J|A|C|S
COMMUNICATIONS

Published on Web 07/13/2002

Cell-Wall Engineering of Living Bacteria

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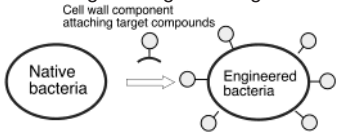
Received March 8, 2002

The chemical engineering of living cells has been the subject of growing interest in biology and biotechnology. The first example was reported by Bertozzi's group,[1−4] who attached a ketone group to *N*-acetyl-D-mannosamine. That compound was then metabolically converted to ketone-attached sialic acid on the surface of mammalian cells. This method is widely applicable to cells that have sialic acid on the surface.

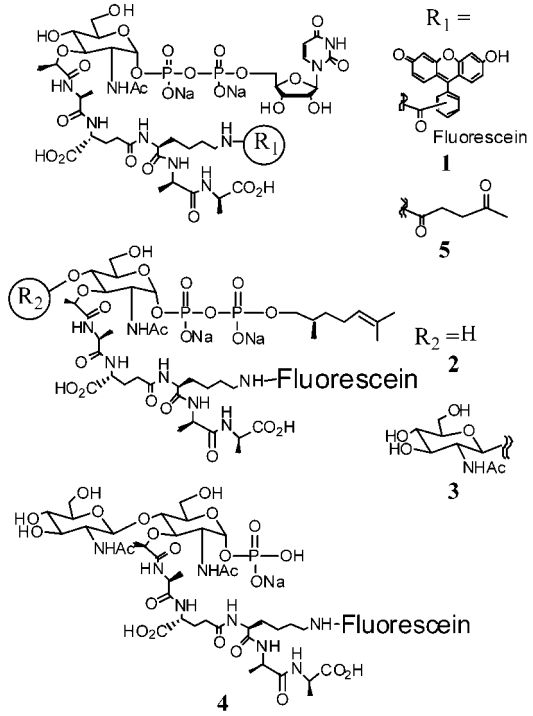
However, when engineering the surface of bacterial cells, proteins can only be expressed genetically on the surface as chimera proteins.[5] The development of a method for the chemical engineering of the bacterial cell surface will allow a large variety of molecular specimens to be displayed, leading to a wide range of applications, such as in discovery of novel drugs or vaccines utilizing lactic acid bacteria.

In this paper, we report a versatile chemical approach for displaying target compounds on the bacterial cell wall (Scheme 1). The bacterial cell wall, which consists of polysaccharides repeating GlcNAc-β(1−4)muramic acid linked with a pentapeptide, is a common structure in all bacteria. Recently, we reported the chemoenzymatic synthesis of bacterial cell-wall precursors.[6] In the present study, we expand this synthetic strategy to establish new method for displaying target molecules on the bacteria. We chemically synthesized target molecules carrying precursors such as UDP-MurNAc, lipid I, and lipid II derivatives **1**−**5**, and tested whether these compounds could be incorporated into the cell wall with the target molecules.

Scheme 1. Cell-Wall Engineering of Living Bacteria



Fluorescein-attached cell wall precursors **1**−**3** were synthesized according to the methods described in the literature[6−9] and the Supporting Information. Compound **4** was also synthesized as a negative control for incorporation. *Escherichia coli*, which is a Gram-negative bacteria, has a thick lipopolysaccharide layer outside



the cell wall. To increase the permeability of the lipopolysaccharide layer, *E. coli* C600 was treated with 50 mM EDTA solution for 30 min[10] and then incubated in the presence of each cell wall precursor (**1**−**4**) for 2 h in a PBS buffer. The cell wall fraction of the bacteria was extracted by a conventional method to measure the fluorescence intensity.

Figure 1 shows the fluorescent spectra from extracted cell walls. The spectra for the cell wall extract of the bacteria grown in the presence of **1** showed higher fluorescence intensity than those of the bacteria grown in the presence of the other three components (Figure 1a). Lipid I and lipid II derivatives, **2** and **3**, gave almost the same intensities as did the negative control, compound **4**, indicating that these two components could not be incorporated into the cell wall through biosynthetic pathway. Similarly, it was also reported that MurNAc pentapeptides with shorter lipids could not be incorporated into the cell wall of *E. coli* using enzymatic methods.[11] As expected, without EDTA treatment, UDP-MurNAc derivative **1** gave the same intensity as the other three components, indicating **1** was not taken up by the bacteria through the thick lipopolysaccharide layer (Figure 1b). These results clearly indicate

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10.1021/ja026133x CCC: $22.00

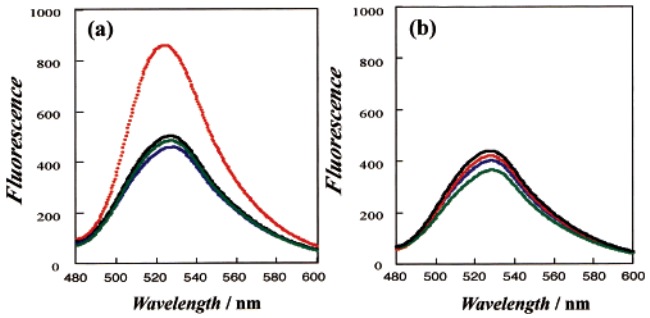

Figure 1. Fluorescent spectra of bacterial cell walls of *E. coli* C600 after incubation with synthetic precursors **1**−**4**. (a) Precursor was added after EDTA treatment. (b) Precursor was added without EDTA treatment. (Red) precursor **1**, (black) **2**, (green) **3**, (blue) **4**.

