Cellular Uptake and Saccharide Chain Elongation of "Fluoro-amphiphilic" Glycosides

Maria Carmelita Z. Kasuya, Ayaka Ito, Reuben Cusi, Toshinori Sato,† and Kenichi Hatanaka*
Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505
†*Graduate School of Science and Engineering, Keio University, 3-14-1 Hiyoshi, Kouhoku-ku, Yokohama 223-8522*

(Received March 23, 2005; CL-050392)

"Fluoro-amphiphilic" glycosides are practical alternatives for the preparation of oligosaccharides. Fluoro-amphiphilic saccharide primers possessing a hydrophilic sugar moiety and a fluorous tag could be taken up by cells and elongated by cellular enzymes. The elongated products were released by cells to the culture medium and could be easily separated from other components by simple extraction with a fluorous solvent.

Fluorous-tagged saccharide primers are viable building blocks for the biocombinatorial synthesis of oligosaccharides.[1] We have reported that 6-(perfluorohexyl)hexyl-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside could be taken in by B16 melanoma cells, the saccharide chain elongated by cellular enzymes, and the elongated product released by the cells to the culture medium.[2] Cellular uptake resulted in sialylation of the terminal galactose residue to afford GM3-type oligosaccharide.

The practical aspect of biocombinatorial synthesis involves not only the easy, simple, and large-scale preparation of oligosaccharides but is also aimed at the efficient recovery of the elongated products in pure form. Traditionally, recovery of products relies on chromatography. However, we have pursued the fluorous approach to address the issue of purification of products via convenient and environment-friendly strategy that eliminates the use of large amounts of organic solvents.[3] We capitalized on the ability of the fluorous-tagged primers to be efficiently separated from the rest of the components of the culture medium by extraction with a fluorous solvent. The practicality of this strategy is critically dependent on the availability of an appropriate fluorous solvent that will dissolve exclusively the elongated product.

In this research various fluorous-tagged saccharide primers were prepared and introduced into B16 melanoma cells as substrates for the synthesis of sialylated oligosaccharides. This study aims not only to evaluate the effects of increasing the number of fluorine on cell viability and glycosylation, but also to demonstrate the utility of the fluorous tag for the purpose of separation.

Fluorous-tagged saccharide primers (LacF10, GluF6, GluF10, GalF6, and GalF10) were prepared by simple methods involving glycosylation and subsequent deacylation.[2] Glycosylation of peracetylated lactoside, galactoside, and glucoside derivatives was carried out in the presence of a Lewis acid using two types of pony tails (perfluorohexylhexanol and perfluorodecylethanol).[4] The fluorous-tagged primers were introduced into B16 melanoma cells to examine their feasibility as substrates for oligosaccharide synthesis.

GalF6 and GluF6 are cytotoxic (Figure 1). Although insoluble in the culture medium, the presence of GalF10 during incubation was harmful to cells. On the other hand, GluF10 that has the same number of fluorine in the aglycon was not cytotoxic. LacF10, having a considerable number of hydroxyl groups, did not impart any adverse effects to cell morphology and viability. This result suggests the significance of the presence of hydroxyl groups to salvage the cells from the toxic effect brought about by the presence of many fluorine atoms. LacF10, GalF6, and GluF6 were taken into by the cells as shown by the presence of the corresponding bands in the cell fraction of the HPTLC results (Figure 2). Cells did not take GalF10 in. Although soluble in

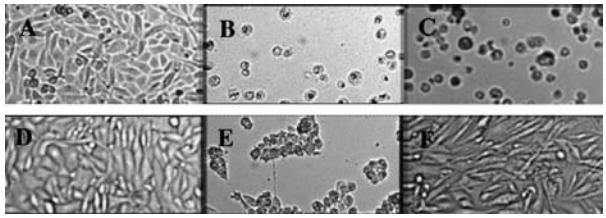

Figure 1. Effect of fluorus-tagged glycosides on B16 melanoma cells. **A**, Control; **B**, GalF6; **C**, GluF6; **D**, LacF10; **E**, GalF10; **F**, Glu F10.

