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Screening for the best detergent for the isolation of placental membrane proteins

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ABSTRACT

Although membrane proteins (MPs) play crucial roles in physiological processes, information on them are insufficient, mostly due to their peculiar nature and surrounding which demand specific procedures for their extraction (using detergents) and analysis. A pallet of ten detergents and β-cyclodextrin was employed to investigate their efficiency in extracting total placental MPs, glycoproteins and insulin-like growth factor receptors (IR/IGF1R/IGF2R). Regardless of detergent used, the identity of major extracted proteins was the same. Glycoproteins extracted with Triton X-100 contained the greatest variety and quantity of glycans recognised by fifteen lectins, pointing to this detergent as universal medium for the extraction of membrane glycoproteins. Glycoproteins extracted using Brij 35 exhibited weak interaction with only seven lectins and were differently recognised by lectins of the similar glycan specificity. Brij 35, Tween 20, saponin and digitonin selectively extracted IGF2R compared to other two receptors. Pilot experiments should be conducted in order to choose adequate detergent for the extraction of specific MP. To obtain preparations enriched in specific receptor of the insulin/IGF system sequential solubilisation of placental MPs can be proposed: to use Brij 35 to extract IGF2R and subject the insoluble remaining suspension to Triton X-114 in order to extract most of IGF1R with small amounts of IR.

1. Introduction

Membrane proteins (MPs) play crucial roles in physiological processes such as cell–cell interaction, adhesion and signal transduction. They are coded by almost one third of a genome [1]. More than one half of MPs are subjected to posttranslational modifications, with glycosylation being the most frequent. Glycosylation exerts modulating role in proper functioning of MPs [2–4]. Although MPs are very important constituents of cells, information on them are rare and insufficient, especially when it comes to their three-dimensional structure. The main reasons are their minute amounts, peculiar nature and natural surrounding of MPs which demands specific procedures for their extraction and analysis.

MPs can be divided into two groups: peripheral, such as phospholipases, and integral, such as receptors. In contrast to peripheral MPs, isolation of the integral MP requires disruption of phospholipid bilayer [5]. A unique feature of MPs is their tendency to aggregate in aqueous solutions. Thus, in order to extract, solubilise and purify integral MP, employment of detergent is required in all widely accepted methods. Molecules of detergent form micelles in aqueous medium. These clusters are organized with polar heads oriented outside and nonpolar tails inside [6]. Detergent extraction of MP is achieved by substitution of phospholipid molecules with detergent micelles, enabling interaction of hydrophobic moieties of detergent and MP.

Some detergents (depending on their nature) are also denaturing agents and harsh extraction conditions might result in protein denaturation and even loss of its function, which should be taken into consideration in functional testing. Although detergents solubilise and extract MPs, their potency is quite different. Natures of detergents and MPs determine stability of micelles and, possibly, select MPs which interact with particular detergent. Depending on experimental steps which follow protein extraction, the choice of detergent may be of great importance [6]. Considering different cell and tissue origin of MPs and diversity of detergents available, pilot experiments should be conducted prior to bulk isolation, to define

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the best detergent and detergent/protein ratio for the experimental purpose (i.e. study of targeted MP).

The importance of a preliminary experiment intended to choose optimal detergent is illustrated in this work on placental MPs. A pallet of ten detergents and β-cyclodextrin (detergent adsorber) was employed to investigate their efficiency in extracting MPs. A robust examination of the total proteome extracted was performed by electrophoresis, while glycoproteins were analysed by lectin-based protein microarray. As targeted MPs for this study, insulin and insulin-like growth factor receptors (IR, IGF1R and IGF2R) were chosen, since they are crucial for proper growth and development of foetus [7].

2. Materials and methods

2.1. Extraction of membrane proteins from placental cells

Healthy human third trimester placenta was obtained from the Clinic of Gynaecology and Obstetrics "Narodni Front", Belgrade, Serbia. The study was approved by the Institutional Review Board of the Clinic "Narodni Front" and the Ethical committee of INEP, with a given oral consent of the placental donor. Placenta was collected immediately after delivery and placed on ice. It was extensively washed in an ice cold 0.1 M phosphate buffered saline, PBS (pH 7.4) within next 2 h, the tissue was cut into pieces and subjected to homogenisation and isolation of cell membranes, according to the procedure of Masnikosa et al. [8]. The placental tissue was homogenised in a 0.25 M sucrose solution supplemented with a protease inhibitor cocktail (Sigma-Aldrich Chemie, Steinheim, Germany). After a 10 min centrifugation at 600*g*, the supernatant was centrifuged at 100,000*g* for 45 min. The resulting pellet, containing all placental cell membranes, was washed in 0.05 M Hepes buffered saline pH 7.4 (HBS) and resuspended in the same buffer. Cell membrane suspension was aliquoted into 10 mg/mL portions which were further treated with detergents (1% v/v in HBS) at 4 °C for 1 h with permanent shaking. Solubilised placental cell membrane (glyco)proteins were recovered in the supernate following centrifugation of the suspension at 100,000*g* at 4 °C for 90 min (the solubilisate). Aliquotes not used immediately were stored at −80 °C. Eleven substances were employed for the extraction of placental MPs (Table 2). They were purchased from Sigma-Aldrich (Steinheim, Germany), except Brij 35 (Merck, Schuchardt, Germany).

