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Manipulating ionic strength to improve single cell electrophoretic separations

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ABSTRACT

A capillary electrophoresis system with ultrasensitive two-color laser-induced fluorescence detection was used to probe the effect of ionic strength on single cell separations of glycosphingolipids. Differentiated PC12 cells were incubated with two ganglioside substrates tagged with different fluorophores within the BODIPY family such that two distinct metabolic patterns could be simultaneously monitored. Aspiration of single differentiated PC12 cells suspended in a phosphate-buffered saline solution showed excessive peak dispersion, poor resolution, and peak efficiencies below 100,000 theoretical plates. Aspiration of single differentiated PC12 cells suspended in deionized water corrected peak dispersion. Average peak efficiencies ranged between 400,000 and 600,000 theoretical plates. Improved performance was due to the dilution of the high salt concentrations inside of single neuronal-like cells to produce field amplified sample stacking. Single cell separations showed the highest resolution when aspiration of single differentiated PC12 cells suspended in deionized water were separated using a running buffer of high ionic strength. The improvement in resolution allowed for the identification of analytes not previously detected in single cell metabolism studies.

1. Introduction

Traditional biological assays utilize aggregate analysis of thousands of cells to study biomolecules of interest. However, this approach eliminates the ability of researchers to study inherent variation among single cells. Even genetically identical cells can display differences in their content due to differences in cell size, cell density within tissue, and differences in their local environment [1,2]. Single cell analysis has been used in studies within the fields of neuroscience [3,4], oncology [5], enzymology [6,7], immunology [8], and genetics [9]. A variety of analytical detection methods have been used to characterize single cells including electrochemistry, mass spectrometry, and fluorescence [10]. Capillary electrophoresis (CE) has been used to separate the contents of single cells since the late 1980s [4,11]. CE offers improvements in single cell analysis due to its small volume requirements and fast, efficient separations [12].

Glycosphingolipids (GSLs) are amphiphilic molecules that are present in moderate concentrations within neuronal cell membranes [13]. GSLs contain a polar headgroup consisting of a diverse suite of carbohydrates combined with a hydrophobic tail composed of a fatty acid and a sphingosine (termed ceramide). Gangliosides are a specific subtype of GSLs that contain at least one sialic acid in the polar headgroup. Gangliosides are involved in a variety of cell functions including cell signaling and differentiation [14–21]. Studying GSL metabolism is of great interest because defects in GSL metabolism play a role in several disorders such as Tay–Sachs Disease and seizure conditions [22–25]. A simplified overview of GSL metabolism is shown in Fig. 1A.

Our group developed the technique termed “metabolic cytometry” that utilizes CE combined with laser-induced fluorescence to study metabolism within single cells [26]. In this approach, cells are incubated with a GSL substrate that has a fluorescent tag covalently bound to its ceramide tail. Carriers such as artificial lipid vesicles [27], de-fatted bovine serum albumin [28], or cyclodextrins [29] are added to the cellular medium to enhance delivery, transfer, and insertion of these fluorescent GSLs into cells. Once inside, these fluorescent GSLs are trafficked and metabolized intracellularly where endogenous anabolic and catabolic enzymes can add and remove (respectively) various sugar moieties within the headgroup [30]. While metabolism occurs within the headgroup of the exogenously added fluorescent GSL, the fluorophore on the ceramide tail remains intact and all metabolic products of the fluorescent substrate will be fluorescent. To assess metabolism, a single cell is then aspirated into a capillary, lysed, and the fluorescently-labeled GSLs are separated by CE with laser-induced fluorescence detection. Cells can also be simultaneously incubated with multiple GSLs labeled with different fluorophores; instruments are then constructed with multiple excitation sources to monitor multiple metabolic pathways in the same cells at the same time [31,32].

Buffer composition plays a pivotal role in the quality of GSL separations by CE. In capillary zone electrophoresis (CZE),