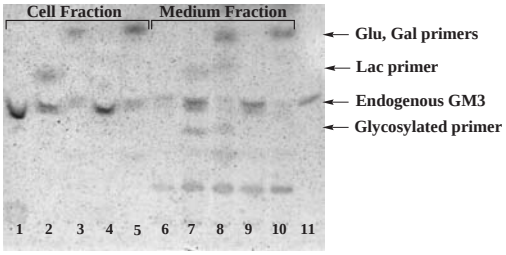

Figure 2. HPTLC result of lipids extracted from the cell and culture medium fractions. B16 melanoma cells (3×10^6, 100 mm dish) were incubated for 48 h in serum free 1:1 DMEM-F12 supplemented with transferrin and insulin in the presence of 50 μM fluorous-tagged saccharide primer. Lanes 1 and 6, Control; Lanes 2 and 7, LacF10; Lanes 3 and 8, GalF6; Lanes 4 and 9, GluF10; Lanes 5 and 10, GluF6; Lane 11, GM3 standard.

Table 1. Results of 48-h incubation of cells with 50 μM fluorous-tagged saccharide primers

Saccharide primer	Glycosylated product
LacF10	
GalF6	
GalF10	No glycosylation product
GluF6	No glycosylation product
GluF10	No glycosylation product

Table 2. Solubility of fluorous-tagged saccharide primers in fluorous solvents

Compound	F-propan-2-ol (F: 68%, OH: 10%)	F-pentanol (F: 53%, OH: 9%)	F-hexanol (F: 65% OH: 6%)	$CF_3(CF_2)_5(CH_2)_6OH$ (F: 59% OH: 4%)
Sialylated LacF6 F: 24%. OH: 16%	○	○	○	×
LacF6 F: 33%. OH: 16%	○	○	○	×
LacF10 F: 45%. OH: 13%	○	○	○	×
LacHC12 F: 0%. OH: 23%	○	×	×	×

LacHC12: dodecyl lactoside; F-propan-2-ol: 1,1,1,3,3,3-hexafluoroisopropyl alcohol; F-pentanol: pentafluoropentyl alcohol; F-hexanol: nonafluorohexyl alcohol
○: soluble ×: insoluble

the medium, GluF10 was not taken in by the cells. The presence of many fluorine atoms seemingly makes the incorporation of the primers into cells difficult. However, the number of hydroxyl groups augments the extent of cellular uptake. Hence, the balance between the number of hydroxyl groups and the number of fluorine atoms is critical on cellular uptake and viability.

Incorporation of LacF10 into B16 melanoma cells resulted in sialylation of the galactose residue to give a GM3-type oligosaccharide (Table 1).[5] Although cytotoxic, GalF6 was surprisingly elongated to give a sialylated galactoside. This suggests that the cells are capable of incorporating GalF6, elongating the saccharide moiety and releasing the elongated products into the culture medium before expiring. The amounts of elongated products obtained from LacF10 and GalF6 primers were almost the same as those obtained from primers with non-fluorous aglycon units. Diffusion of saccharide primers through the cell membrane to the Golgi where the sialyl transferases reside is a prerequisite for elongation by cellular enzymes. Consequently, GalF10 and GluF10 that were not taken in by cells did not give any glycosylation products. Although sialyl transferases are amenable to acceptor modifications, the presence of a galactose residue to effect sialylation is essential. Thus, GluF6 although taken in by the cells was also not elongated.

The simple yet efficient separation of the elongated products from the rest of the components of the culture medium without resorting to chromatography requires an appropriate fluorous solvent for extraction. We drew our attention on careful consideration of the appropriate combination of the amounts of hydroxyl and fluorine groups of the primers as well as the fluorous solvent. As shown in Table 2, the primers have 13–16% hydroxyl groups and 33–45% fluorine. Fluorous-tagged primers and sialylated primer were found to be insoluble in solvents having relatively low content of fluorine and hydroxyl groups such as perfluorohexylhexanol (4% hydroxyl groups and 59% fluorine). Although they were soluble in perfluoropropan-2-ol (F-propan-2-ol) having 10% hydroxyl groups and 68% fluorine, F-propan-2-ol is not a likely candidate as solvent for efficient separation because the relatively high OH content renders it miscible in water and thus, the primers could not be efficiently extracted from the rest of the components of the aqueous culture medium. Primers without fluorous tag were also found to be soluble in F-propan-2-ol.

