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THEMATIC REVIEW

Metabolic Cytometry: Monitoring Oligosaccharide Biosynthesis in Single Cells by Capillary Electrophoresis

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Metabolism is the sum total of chemical changes that occur in living organisms. Metabolic conversions can be monitored by analytical methods such as spectroscopy, chromatography, and electrophoresis; however, these methods generally require large numbers of cells and therefore do not provide information on cell to cell metabolic variation. The chemical or physical properties of individual cells can be probed with flow cytometry. It can be used for quantitative analysis of cellular constituents such as DNA, protein, enzyme activity, or phenotyping on an individual cellular basis. For example, cell-surface antigens can be detected with fluorescently labeled antibodies, cell cycle phase with DNA intercalating dyes, and enzyme activities with fluorogenic substrates. Multiple properties can be simultaneously identified up to the total number of channels available on the flow cytometer. However, metabolic cascades are difficult to investigate by flow cytometry since enzymatic conversions yield numerous products. Metabolic cytometry, the analysis of metabolic conversions in individual cells, requires ultrasensitive multicomponent analysis. This can be achieved by combining capillary electrophoresis (CE)[4] for the high-speed separation of analytes in a single cell with ultrasensitive laser-induced fluorescence detection (LIF) (1–3).

In metabolic cytometry, cells are grown in the presence of a fluorescently tagged substrate. Substrate is taken up by the cells and intracellularly converted to a series of reaction products. Conversions are monitored by CE-LIF after introducing a single cell into the separation capillary with a micromanipulator. The application of metabolic cytometry to monitor oligosaccharide biosynthesis is reviewed.

OLIGOSACCHARIDE LABELING AND DETECTION

A variety of fluorescent tags have been employed for carbohydrate labeling for LIF detection. These include 8-aminonaphthalene-1,3-6-trisulfonic acid (ANTS), 9-aminopyrene-1,4,6 trisulfonic acid (APTS), 2-amino pyridine (PA), 3-(4-carboxybenzoyl)-2-quinoline carboxyaldehyde (CBQCA), and 5-carboxytetramethylhrhodamine succinimidyl ester (TMR) (4–8). For metabolic cytometry studies TMR was selected as the fluorophore since it is pH insensitive, photostable, has a high quantum yield and good cell uptake properties, and the molar absorptivity maximum (ϵ = 65,000 M^{-1} cm^{-1}) is well matched for excitation at 543.5 nm with an inexpensive and stable He–Ne laser. The fluorescence background signals due to solvent or cellular constituents or light scatter decrease as the excitation wavelength is shifted to red; therefore, the detection limit for TMR is about an order of magnitude superior to the detection limits for fluorescein or CBQCA. TMR can be incorporated into saccharides via reductive amination of reducing sugars and then reaction with 5-carboxytetramethylrhodamine succinimidyl ester (9). Alternatively, a hydrophobic aglycon such as a methoxycarbonyloctyl group can be attached to the oligosaccharide. TMR label can be incorporated by reaction of the aglycon with ethylenediamine and then coupling with 5-carboxytetramethyl-rhodamine succin-

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[4] Abbreviations used: CE, capillary electrophoresis; LIF, laser-induced fluorescence; CBQCA, 3-(4-carboxybenzoyl)-2-quinoline carboxyaldehyde; TMR, carboxytetramethylrhodamine; LacNAc-TMR, Galβ1-4GlcNAcβ- $O(CH_2)_8CONH(CH_2)_2NHCO$-TMR; PBS, phosphate-buffered saline.

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imide ester (10–12). In this case the oligosaccharide remains intact.

These intact TMR-labeled compounds are good substrates for glycosidases and glycosyltransferases involved in biosynthetic conversions of oligosaccharides. As few as 60 molecules of enzyme reaction product can be detected and quantitated by CE-LIF (12). The instrument employed in these studies is locally constructed with a 1.0-mW He–Ne laser, $\lambda = 543.5$ nm, focused into a postcolumn sheath flow cuvette for LIF detection at 580 nm (13–15). Separations were carried out at an electric field of 400 V/cm in a 40-cm-long, 20-μm-i.d. bare fused silica capillary. The CE running buffer contained 10 mM each of disodium hydrogen phosphate, tetraborate, phenylboronic acid, and SDS at pH 9.0.

