Journal of Chromatography B 1061–1062 (2017) 334–341

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Proteome analysis of vitreous humor in retinal detachment using two different flow-charts for protein fractionation

Leonor M. Gaspar[a,b,1], Fátima M. Santos[a,b,c,1], Tânia Albuquerque[a], João P. Castro-de-Sousa[d], Luís A. Passarinha[a,c], Cândida T. Tomaz[a,b,*]

[a] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal
[b] Chemistry Department, Faculty of Sciences, University of Beira Interior, 6201-001 Covilhã, Portugal
[c] Laboratory of Pharmacology and Toxicology – UBIMedical, University of Beira Interior, 6200-284, Covilhã, Portugal
[d] Ophthalmology Service, Leiria Hospital Center, 2410 Leiria, Portugal

ARTICLE INFO

Keywords:
Electrophoresis
Liquid chromatography
Mass spectrometry
Proteomic analysis
Retinal detachment
Vitreous humor

ABSTRACT

The deeper understanding of retinal detachment (RD) pathogenesis may improve the visual outcome after surgery. Given the main role of the vitreous in retinal eye diseases, two strategies were explored to identify its proteome in RD. Fractionation techniques such as anion exchange chromatography (IEX) and SDS-PAGE combined with MALDI-TOF/TOF analysis allowed to identify 127 proteins in vitreous of RD patients. From these proteins, 19 were identified using only the IEX fractionation strategy, and 117 using a bidimensional (IEX and SDS-PAGE) fractionation. Of these proteins, 68 had not yet been found in other vitreous proteomic studies. The fractionation with IEX and SDS-PAGE largely improved the number of identified proteins proving that it is crucial to combine several methodologies to cover vitreous proteome.

1. Introduction

The human eye is a highly organized and complex organ, responsible for visual perception [1]. It is composed by different fluids and structures that may be subjected to biochemical changes during pathological states of the eye [2]. Vitreous humor is a gel-like fluid that fills the cavity behind the lens and the retinal pigment epithelium (RPE) and helps to stabilize the eye structure. Although vitreous is remarkably stable, it may suffer biochemical, proteomic and structural changes overtime according to the physiological and pathological state of the retina. This occurrence can lead to severe ocular pathologies, such as retinal detachment (RD) [1,3].

RD is an ocular disease characterized by a retinal accumulation of fluid between the neurosensory retina and the underlying RPE [4]. It may result of liquefaction of vitreous due to the weakening of vitreoretinal adhesion, and to alterations of the structure of collagen fibers. When this occurs, vitreous falls upon itself causing the physical separation between the photoreceptor layer and the RPE of the retina, leading to severe and permanent loss of vision [4,5]. Currently, the treatment for RD is exclusively surgical but its combination with new therapeutic may improve the visual outcome after surgery [4]. The suitable management of RD is critical to allow the retinal reattachment after surgery and avoid its evolution for more severe diseases such as proliferative vitreoretinopathy (PVR), characterized by enhanced photoreceptor degeneration [4,6]. Vitreous is a suitable matrix for study RD pathogenesis because, due to its location, its proteome and biochemical properties are directly affected by physiological and pathological conditions of the retina [7]. Therefore, it is extremely crucial to explore the vitreous proteome in the rhegmatogenous RD (RRD), the less severe form of RD, in order to make advances in the earlier diagnosis, prognosis, and improvement of visual outcome of the patients after surgery [4,6].

Although the application of proteomics technology in ophthalmic research is much more frequent nowadays [8], there are only a limited number of publications about RD [6,9,10]. In 2008, Shitama and colleagues observed substantially higher expression levels of proteins, such as pigment epithelium derived factor and proapolipoprotein A1 in RD, when compared to other ocular diseases [9]. Yu and colleagues found

Abbreviations: AB, ammonium bicarbonate; ACN, acetonitrile; GO, gene ontology; IEX, Ion exchange chromatography; pI, isoelectric point; LC-ESI–MS/MS, nano-liquid chromatography–electrospray ionization mass spectrometry; MALDI-TOF-MS, matrix-assisted laser desorption ionization/time-of-flight mass spectrometry; RD, retinal detachment; RRD, rhegmatogenous retinal detachment SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis; STRAP 1.5, Software Tool for Rapid Annotation of Proteins; STRING 10, Search Tool for the Retrieval of Interacting Genes/Proteins

* Corresponding author at: CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-001 Covilhã, Portugal.
E-mail address: ctomaz@ubi.pt (C.T. Tomaz).