Concentrations of the extracted MPs were determined using BCA protein kit (Pierce Biotechnology, Rockford, IL, USA). Each extraction procedure was performed in triplicate and all samples underwent electrophoretic, immunoblotting and lectin-based protein microarray analysis.

2.2. Electrophoresis and western immunoblotting (WIB)

Extracted MPs were subjected to reducing SDS-PAGE (on 8% gels) and WIB. Proteins in gels were stained with Coomassie brilliant blue G-250 (CBB). Placental IR, IGF1R and IGF2R were detected using mouse monoclonal anti-IR (Calbiochem, Merck KGaA, Darmstadt, Germany), anti-IGF1R (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit polyclonal anti-IGF2R antibodies (Calbiochem, Merck KGaA, Darmstadt, Germany). Receptors were visualised using goat anti-mouse (BioSource International, Camarillo, CA, USA) and donkey anti-rabbit HRP-conjugated antibodies (Pierce Biotechnology, Rockford, IL, USA), chemiluminescent substrate (Pierce Biotechnology, Rockford, IL, USA) and autoradiography. Densitometric analysis of protein bands was performed using Image Master Total Lab v2.01 software (Amersham BioSciences, Buckinghamshire, UK) and the results expressed in arbitrary densitometric units.

Table 1
Overview of lectins used for the examination of glycoproteins with specific glycans.

Carbohydrate specificity	Lectin
Sialic acid	*Sambucus nigra* lectin (SNA) *Maackia amurensis* lectin I (MAL-I) *Maackia amurensis* lectin II (MAL-II)
Mannose	*Canavalia ensiformis* lectin (ConA) *Glanthus nivalis* lectin (GNL) *Lens culinaris* lectin (LCA) *Narcissus pseudonarcissus* lectin (DFL/NPL) *Hippeastrum hybrid (Amaryllis)* lectin (HHL)
N-acetylglucosamine	Wheat germ lectin (WGA) *Griffonia simplicifolia* lectin II (GSL)
Galactose	*Ricinus communis* lectin (RCA) *Phaseolus vulgaris* erythroagglutinin (PHA-E) *Phaseolus vulgaris* leucoagglutinin (PHA-L)
Fucose	*Aleuria aurantia* lectin (AAL) *Pholiota squarrosa* lectin (PhosL)

2.3. Lectin-based protein microarray

Extracted MPs (0.1 mg/mL in PBS) were spotted on the epoxy coated glass slides (Nexterion Slide E, Schott, Jena, Germany) using high-speed robotic printing platform with a non-contact piezoelectric printer sciFLEXARRAYER S1 (Scienion, Berlin, Germany), at the temperature of 14 °C and humidity of 60%. The printing was performed into 16 identical arrays on the slides. Each sample was spotted in triplicate within each array. The printed slides were incubated overnight at 4 °C. Unreacted epoxy groups were blocked with 1 M ethanolamine in PBS with 0.05% Tween 20 (PBST) at 4 °C for 1 h. The slides were washed and each array was loaded with one of the biotinylated lectins at the concentration of 12.5 μg/mL in PBST using a multiwell microarray mask. All biotinylated lectins were from Vector (Vector, Burlingame, USA) except PhosL which was a kind gift from Dr Yuka Kobayashi (J-Oil mills, Inc., Yokohama, Japan). The slides were incubated with lectins at room temperature for 1 h. The employed lectins with their carbohydrate specificity are presented in Table 1. Slides were washed again and allowed to interact with streptavidin conjugated with a fluorescent dye CF647 (Biotium, Hayward, USA, 0.5 μg/mL in PBS), at room temperature for 15 min. Slides were thoroughly washed, centrifuged to remove residual water and scanned using InnoScan® 710 fluorescent scanner (Innopsys, Carbonne, France). Fluorescent signals were analysed by Mapix® 5.5.0 software (Innopsys), background signal was subtracted and the intensities of the specific interactions were expressed in arbitrary units (a.u.).

3. Results

3.1. Extraction of placental membrane proteins

Placental MPs were extracted using ten different detergents and β-cyclodextrin. The efficiency of MP extraction varied, as can be seen from the concentrations of extracted MPs in Table 2.

Extracted proteins were separated by SDS-PAGE and stained with CBB (Fig. 1A). Extracts were used as obtained, without adjustment to the same protein concentration. Results in Fig. 1A confirmed different solubilisation efficiency of detergents, as electrophoretic separation also enabled visualisation of the major MPs in extracts. All detergents solubilised the same MPs, but not in the same ratio. Regardless of the protein concentration, detergents 2–10 solubilised similar amounts of protein "X". In extracts 6, 8 and