Biochemical Pharmacology 152 (2018) 11–20

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Differential patterns of inhibition of the sugar transporters GLUT2, GLUT5 and GLUT7 by flavonoids

Julia S. Gauer[a], Sarka Tumova[a], Jonathan D. Lippiat[b], Asimina Kerimi[a], Gary Williamson[a,*]

[a] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK
[b] School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

ARTICLE INFO

Keywords:
Polyphenols
Xenopus laevis oocytes
Sugar transporters
Flavonoids
Caco-2 cells

ABSTRACT

Only limited data are available on the inhibition of the sugar transporter GLUT5 by flavonoids or other classes of bioactives. Intestinal GLUT7 is poorly characterised and no information exists concerning its inhibition. We aimed to study the expression of GLUT7 in Caco-2/TC7 intestinal cells, and evaluate inhibition of glucose transport by GLUT2 and GLUT7, and of fructose transport by GLUT2, GLUT5 and GLUT7, by flavonoids. Differentiated Caco-2/TC7 cell monolayers were used to investigate GLUT7 expression, as well as biotinylation and immunofluorescence to assess GLUT7 location. For mechanistic sugar transport studies, *X. laevis* oocytes were injected with individual mRNA, and GLUT protein expression on oocyte membranes was confirmed. Oocytes were incubated with D-[^{14}C(U)]-glucose or D-[^{14}C(U)]-fructose in the presence of flavonoids, and uptake was estimated by liquid scintilation counting.

In differentiated Caco-2/TC7 cell monolayers, GLUT7 was mostly expressed apically. When applied apically, or to both compartments, sorbitol, galactose, L-glucose or sucrose did not affect GLUT7 mRNA expression. Fructose applied to both sides increased GLUT7 mRNA (13%, $p \leq 0.001$) and total GLUT7 protein (2.7-fold, $p \leq 0.05$), while the ratio between apical, basolateral and total GLUT7 protein was unchanged. In the *X. laevis* oocyte model, GLUT2-mediated glucose and fructose transport were inhibited by quercetin, (−)-epigallocatechin gallate (EGCG) and apigenin, GLUT5-mediated fructose transport was inhibited by apigenin and EGCG, but not by quercetin, and GLUT7-mediated uptake of both glucose and fructose was inhibited by apigenin, but not by quercetin nor EGCG. Expression of GLUT7 was increased by fructose, but only when applied to Caco-2/TC7 cells both apically and basolaterally. Since GLUT2, GLUT5 and GLUT7 show different patterns of inhibition by the tested flavonoids, we suggest that they have the potential to be used as investigational tools to distinguish sugar transporter activity in different biological settings.

1. Introduction

GLUT proteins are members of the SLC2 family and transport monosaccharides and polyols across eukaryotic cell membranes by a facilitative mechanism but with different affinity and specificity. Intestinal glucose absorption across the apical membrane involves both sodium-glucose cotransporter 1 (SGLT1) and GLUT2 [1,2], while transport of glucose to the blood is catalysed by GLUT2 on the basolateral membrane [3]. In the presence of high glucose concentrations, transport is mediated primarily by GLUT2 rather than SGLT1, through trafficking of additional GLUT2 to the apical surface [4,5]. This mechanism is supported by evidence showing, for example, that GLUT2 is localised to the apical membrane in rat intestinal models with elevated sugar concentrations [6]. Fructose uptake in the gut is primarily mediated by GLUT5 which recognizes all forms of the sugar [7–9]. Secondary fructose transport is facilitated by GLUT2, which is able to recognize fructose in its furanose form [10], and is responsible for transporting absorbed fructose across the basolateral membrane of enterocytes and into the blood [11]. GLUT7 is expressed in the intestine and very few other tissues, and is the closest relative to GLUT5, sharing 53% sequence homology and 68% amino acid identity [12,13]. GLUT7 has a notably high affinity for both glucose and fructose, and due to high levels of expression in the ileum, may be responsible for sugar uptake at the end of a meal, when sugar concentrations gradually