SINGLE-CELL STUDIES

Single-cell biosynthesis and hydrolysis employing Galβ1-4GlcNAcβ-O$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR (LacNAc-TMR) have been investigated in HT-29 cells (1, 2). Cells were grown for 18 h with 25 μM LacNAc-TMR added to the cell culture medium. After incubation the cells were washed with phosphate-buffered saline (PBS) to remove residual substrate and diluted with PBS to 10^4 cells/ml. A 10-μl drop of cell suspension was placed in the center of a polyvinyl alcohol-coated glass slide (16) and viewed with an inverted fluorescence microscope. One end of the capillary was placed over a cell with a micromanipulator (2, 3). The cell was drawn about 0.2 mm into the capillary with an 11-kPa $\times$ 1 s siphoning pulse applied to the opposite end of the capillary. The capillary tip was placed in a vial containing CE running buffer; the SDS in the running buffer lysed cells within 30 s (2). High voltage was applied and the TMR-labeled species were separated by electrophoresis and detected using LIF of the TMR label at 580 nm.

Figure 1 shows electropherograms from the analysis of three different HT-29 cells. The peak identities were confirmed by bulk analysis of whole-cell extracts, specifically by their comigration with authentic standards (1). All of the cells contain some unmodified LacNAc-TMR (peak 3). Hydrolytic products include GlcNAcβ-TMR (peak 2) and the linking arm HO$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR (peak 1). The monofucosylated trisaccharide Galβ1-4[Fucα1-3]-GlcNAcβ-TMR (Le^x-TMR) (peak 4) was produced in all of the cells while the difucosylated tetrasaccharide Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ-TMR (Le^y-TMR) (peak 5) was seen in one of the cells. These fucosylated oligosaccharides are synthesized by fucosyltransferases that are located in the Golgi. While the major products are hydrolytic, about 1000 molecules of Galβ1-4[Fucα1-3]GlcNAcβ-TMR (Le^x-TMR) (peak 4) and Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ-TMR (Le^y-TMR) (peak 5) are formed. Simultaneous measurements of DNA cell ploidy with Hoechst 33342 by fluorescence image cytometry prior to injection into the capillary allowed the correlation of cell cycle phase with the biosynthetic transformations that occurred (1). The rapid (30 s) lysis within the capillary also reduces chemical and enzymatic modifications that might occur during cellular extract preparation, avoiding the biasing of metabolic data due to sample handling (17).

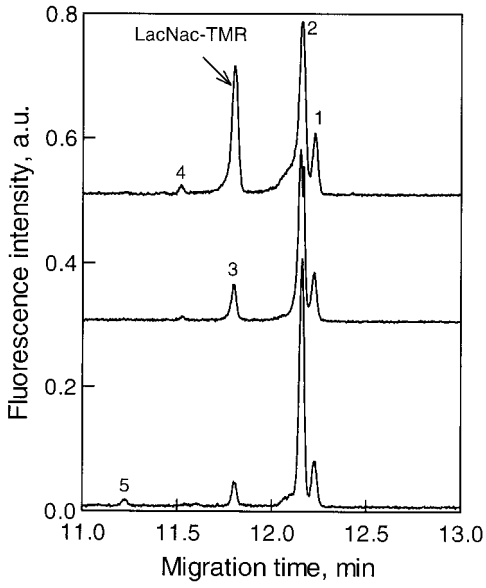

FIG. 1. Electropherograms obtained from CE-LIF analysis of individual HT-29 cells incubated with 25 μM Galβ1-4GlcNAcβ-O$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR (LacNAc-TMR) for 18 h. The peaks correspond to the hydrolytic products GlcNAcβ-TMR (peak 2) and the linking arm HO$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR (peak 1), unmodified LacNAc-TMR (peak 3), and about 1000 molecules of Galβ1-4[Fucα1-3]GlcNAcβ-TMR (Le^x-TMR) (peak 4) and Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ-TMR (Le^y-TMR) (peak 5).