Among the perfluorous solvents tested, nonafluorohexanol (3,3,4,4,5,5,6,6,6-nonafluorohexan-1-ol) and pentafluoropentanol (4,4,5,5,5-pentafluoropentan-1-ol) were found most suitable to meet the requirements for separation of the fluorous-tagged lactoside primers and the elongated products. The primers and products are soluble in these solvents that have about 6–9% hydroxyl groups and 53–65% fluorine. More importantly, these fluorous solvents were immiscible in water. Thus, the primers and product can partition out of the aqueous phase and into the fluorous phase during extraction. Investigation showed that the fluorous-tagged saccharide primer and the glycosylated product could be separated from the other components of the culture medium by extraction with these solvents.[6]

In the light of increased demand for sialylated oligosaccharides, the use of fluoro-amphiphilic glycosides possessing a hydrophilic sugar moiety and a fluorous tag is a welcome approach. Fluoro-amphiphilic glycosides could be incorporated into cells and act as substrates for glycosylation to give elongated products that could be purified by employing the fluorous approach as an alternative to the usual separation by chromatographic methods. Finding the appropriate fluoro-amphiphilic solvent is crucial but this strategy allows the simple separation of mixtures into fluorous-tagged and untagged fractions by partitioning between fluorous and aqueous liquids, respectively.

We express our heartfelt gratitude to the Noguchi Fluorous Project for the financial support extended to this research.

References and Notes

1 M. C. Kasuya, L. Wang, Y. C. Lee, M. Mitsuki, N. Nakajima, T. Sato, K. Hatanaka, S. Yamagata, and T. Yamagata, *Carbohydr. Res.*, **329**, 755 (2000); H. Nakajima, Y. Miura, and T. Yamagata, *J. Biochem.*, **124**, 148 (1998); Y. Miura and T. Yamagata, *Biochem. Biophys. Res. Commun.*, **241**, 698 (1997).
2 M. C. Kasuya, R. Cusi, O. Ishihara, A. Miyagawa, K. Hashimoto, T. Sato, and K. Hatanaka, *Biochem. Biophys. Res. Commun.*, **316**, 599 (2004).
3 Z. Luo, Q. Zhang, Y. Oderaotoshi, and D. Curran, *Science*, **291**, 1766 (2001); A. Studer, S. Hadida, R. Ferritto, S.-Y. Kim, P. Jeger, P. Wipf, and D. Curran, *Science*, **275**, 823 (1997); D. Curran and Z. Luo, *J. Am. Chem. Soc.*, **121**, 9069 (1999); D. Curran and S. Hadida, *J. Am. Chem. Soc.*, **118**, 2531 (1996); H. Matsubara, S. Yasuda, H. Sugiyama, I. Ryu, Y. Fujii, and K. Kita, *Tetrahedron*, **58**, 4071 (2002); O. Paleta, I. Dlouha, R. Kaplanek, K. Kelfurt, and M. Kodicek, *Carbohydr. Res.*, **337**, 2411 (2002); M. Wende and J. A. Gladysz, *J. Am. Chem. Soc.*, **125**, 5861 (2003); A. Dobbs and M. Kimberley, *J. Fluorine Chem.*, **118**, 3 (2002); J. Gladysz and D. Curran, *Tetrahedron*, **58**, 3823 (2002).
4 The synthesis of fluorous-tagged saccharide primers and their incorporation into B16 cells were carried out according to literature. The NMR and MALDI TOF mass spectral data are provided as Supporting Information.
5 The structures of the sialylated products were determined from the MALDI TOF mass spectral results. Treatment of the sialylated products with α2-3 sialidase (cloned from *S. typhimurium LT2* and expressed in *E. coli*) confirmed the structures of the elongated saccharides.
6 HPTLC result confirmed that only the fluorous-tagged saccharide primer and the glycosylated product joined the fluorous phase.

Published on the web (Advance View) May 21, 2005; DOI 10.1246/cl.2005.856