Abbreviations: GLUT2, solute carrier family 2, member 2 (SLC2A2), human; GLUT5, solute carrier family 2, member 5 (SLC2A5), human; GLUT7, solute carrier family 2, member 7 (SLC2A7), human; SGLT1, Sodium-dependent sodium-glucose cotransporter 1; EGCG, (−)-epigallocatechin-gallate; IVT, *in vitro* transcription; ddPCR, digital droplet polymerase chain reaction; FBS, fetal bovine serum; DAPI, 4′,6-diamidino-2-phenylindole; WGA, Wheat Germ Agglutinin

* Corresponding author.
E-mail address: g.williamson@leeds.ac.uk (G. Williamson).

https://doi.org/10.1016/j.bcp.2018.03.011
Received 19 January 2018; Accepted 9 March 2018
Available online 14 March 2018

decrease [14–16]. Although there is still controversy about the ability of GLUT7 to transport sugars [17,18], two studies on expression of human GLUT7 in *X. laevis* oocytes reported that this protein was able to transport fructose and glucose, but not galactose [12,15]. It has been hypothesised that a conserved motif, present in the sequences of GLUT2, GLUT5 and GLUT7, is responsible for their ability to transport fructose [18–20].

Many flavonoids have been shown to inhibit GLUT2, including tiliroside, myricetin, phloretin, EGCG and apigenin [21–24]. Quercetin is also a potent inhibitor of both glucose and fructose uptake by GLUT2, through binding to a non-sugar binding site [23,25]. Unlike GLUT2, inhibition of GLUT5 sugar uptake has only been shown for a limited number of flavonoids: (–)-epicatechin-gallate (ECG) and (−)-epigallocatechin-gallate (EGCG) [26]. The sugar analogue L-sorbose-Bn-OZO also inhibits GLUT5, and contains a bulky benzyl group thought to bind to a position out of the binding site. The oxygen molecule in the OZO moiety increases hydrogen interactions with the protein, allowing for tighter binding [8]. No inhibitors of GLUT7 of any type have yet been reported, and, in fact, even the ability of GLUT7 to transport sugars has been questioned [17,18].

2. Materials and methods

2.1. Chemicals

D-[^{14}C(U)]-glucose was from Perkin Elmer (Boston, USA), D-[^{14}C(U)]-fructose was from Hartmann Analytic (Braunschweig, Germany), and D-fructose, D-glucose and Glutamax™ were from Thermo Fisher Scientific (Paisley, UK). Dulbeccos's modified Eagle's Medium, fetal bovine serum (FBS), non-essential amino acids, penicillin, protease inhibitor cocktail and streptomycin were from Sigma-Aldrich, UK. L-sorbose-Bn-OZO was kindly provided by Professor Arnaud Tatibouet, Université d'Orléans, France.

2.2. Cell culture

Caco-2/TC7 cells, at passage number 30 and kindly donated by Dr M. Rousset (U178 INSERM, Villejuif, France), were routinely cultured in 25 mM glucose Dulbeccos's modified Eagle's Medium supplemented with 20% (v/v) fetal bovine serum (FBS), 2% (v/v) Glutamax™, 2% (v/v) non-essential amino acids, 100 U/mL penicillin and 0.1 mg/mL streptomycin at 37 °C with 10% CO_2 in a humidified atmosphere. Cells were used between passage numbers 30 and 40.

2.3. Gene expression analysis using digital droplet PCR

Caco-2/TC7 cells were seeded on 6-well Transwell plates (0.4 μm pore size, polycarbonate, Corning, UK) at a density of 6×10^4 cm^{-2} and maintained for 21 d in the conditions indicated above. After 7 d post-seeding and up to 21 d cells were grown in asymmetric conditions, with FBS included only in the medium added to the basolateral side of each well. Throughout the differentiation period (7–21 d), cells were grown in the standard glucose medium or in medium supplemented with 25 mM of one of the following sugars; fructose, sorbitol, galactose, L-glucose and sucrose on apical side only, or on both apical and basolateral sides. At day 21 cells were lifted and mRNA was extracted using the Aurum Total RNA Mini Kit (Bio-Rad, UK), following manufacturer's instructions. Reverse transcription of RNA to cDNA was performed with a high capacity RNA to cDNA kit (Applied Biosystems, Life Technologies, USA). Droplet digital PCR (ddPCR) was used to quantitatively determine gene expression using TaqMan duplexed FAM/VIC assays in a QX100 system (Bio-Rad), as previously described [27]. Triplicate reactions of 20 μL stock sample solution were prepared by adding 8 μL total transcribed nucleic acids (5 ng) diluted with MilliQ water, 1 μL GLUT7 (SLC2A7) FAM™-labelled TaqMan primer (Hs01013553_m1, Thermo Fisher Scientific, UK) and 10 μL of ddPCR Supermix for Probes (Bio-Rad). In addition, 1 μL VIC™-labelled probe for TBP (TATA box binding protein, Hs00427620_m1) (Thermo Fisher Scientific, UK) was added to final sample solution to act as a reference. All data were analysed with the QuantaSoft software (Kosice, Slovakia). Concentrations of target and reference cDNA (Tata Binding Box Protein 1, TBP) measured as copies/μL are presented as a ratio.