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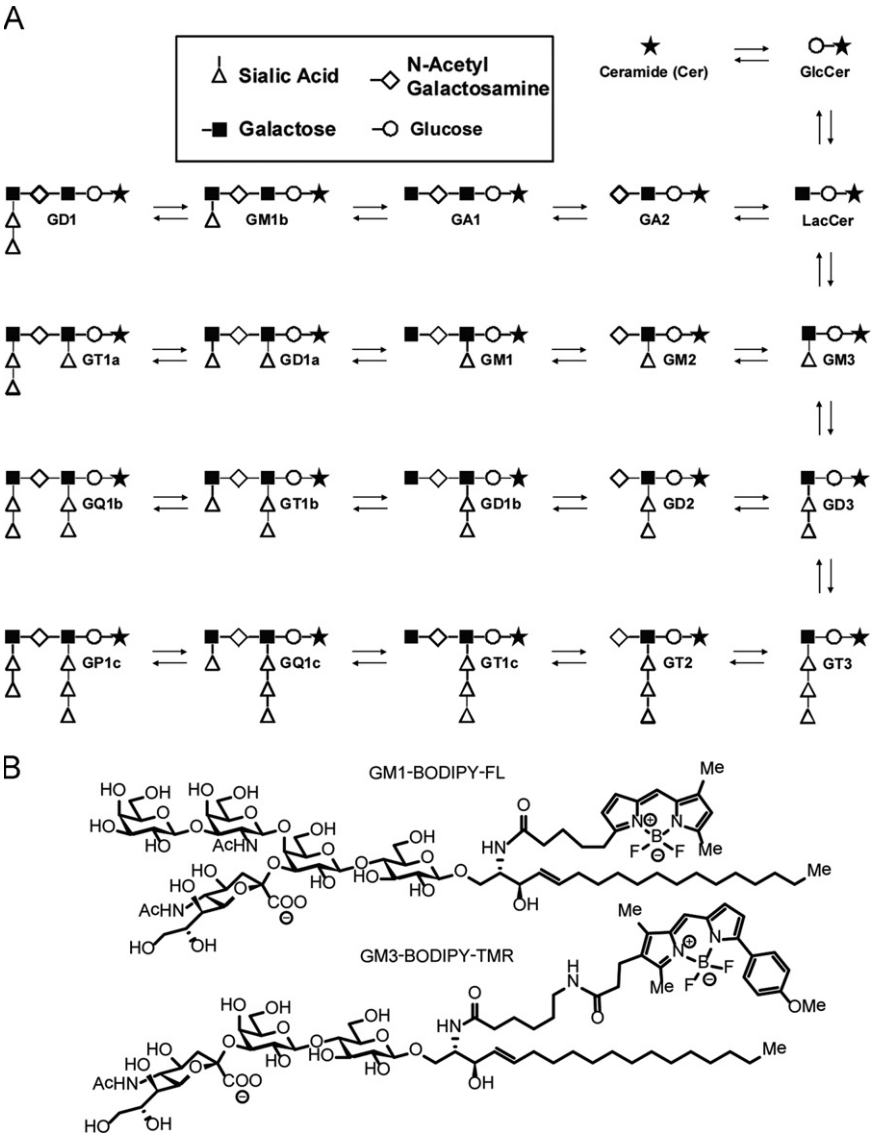

Fig. 1. (A) Simplified schematic of GSL metabolism. (B) Structures of the synthetically prepared GM1-BODIPY-FL and GM3-BODIPY-TMR substrates.

borate/phosphate buffers containing the additive α-cyclodextrin (α-CD) have been used to separate GSLs [33–35]. The ionic strength of the separation buffer can also affect the separation of GSLs [36]. In CZE-based separations, buffers with low ionic strengths are preferred because increases in the ionic strength have been shown to cause peaks shapes to become more asymmetric as the migration times increase [36]. CZE is not a preferred method of separating GSLs because the amphiphilic nature of GSLs causes them to form heterogeneous multi-analyte mixed micelles in solution [37], confounding their detection [36]. In micellar electrokinetic capillary chromatography (MECC), the presence of surfactant breaks up these mixed micelles and generally yields very high peak efficiencies (>400,000 theoretical plates) [38].

Varieties of experimental and instrumental approaches have been used to improve single cell electrophoretic separations focusing on sample preparation, sample manipulation, and cellular lysis. The cell suspension solution should both maintain cell integrity while also preventing cell lysis, allowing for an intact cell to be injected into the capillary [11]. When studying single cell metabolism, fixation of the cells is needed to arrest their metabolic profiles at the time of harvesting to eliminate artifacts associated with storing the cells long-term [36]. The manual isolation of a single cell and introduction of that cell into the capillary also affects analyte separation [39]. Intact cells are traditionally introduced into the capillary using negative pressure and lysed once inside to release their contents [12,39,40]. In addition, the inner diameter of the capillary influences the injection of a single cell. Having an etched capillary tip similar to the size of the cell to be separated can improve the injection of a single cell into the capillary [12]. The running buffer can be used to both lyse the cell and separate the components inside the cell [12]. Otherwise, plugs of detergent such as sodium dodecylsulfate (SDS) can be aspirated to assist in cell lysis [41].

Here, we demonstrate that the manipulation of ionic strength can also be used to improve the quality of single cell separations. The PC12 cell line, originating from rat adrenal tissue, was used as a model neuronal system. Upon addition of neuronal growth factor (NGF), PC12 cells differentiate into neuronal-like cells (dPC12s) [42]. dPC12 cells were incubated with two fluorescent GSL substrates shown in Fig. 1B, GM1-BODIPY-FL ($\lambda_{ex}/\lambda_{em}$ 505 nm/513 nm) [43] and GM3-BODIPY-TMR ($\lambda_{ex}/\lambda_{em}$ 542 nm/574 nm) [43]. GSL metabolism was then monitored in single cells using capillary electrophoresis with two-color laser-induced fluorescence detection.