S. Y. *Appl. Environ. Microbiol.* **1999**, *65*, 5142.
(5) Sousa, C.; Cebolla, A.; de Lorenzo, V. *Nat. Biotechnol.* **1996**, *14*, 1017.
(6) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125.
(7) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.
(8) Saxon, E.; Luchansky, S. J.; Hang, H. C.; Yu, C.; Lee, S. C.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2002**, *124*, 14893.
(9) Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735.
(10) Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 19.
(11) Puente, J. L.; Alvarez-Scherer, V.; Gosset, G.; Calva, E. *Gene* **1989**, *83*, 197.
(12) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
(13) Wang, Q.; Chan, T.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192.
(14) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686.
(15) The mutations introduced were V50M, N88M, T187M, A230M, L271M, L306M. The exposed methionine in wild-type OmpC is at residue 142.
(16) Kiick, K. L.; van Hest, J. C. M.; Tirrell, D. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 2148.
(17) Cells subjected to overexpression of either OmpC-met or OmpC-AHA, and then to the [3+2] reaction conditions described here, are unable to divide after transfer to rich medium. Overexpression of recombinant outer membrane proteins is known to adversely affect cell viability (see ref 2 and Christmann et al. *Protein Eng.* **1999**, *12*, 797). We are currently exploring conditions for maintaining viability, and for use of nonviable cells in screening experiments.

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