MethodsX 3 (2016) 251–260

Contents lists available at ScienceDirect

MethodsX

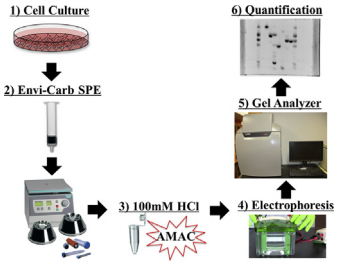
journal homepage: www.elsevier.com/locate/mex

Isolation and analysis of sugar nucleotides using solid phase extraction and fluorophore assisted carbohydrate electrophoresis

Jarrod Barnes[a,1], Liping Tian[a,1], Jacqueline Loftis[a], James Hiznay[c], Suzy Comhair[a], Mark Lauer[a,2], Raed Dweik[a,b,*]

[a] Departments of Pathobiology, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA
[b] Pulmonary Critical Care Medicine, Respiratory Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA
[c] Molecular Medicine, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, USA

GRAPHICAL ABSTRACT



ABSTRACT

The building blocks of simple and complex oligosaccharides, termed sugar nucleotides, are often overlooked for their role in metabolic diseases and may hold the key to the underlying disease pathogenesis. Multiple reasons may account for the lack of analysis and quantitation of these sugar nucleotides, including the difficulty in isolation and purification as well as the required expensive instrumentation such as a high performance liquid

Abbreviations: HPLC, high performance liquid chromatography; SPE, solid phase extraction; FACE, fluorophore assisted carbohydrate electrophoresis; UDP, uridine diphosphate; GDP, guanosine diphosphate; CMP, cytosine monophosphate; TEAA, triethylamine acetate; APS, ammonium persulfate; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Man, Mannose; NeuAc, sialic acid; GlcUA, glucuronic acid; AMAC, 2-aminoacridone; TEMED, *N′,N′,N′N′*-tetramethylenediamine.

* Corresponding author at: Departments of Pathobiology, Cleveland Clinic, 9500 Euclid Ave, Cleveland, Ohio 44195, USA.
E-mail address: dweikr@ccf.org (R. Dweik).

[1] Authors contributed equally to this work.

[2] In honor of Dr. Mark Lauer who passed away October 12, 2015.

http://dx.doi.org/10.1016/j.mex.2016.03.010

chromatography (HPLC), mass spectrometer, or capillary electrophoresis. We have established a simple yet effective way to purify and quantitate sugar nucleotides using solid phase extraction (SPE) chromatography combined with fluorophore assisted carbohydrate electrophoresis (FACE). The simplicity of use, combined with the ability to run multiple samples at one time, give this technique a distinct advantage over the established methods for isolation and analysis of sugar nucleotides from cell culture models.

- Sugar nucleotides can be easily purified with solid phase extraction chromatography.
- FACE can be used to analyze multiple nucleotide sugar extracts with a single run.
- The proposed method is simple, affordable, and uses common everyday research labware.

ARTICLE INFO

Method name: Sugar nucleotide analysis by SPE and FACE

Keywords: Sugar nucleotides, Face, HPLC, Carbohydrate, Electrophoresis

Article history: Received 18 December 2015; Accepted 11 March 2016; Available online 14 March 2016

Method details

The biosynthesis of polysaccharides and the N- and O-linked glycosylation of proteins depend the charged substrates of glycosyltransferases called sugar nucleotides. These substrates are sugars activated by the addition of a nucleoside mono- or diphosphate (UDP, GDP, or CMP), which forms the sugar nucleotide. A large proportion of the sugar nucleotides are synthesized in the cytosol, while their respective enzymes are located within the lumen of the Golgi apparatus or the endoplasmic reticulum. Thus, sugar nucleotides are translocated from the cytosol to the lumen of the Golgi apparatus and endoplasmic reticulum by multiple spanning domain proteins known as sugar nucleotide transporters. It is in these compartments that glycoconjugates, including polysaccharides, glycoproteins, and glycolipids are synthesized and glycosylated by glycosyltransferases. Glycosylation and their respective glycosyltransferases have become the predominant focus of the literature when glycan alterations are investigated in growth, development, and disease processes such as cancer and pulmonary arterial hypertension [1–6] with little focus on the role of nucleotide sugars.

Sugar nucleotides were first discovered by Leloir and colleagues [7–9]. It is now known that the vast majority of sugar nucleotides is derivatized from UDP-glucose (glc); a reaction that take places predominantly in the cytosol and gives rise to several sugar nucleotides including UDP-galactose (Gal), UDP-N-Acetylglucosamine (GlcNAc), UDP-N-Acetylgalactosaime (GalNAc), GDP-Mannose (Man), and CMP-Sialic Acid (NeuAc). Multiple reports have demonstrated methods that generate or synthesize sugar nucleotides [14–20] as well as high-throughput assays for sugar nucleotide formation and glycosyl transfer [21,22]. Others have shown great promise with the use of sugar nucleotide derivatives and sugar analogs for the inhibition of glycosyltransferases [22,23], which may be useful in disease such as cancer. Since the late 1970s, several HPLC methods have been developed to purify and analyze sugar nucleotides [10–13]. In addition, Mass Spectrometry [11,24,25], capillary electrophoresis [26,27], and NMR methods [11,28,29] have been put forth to study sugar nucleotide levels. However, these instruments are expensive and require extensive expertise to operate.

Fluorophore Assisted Carbohydrate Electrophoresis (FACE) was created as a simple alternative to Mass Spectrometry (MS), NMR, and HPLC for determining carbohydrates and oligosaccharides [30–34]. Two different fluorophores, 8-aminonaphthalene- 1,3,6-trisulphonic acid (ANTS) and 2-aminoacridone (AMAC), have routinely been used to fluorescently label carbohydrates (Fig. 1) for visualization [30,31]. There are a few studies that have used FACE to analyze sugar nucleotides in combination with anion exchange chromatography [35,36]. However, the anion exchange columns also bind monosaccharides modified with phosphates and other charged entities as well as oligosaccharides. Therefore, multiple steps are required to purify the sugar nucleotides from these other glycans.

Previously published reports have shown that the Solid Phase Extraction (SPE) ENVI-carbon columns bind tightly to sugar nucleotides and not other monosaccharides or glycoconjugates [12,13]. Bound sugar nucleotides can be eluted using an ion-pairing reagent such as TEAA [12,37].