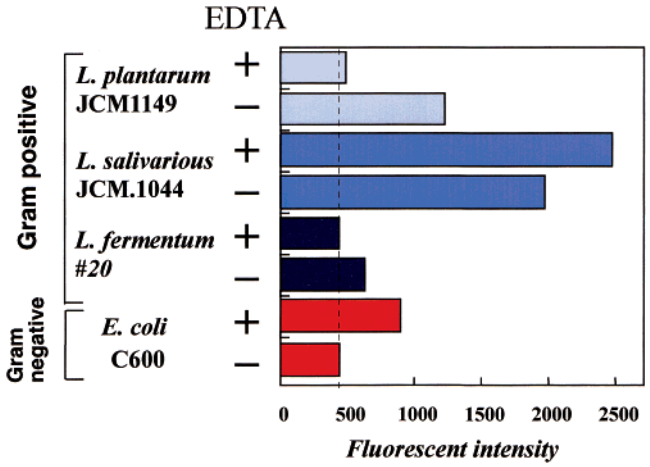

Figure 2. The metabolic incorporation of **1** into cell wall of various bacteria with or without EDTA treatment.

that the UDP-MurNAc derivative **1** can be metabolically incorporated into the living bacterial cell wall.

Since Gram-positive bacteria have thick cell walls with no outer membrane, more efficient incorporation of the target compounds into the cell wall was expected due to the higher permeability. Next we tested three strains of lactic acid bacteria: *Lactobacillus plantarum* JCM1149, *L. salivarius* JCM1044, and *L. fermentum* no. 20. The same experiment as that performed with *E. coli* was repeated for these three bacteria. Fluorescent intensity of the extracted cell wall is shown in Figure 2. All three *Lactobacillus* strains showed incorporation without EDTA treatment. The amount of incorporated fluorescein for two strains was larger than that for *E. coli*.

Lactic acid bacteria displaying fluorescein on the surface were confirmed by fluorescent microscopy (Figure 3). Lactic acid bacteria JCM1149 incubated in MRS Lactobacilli medium containing **1** provided a strong fluorescent image. On the other hand, bacteria incubated with **4** gave a faint fluorescent image (Figure 3,a and b). These results indicate that **1** is incorporated into the cell wall, not simply bound in a nonspecific manner.

Finally, we tested the metabolic synthesis of the cell wall using a ketone-attached UDP-MurNAc pentapeptide **5**. As above, lactic

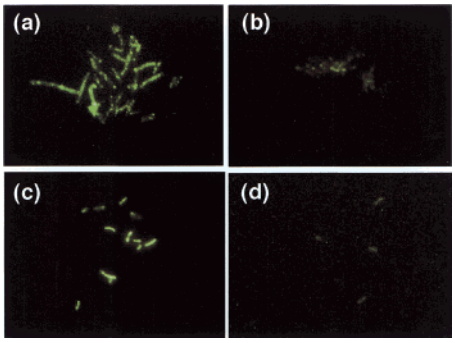

Figure 3. Fluorescent microscopy images of lactic acid bacteria displaying dyes on the cell wall. (a) Lactic acid bacteria after incubation with **1**. (b) Lactic acid bacteria after incubation with **4** (control). (c) Lactic acid bacteria after incubation with **5** and coupled with Alexa 488 hydrazide. (d) Lactic acid bacteria after treatment with Alexa 488 hydrazide (control).

acid bacteria was incubated with **5** and then treated with a dye, Alexa 488 hydrazide, which specifically binds to the ketone group (Figure 3c). After treatment with the dye, the bacteria incubated with **5** generated a strong fluorescent image, suggesting that the ketone group was efficiently displayed on the cell wall. As a control, the hydrazide dye was added to native bacteria, but no fluorescence was observed. This method should be widely applicable to the attachment of hydrazine-carrying large molecules onto the cell wall.

In conclusion, using cell wall precursors **1** and **5**, the bacterial cell wall has been modified through the biosynthetic pathway. We are now exploring the practical uses of this method by attaching a variety of compounds to the bacterial surface.

Acknowledgment. This work was supported by a grant for the "Glycocluster Project" from NEDO. We thank Ms. S. Oka for measuring ESI mass spectrometry at Center for Instrumental Analysis, Hokkaido University.

Supporting Information Available: Experimental procedures and analytical data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hang, H. C.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1242−1243.
(2) Jacobs, C. L.; Goon, S.; Yarema, K. J.; Hinderlich, S.; Hang, H. C.; Chai, D. H.; Bertozzi, C. R. *Biochemistry* **2001**, *40*, 12864−12874.
(3) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007−2010.
(4) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125−1128.
(5) Ståhl, S.; Uhlén, M. *Trends Biotechnol.* **1997**, *15*, 185−192.
(6) Liu, H.; Sadamoto, R.; Sears, P. S.; Wong, C.-H. *J. Am. Chem. Soc.* **2001**, *123*, 9916−9917.
(7) Hitchcock, S. A.; Eid, C. N.; Aikins, J. A.; Zia-Ebrahimi, M.; Blaszczak, L. C. *J. Am. Chem. Soc.* **1998**, *120*, 1916−1917.
(8) Men, H.; Park, P.; Ge, M.; Walker, S. *J. Am. Chem. Soc.* **1998**, *120*, 2484−2485.
(9) Ha, S.; Chang, E.; Lo, M.-H.; Men, H.; Park, P.; Ge, M.; Walker, S. *J. Am. Chem. Soc.* **1999**, *121*, 8415−8426.
(10) Amro, N. A.; Kotra, L. P.; Wadu-Mesthrige, K.; Bulychev, A.; Mobashery, S.; Liu, G. Y. *Langmuir* **2000**, *16*, 2789−2796.
(11) Ye, X.-Y.; Lo, M.-C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. *J. Am. Chem. Soc.* **2001**, *123*, 3155−3156.

JA026133X