2.4. Protein expression analysis by cell surface biotinylation

Cell surface biotinylation was performed using the Pierce Cell Surface Protein Isolation Kit (89881, Thermo Fisher Scientific, UK) according to the manufacturer's instructions. Briefly, cells were washed twice with ice cold phosphate buffered saline with calcium chloride and magnesium chloride (PBS+) (D1283, Sigma-Aldrich, UK) before incubation with 0.25 mg/ml Sulfo-NHS-SS-Biotin for 30 min, on ice, on a low speed shaker. Quenching solution was added to each well, and cells were washed twice with Tris buffered saline (TBS) buffer (1706435, Bio-Rad, UK). Cells were scraped and lysed on ice for 30 min in 60 mM octylglucoside/150 mM NaCl/20 mM Tris solution (pH 7.4), containing protease inhibitors. Following centrifugation at 14,000*g* for 5 min, supernatant was transferred into a new tube and protein concentration for each cell lysate was determined with a ND100 Nanodrop spectrophotometer (Thermo Fisher Scientific, UK). NeutrAvidin Agarose beads in Pierce Spin Columns (Thermo Fisher Scientific, UK) were washed twice with TBS and lysis buffer. Lysates were added to the filter columns at comparable protein concentrations, and incubated at room temperature on a rotator at low speed for 1 h. Filter columns were washed twice with TBS and then treated with Rapid PNGase F (P0710S, New England Biolabs, USA) for 15 min at 37 °C to remove N-glycosylation. Biotinylated membrane fractions were eluted with SDS-PAGE buffer containing 0.5 M dithiothreitol following a 20 min incubation at 37 °C.

2.5. Immunofluorescence staining

Caco-2/TC7 cells were seeded at a density of 6 x 10^4 cm^2 on Millicell cell culture inserts (12-well, PET 0.4 mm pore size, Millipore) and maintained as described above for 21 d. Cells were fixed with 4% *para*-formaldehyde in phosphate buffered saline (PBS) and incubated with 5 μg/mL fluorescein labelled Wheat Germ Agglutinin (WGA) (Fl-1021, Vector Laboratories, Peterborough, UK) for 10 min at 37 °C. Cells were then washed three times with PBS + and permeabilised with 0.1% Triton-X100 for 20 min at room temperature before incubation with GLUT7 primary antibody (NBP1-81821, Novus Biologicals, USA) at a 1:50 dilution for 1 h at room temperature. After three washes with PBS, cells were incubated with Cy3-conjugated AffiniPure donkey anti-rabbit IgG (711-165-152, Jackson ImmunoResearch, USA) secondary antibody at a dilution of 1:300. Cells were washed three times with PBS, stained with 2 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) for 5 min, rinsed with water, and mounted onto microscopy slides using ProLong Gold antifade reagent mounting medium (Thermo Fisher Scientific, UK). Images were obtained with a Zeiss LSM 700 Inverted Confocal Microscope using the 63x lens objective. Cells imaged without WGA were permeabilized once fixed and processed for imaging in the same way as described above, with the exception that Alexa Fluor 488-conjugated AffiniPure donkey anti-rabbit IgG (711-545-152, Jackson ImmunoResearch, USA), at a dilution of 1:300, was used in the secondary antibody incubation step.

2.6. Protein expression analysis in Caco-2/TC7 cells by automated western blotting

Expression of membrane-localised GLUT7 after biotinylation was determined using automated capillary Western blotting (WES, ProteinSimple, Bio-Techne, UK). Cell lysates were treated with Rapid PNGase F for 15 min prior to the denaturation step (37 °C, 15 min).