Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification and characterization of UDP-mannose in human cell lines and mouse organs: Differential distribution across brain regions and organs

Kazuki Nakajima [a, b], Yasuhiko Kizuka [b], Yoshiki Yamaguchi [c], Yoshio Hirabayashi [d], Kazuo Takahashi [e], Yukio Yuzawa [e], Naoyuki Taniguchi [b, *]

[a] Department of Academic Research Support Promotion Facility, Center for Research Promotion and Support, Fujita Health University, Toyoake, Aichi 470-1192, Japan
[b] Disease Glycomics Team, Systems Glycobiology Research Group, RIKEN-Max Planck Joint Research Center, RIKEN Global Research Cluster, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[c] Structural Glycobiology Team, Systems Glycobiology Research Group, RIKEN-Max Planck Joint Research Center, RIKEN Global Research Cluster, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[d] Molecular Membrane Neuroscience, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[e] Department of Nephrology, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan

ARTICLE INFO

Article history:
Received 19 October 2017
Accepted 30 October 2017
Available online xxx

Keywords:
Nucleotide sugar
UDP-mannose
Mannosylation
LC-ESI-MS/MS
Hypothalamus
Neocortex

ABSTRACT

Mannosylation in the endoplasmic reticulum is a key process for synthesizing various glycans. Guanosine diphosphate mannose (GDP-Man) and dolichol phosphate-mannose serve as donor substrates for mannosylation in mammals and are used in *N*-glycosylation, *O*-mannosylation, *C*-mannosylation, and the synthesis of glycosylphosphatidylinositol-anchor (GPI-anchor). Here, we report for the first time that low-abundant uridine diphosphate-mannose (UDP-Man), which can serve as potential donor substrate, exists in mammals. Liquid chromatography-mass spectrometry (LC-MS) analyses showed that mouse brain, especially hypothalamus and neocortex, contains higher concentrations of UDP-Man compared to other organs. In cultured human cell lines, addition of mannose in media increased UDP-Man concentrations in a dose-dependent manner. These findings indicate that in mammals the minor nucleotide sugar UDP-Man regulates glycosylation, especially mannosylation in specific organs or conditions.

1. Introduction

Glycosylation of proteins and lipids is vitally important in many biological processes. Dysregulation of glycosylation is associated with various diseases, such as diabetes mellitus, cancer, and degenerative neuromuscular disease [1–4]. Mannosylation occurring in the endoplasmic reticulum (ER) plays a key role in the synthesis of multiple types of glycans. Dolichol-linked mannose (Dol-P-Man) and GDP-mannose (GDP-Man) are donor substrates for mannosylation. Dol-P-Man is synthesized from mannose-6-phosphate via several intermediates (e.g., GDP-Man) of mannose metabolism [5]. These intermediates are also used in *N*-glycosylation, synthesis of GPI-anchor, *O*-mannosylation, and *C*-mannosylation.

With *N*-glycosylation Dol-P-Man and GDP-Man concentrations regulate mannosylation in the highly organized biosynthesis of *N*-glycan precursors, dolichol-linked oligosaccharides (DLOs). DLO biosynthesis is sensitive to the cellular metabolic states of monosaccharides. For example, glucose deprivation reduces GDP-Man concentrations, which in turn leads to the biosynthetic arrest of DLOs [6,7]. This facilitates the premature degradation of DLOs by pyrophosphatase [8]. This serves as a quality control system that prevents abnormal *N*-glycosylation [8].

Dol-P-Man deficiencies related to genetic factors cause congenital disorders of glycosylation (CDG), resulting in severe clinical symptoms [9]. For example, PMI-CDG and PMM2-CDG subtypes are caused by genetic mutations in phosphomannose isomerase and phosphomannomutase 2. Both of these enzymes are

* Corresponding author.
E-mail addresses: dglycotani@riken.jp, rvw.tani@sanken.osaka-u.ac.jp (N. Taniguchi).

https://doi.org/10.1016/j.bbrc.2017.10.173
0006-291X/

The abbreviations used

CDG	congenital disorders of glycosylation
CMP-NeuAc	cytidine monophospho N-acetyl-D-neuraminic acid
Dol-P-Man	dolichol-phosphate-mannose
DLO	dolichol-linked oligosaccharide
ESI-MS/MS	electrospray ionization-tandem mass spectrometry
GDP-Man	guanosine diphosphate mannose
GDP-Fuc	guanosine diphosphate fucose
GPI-anchor	glycosylphosphatidylinositol-anchor
LC-MS	liquid chromatography-mass spectrometry
MEF	mouse embryonic fibroblast
NMR	nuclear magnetic resonance
UDP-Gal	uridine diphosphate galactose
UDP-Glc	uridine diphosphate glucose
UDP-GlcA	uridine diphosphate glucuronic acid
UDP-HexNAc	uridine diphosphate N-acetyl hexosamine
UDP-Man	uridine diphosphate mannose
UDP	uridine diphosphate
UMP	uridine monophosphate

involved in GDP-Man biosynthesis [10,11]. In PMI-CDG patients, oral mannose supplementation restores normal glycosylation. Symptoms are relieved, because mannose can be converted to GDP-Man [10]. In mouse models of autoimmune diabetes and airway inflammation, oral mannose supplementation induces regulatory T cells and suppresses the immunopathology [12]. Genetic mutations associated with Dol-P-Man biosynthesis give rise to a high incidence of α-dystroglycanopathy [13–15]. This is caused by a deficiency of O-mannosyl glycans on α-dystroglycan in brain, peripheral nerves, and skeletal muscle [16,17]. Indeed, mannosylation is vital for mammalian development and cellular homeostasis. However, the precise roles of nucleotide sugar metabolism in regulating mannosylation are still not fully understood, particularly in *O*-mannosylation, GPI-anchor synthesis, and *C*-mannosylation.