[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jchromb.2017.07.049
Received 14 June 2017; Received in revised form 24 July 2017; Accepted 27 July 2017
Available online 29 July 2017
1570-0232/

Table 1
Proteomic studies developed with vitreous humor matrices in several eye diseases.

Techniques	Identified proteins	Ocular pathology	Report
- Immunoaffinity depletion - SDS-PAGE - LC-MALDI-MS/MS	54 proteins	Proliferative diabetic retinopathy (PDR)	[16]
- 2D-PAGE - MALDI-TOF-MS	56 proteins	Diabetic retinopathy	[13]
- SDS-PAGE - LC-ESI–MS/MS	252 proteins	Proliferative diabetic retinopathy (PDR)	[14]
- IEF - SDS-PAGE - LC-ESI–MS/MS	1111 proteins	Epiretinal gliosis	[15]
- Affinity depletion - SDS-PAGE - LC–MS/MS	1205 proteins	Vitreoretinopathy	[12]
- 2D-PAGE - MALDI-TOF-MS/MS	38 proteins	Proliferative diabetic retinopathy (PDR)	[11]
- LC–MS/MS	2482 proteins	Proliferative diabetic retinopathy (PDR)	[17]
- iTRAQ - ESI–MS/MS	431 proteins	Retinoblastoma	[18]

Electrospray Ionization with tandem Mass Spectrometry (ESI–MS/MS); Isobaric Tag For Relative And Absolute Quantitation (iTRAQ); Isoelectric Focusing (IEF); Liquid Chromatography coupled to Electrospray Ionization with tandem Mass Spectrometry (LC-ESI–MS/MS); Liquid Chromatography coupled to Matrix-Assisted Laser Desorption/Ionization with tandem Mass Spectrometry (LC-MALDI-MS/MS); Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS); Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF-MS) and tandem Mass Spectrometry (MALDI-TOF-MS/MS); Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE); Two-Dimensional Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (2D-PAGE).

516 proteins in vitreous of RRD patients with PVR using SDS-PAGE and reversed-phase liquid chromatography tandem mass spectrometry [10]. More recently, Wu and co-workers identified 750 proteins by iTRAQ-mass spectrometry, from which 103 were found differentially expressed in RD with choroidal detachment when compared with RD [6].

Many researchers [11–18] have contributed to the enrichment of our knowledge about human vitreous proteome in several ocular pathologies, using different techniques, as displayed in Table 1. The overview of proteomic strategies applied in the last years emphasize that no individual technology can cover completely the vitreous proteome. So, as the diversity and complexity of the human vitreous proteome is overwhelming, it is important to take into consideration all reported studies to know the entire proteome and understand its change in several pathologic environment [19].

The present study aims to characterize the vitreous proteome of patients with RRD, which is the most common type of RD and is characterized by a full thickness retinal break [4]. For this purpose, vitreous samples from RRD patients, with ages comprised between 26 and 82 years old, were pooled together and analyzed using two different approaches, with and without SDS-PAGE, according to the experimental workflow represented in Fig. 1. The first approach included protein fractionation through ion-exchange chromatography (IEX) and identification of the target fractions by MALDI-TOF/TOF. Alternatively, in the second approach, a SDS-PAGE step was introduced after the IEX to improve the fractionation level of vitreous proteins before its identification by MALDI-TOF/TOF.