Another application of metabolic cytometry utilized Glcα1-2Glcα1-3Glcα-O$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR substrate to monitor hydrolytic pathways in yeast cells (3). This trisaccharide substrate was taken up by the cells and was converted to the disaccharide Glcα1-3Glcα-O$(CH_2)_8$CONH$(CH_2)_2$NHCO-TMR by the endoplasmic reticulum-localized α-glucosidase I. The disaccharide was further hydrolyzed to monosaccharide αGlc-TMR and the TMR-linking arm. Electropherograms of single-yeast spheroplasts showed approximately 500–1000 molecules of TMR-labeled trisaccharide and the free linker arm with less than 1000 molecules of the intermediate disaccharide and monosaccharide (3). These conversions were inhibited by preincubation of the yeast cells with castanospermine, a competitive inhibitor of α-glucosidase I (3).

Metabolic cytometry is not restricted to oligosaccharide metabolism. It can be used to monitor any transformations of a fluorescent enzyme substrate provided it is taken up by cells. This methodology can also be applied to samples where limited cell numbers (embryogenesis, biopsy) have precluded analysis.

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REFERENCES

1. Krylov, S. N., Zhang, Z., Chan, N. W. C., Arriaga, E., Palcic, M. M., and Dovichi, N. J. (1999) *Cytometry* **37,** 14–20.
2. Krylov, S. N., Starke, D. A., Arriaga, E. A., Zhang, Z., Chan, N. W. C., Palcic, M. M., and Dovichi, N. J. (2000) *Anal. Chem*. **72,** 872–877.
3. Le, X. C., Tan W., Scaman, C. H., Szpacenko, A., Arriaga, E., Zhang, Y., Dovichi, N. J., Hindsgaul, O., and Palcic, M. M. (1999) *Glycobiology* **9,** 219–225.
4. Suzuki, S., and Honda, S. (1998) *Electrophoresis* **19,** 2539–2560.
5. Novotny, M. V. (1998) *in* High Performance Capillary Electrophoresis (Khaledi, M. G., Ed.), Vol. 146, pp. 729–765, Chemical Analysis Series.
6. Paulus, A., and Klockow, A. (1996) *J. Chromatogr. A* **720,** 353–376.
7. Rassi, Z. E., and Mechref, Y. (1996) *Electrophoresis* **17,** 275–301.
8. Oefner, P. J., and Chiesa, C. (1994) *Glycobiology* **4,** 397–412.
9. Zhao, J. Y., Diedrich, P., Zhang, Y., Hindsgaul, O., and Dovichi, N. J. (1994) *J. Chromatogr. B* **657,** 307–313.
10. Zhang, Y., Le, X., Dovichi, N. J., Compston, C. A., Palcic, M. M., Diedrich, P., and Hindsgaul, O. (1995) *Anal. Biochem*. **227,** 368–376.
11. Scaman, C. H., Hindsgaul, O., Palcic, M. M., and Srivastava, O. P. (1996) *Carbohydr. Res.* **296,** 203–213.
12. Zhao, J. Y., Dovichi, N. J., Hindsgaul, O., Gosselin, S., and Palcic, M. M. (1994) *Glycobiology* **4,** 239–242.
13. Cheng, Y. F., and Dovichi, N. J. (1988) *Science* **242,** 562–564.
14. Chen, D. Y., and Dovichi, N. J. (1994) *J. Chromatogr. B* **657,** 265–269.
15. Wu, S., and Dovichi, N. J. (1989) *J. Chromatogr.* **480,** 141–155.
16. Krylov, S. N., and Dovichi, N. J. (2000) *Electrophoresis* **21,** 767–773.
17. Krylov, S. N., Arriaga, E. A., Zhang, Z., Chan, N. W. C., Palcic, M. M., and Dovichi, N. J. (2000) *J. Chromatogr. B* **241,** 31–35..