In mammals, at least 12 types of nucleotide sugars are known. In contrast many more nucleotide sugars have been identified in plants and bacteria. At least 30 types of low-abundant nucleotide sugars exist in plants, and 70 nucleotide sugars have been identified in bacteria [18–21]. This disparity between mammals and plants and bacteria raises the possibility that minor nucleotide sugars remain to be identified in mammals. Indeed, low-abundant nucleotide sugars like UDP oligosaccharides are found in human milk [22,23].

We previously developed two methods for monitoring nucleotide sugar metabolism by using ion-pair reversed-phase LC and LC-electrospray ionization-tandem mass spectrometry (ESI-MS/MS) [24,25]. With these methods, cellular concentrations of abundant nucleotide sugars in mammalian cell lines were determined simultaneously [24]. In the present study, we sought to identify and measure low-abundant nucleotide sugars using these methods. We detected a nucleotide sugar that increases in concentration in mannose-rich media and found this nucleotide to be UDP-Man. We also determined UDP-Man concentrations in several human cell lines and mouse tissues, which led to the revelation that UDP-Man likely plays a unique role in glycosylation in specific mammalian organs, including brain.

2. Materials and methods

2.1. Materials

Uridine-5-diphospho-α-D-mannopyranoside (UDP-Man) was purchased from Sigma Aldrich Japan (Tokyo, Japan). Ammonium bicarbonate, acetonitrile, and distilled water were of LC-MS grade (Thermo Fisher Scientific, Waltham, MA). Triethylamine and formic acid of LC-MS grade were purchased from Wako Chemicals (Osaka, Japan). The sources of other materials were as follows: high-glucose DMEM (Sigma Aldrich, Japan); fetal bovine serum (Biowest, Nuaillé, France); glucose-free DMEM, penicillin, and streptomycin sodium salt (Life Technologies, Carlsbad, CA); all other chemicals (Wako Chemicals, Osaka, Japan).

2.2. Cell lines and animals

The human hepatoma cell line Hep3B, human breast cancer cell line MCF7, and the human lung adenocarcinoma epithelial cell line A549 were obtained from the ATCC (Manassas, VA). Human pancreatic cancer cell line PK8 was obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging, and Cancer (Tohoku University, Miyagi, Japan). Mouse embryonic fibroblast (MEF) cells were cultured and maintained as described previously [26]. Inadvertent mycoplasma infection of the MEF cell line was detected using e-MycoTM plus Mycoplasma PCR detection kit (iNtRON Biotechnology Inc, Jungwong-gu, Seongnam, Korea). Genomic DNA in harvested MEF cells was extracted using the i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology Inc., Korea).

Cell lines were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin until they reached approximately 60–70% confluence. To produce nucleotide sugars in the cell cultures, the culture medium was replaced with low glucose DMEM supplemented with mannose at various concentrations, ranging up to 20 mM. Then, the cells were maintained in culture for 3, 6, 12, or 24 h, followed by cell extraction and analysis (below).

All animal experiments were performed in compliance with the Institutional Guidelines for Animal Experiments of RIKEN. RIKEN institutional policies are consistent with ARRIVE Guidelines and follow international standards. To assess the regional distribution of UDP-Man in different organs, we used four 10-week-old male C57BL/6N mice (Charles River, MA). Mice were deeply anesthetized, and the liver, lung, brain, and kidney were extirpated immediately, and then placed into liquid nitrogen. The brain was further divided into seven parts according to standard anatomical regions: olfactory bulb, hypothalamus, cerebellum, medulla oblongata, hippocampus, neocortex, and “other” brain regions not included in the first six.

2.3. Preparation of cellular extracts from cultured cells and mouse tissues

Nucleotide sugars were prepared from the cultured cells (6-cm diameter dish), according to a previous report [24]. Cells were collected in ice-cold 70% ethanol (2 ml); and GDP-Glc (500 pmol) was added as an internal standard. The extract was centrifuged at 16,000 g for 15 min at 4 °C, and the supernatant was lyophilized. The freeze-dried samples were subjected to solid-phase extraction using an Envi-Carb column (100 mg; Supelco Inc, Bellafonte, PA). For mouse tissue samples, blocks of same-organ samples (30–90 mg) were manually homogenized in ice-cold 75% ethanol, and the tissue homogenates were prepared similarly to that described for cells. An Envi-Carb column (250 mg) was used also for

Please cite this article in press as: K. Nakajima, et al., Identification and characterization of UDP-mannose in human cell lines and mouse organs: Differential distribution across brain regions and organs, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.10.173