2. Materials and methods

2.1. Vitreous sample collection

All vitreous samples were obtained via pars plana vitrectomy at Ophthalmology service of Leiria Hospital Center (Portugal). This study was conducted according to the principles of the Declaration of Helsinki. The sample collection protocol was approved by the hospital ethics committee and an informed consent was obtained from all patients. Characteristics of patients, including age at presentation, sex and disease classification are summarized in Table 2. Vitreous samples contaminated with plasma and associated with other diseases were excluded. Samples from patients subjected to previous intraocular surgeries (including vitrectomy, glaucoma surgery and laser coagulation), intravitreal drug treatments or with other vitreoretinal diseases were also excluded. Vitreous samples from 25 patients with RRD were selected, with ages ranging between 26 and 82. Undiluted samples (500–1000 µL) were collected in sterilized tubes during surgical procedure, placed on ice immediately and stored at −80 °C until further processing.

2.2. Sample preparation

Vitreous samples were centrifuged at 18 620 × *g* for approximately 10 min at 4 °C, to separate the structural component from the soluble phase. Total protein concentration was measured by Pierce™ BCA Protein Assay Kit (Thermo Scientifics, USA) according to the kit manufacturer's protocol, using BSA as standard and calibration control samples (125–8000 µg/mL). In order to exclude samples with plasma contamination, hemoglobin levels were measured using Hemoglobin Colorimetric Assay Kit (Cayman Chemical, Michigan, USA), following the kit manufacturer's protocol. Then highly abundant proteins, such as human serum albumin and IgG were depleted from vitreous using a HiTrap™ Albumin & IgG Depletion 1 mL column (GE Healthcare, Uppsala, Sweden). Briefly, the depletion was performed at room temperature in an ÄKTA Pure system with UNICORN 6 software (GE Healthcare, Uppsala, Sweden) equipped with a 2 mL injection loop. All buffers pumped in the system were prepared with Mili-Q system water, filtered through a 0.20 µm pore size membrane (Schleicher Schuell, Dassel, Germany) and degassed ultrasonically. Vitreous samples (0.5–4 mg) were loaded in column, previously equilibrated with 20 mM sodium phosphate, 0.15 M NaCl at pH 7.4, at a flow rate of 1 mL/min. After the elution of low abundant proteins, the mobile phase conditions were changed to 0.1 M glycine buffer at pH 2.7 to allow the elution of highly abundant proteins. In all chromatographic runs, conductivity, pH, and absorbance, at 280 and 214 nm, were continuously monitored. The collected samples were concentrated and desalted using Vivapsin 6 with a 3.000 MW cut-off, and stored at −20 °C. Depleted samples were pooled together and concentrated to a volume of 2 mL before fractionation by IEX.

2.3. Protein fractionation by ion-exchange chromatography

Ion-exchange chromatography was performed at room temperature in an ÄKTA Pure, equipped with a 500 µL injection loop. Proteins were fractionated according to their isoelectric point (pI) using a HiScreen™ Capto™ Q 4.7 mL column (GE Healthcare, Uppsala, Sweden), containing a strong anion exchanger quaternary ammonium coupled to Sepharose (Q-Sepharose®). Buffer A was prepared by combining 20 mM L-histidine, 20 mM ethanolamine, 20 mM di-ethanolamine, 20 mM tri-ethanolamine, and 20 mM Tris and adjusting the pH to 10 using sodium hydroxide. Buffer B was prepared likewise but by adding 5 mM sodium chloride and adjusting the pH to 5 using chloridric acid. Detailed description of buffers is presented in Supplementary Table S1. Buffers were prepared with Mili-Q system water, filtered through a 0.20 µm pore size membrane (Schleicher Schuell, Dassel, Germany) and degassed ultrasonically. Samples (2–4 mg) were injected onto the column, previously equilibrated with buffer A, at a flow rate of 1.5 mL/min. In order to promote a gradual elution of adsorbed proteins, a linear pH gradient until 100% of buffer B was applied during 15–20 CV, at the same flow rate. The absorbance was continuously monitored at 280 nm, as well as conductivity and pH. Fractions of 1.8 mL were collected and evaporated to a volume near 200 µL, at room temperature, using a