European Journal of Pharmaceutical Sciences 57 (2014) 34–40

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Identification of human cerebrospinal fluid proteins and their distribution in an in vitro microdialysis sampling system

Magnus Wetterhall [a], Jonas Bergquist [a], Lars Hillered [b], Klas Hjort [c], Andreas P. Dahlin [c,*]

[a] Analytical Chemistry, Department of Chemistry – BMC and SciLifeLab, Uppsala University, PO Box 599, SE-751 24 Uppsala, Sweden
[b] Department of Neuroscience, Neurosurgery, Uppsala University, Uppsala University Hospital, SE-751 85 Uppsala, Sweden
[c] Department of Engineering Sciences, Uppsala University, PO Box 534, SE-751 21 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 8 September 2013
Accepted 7 December 2013
Available online 20 December 2013

Keywords:
Protein adsorption
Surface analysis
on-Surface enzymatic digestion (oSED)
Microdialysis (MD)
Cerebrospinal fluid (CSF)
Mass spectrometry (MS)

ABSTRACT

A qualitative study is presented on how proteins from a complex biological sample are distributed in a microdialysis sample system. A comparison between proteins identified in the human ventricular cerebrospinal fluid (CSF) sample, the collected dialysate and the proteins adsorbed onto the membrane was conducted. The microdialysis experiment was performed in vitro at 37 °C for the duration of 24 h. Thereafter, the membranes were removed from the catheter and the adsorbed proteins were tryptically digested using the on-surface enzymatic digestion (oSED) protocol. The CSF samples and the dialysates were digested using a standard in-solution trypsin digestion protocol. In the final phase, the samples were analysed using nano-liquid chromatography in combination with tandem mass spectrometry. In the four sample compartments analysed (CSF start, Membrane, Dialysate, CSF end) a total of 134 different proteins were found. However, most of the identified proteins ($n = 87$) were uniquely found in one sample compartment only. Common CSF proteins such as albumin, apolipoproteins and cystatin C together with plasma proteins such as hemoglobin and fibrinogen were among the 11 proteins that were found in all samples. These proteins are present in high concentrations in CSF, which means that they effectively block out the detection signal of less abundant proteins. Therefore, only 25% of the proteins adsorbed onto the membrane were detected in the CSF compared with the dialysate that shared 44% of its proteins with the CSF. The proteins adsorbed onto the membrane were significantly more hydrophobic, had a lower instability index and more thermostable compared to the proteins in the CSF and the dialysate. The results suggest that proteins adsorbed onto the microdialysis membranes may escape detection because they are prevented from passing the membrane into the dialysate. Thus, the membrane needs to be examined after sample collection in order to better verify the protein content in the original sample. This is particularly important when searching for new protein biomarkers for neurodegenerative diseases.

1. Introduction

Microdialysis is an in vivo sampling technique where a hollow fiber is immersed or implanted into biological matrices of interest, such as animal models or humans, from which a dialysate is collected by diffusive transfer over the membrane. Ideally, the inserted microdialysis probe should be well tolerated with minimal tissue reactions (e.g. biofouling), the fluid recovery (FR) should be close to 100%, i.e. all the fluid in the perfusate of the microdialysis probe should be recovered in the collected dialysate. Finally, the dialysate obtained from microdialysis sampling should reflect the microenvironment in which the membrane is placed. Microdialysis is one of the few real time sample collection methods and is widely used in clinical neurosurgery today (Hillered et al., 2005, 2006). This approach allows continuous monitoring of complex neurochemical processes in the neurointensive care setting (Oddo et al., 2012) and the recent availability of high molecular cut-off membranes for clinical use has opened a new field of research on brain injury protein biomarkers (Helmy et al., 2011a). However, the wide diversity in the physical, chemical and biological properties of proteins poses several challenges to the method of sampling proteins using microdialysis; resulting in low protein extraction efficiency, reproducibility and repeatability (Schutte et al., 2004; Helmy et al., 2009). For example, since the sampling method is dependent on the diffusivity of an analyte, according to Stoke–Einstein's equation larger size and weight of a protein reduces the diffusion velocity, which in turn resulting in decreased extraction efficiency (Wang and Stenken, 2006). The sampling of proteins also requires larger membrane pore sizes (>100 kDa) which makes the

* Corresponding author. Tel.: +46 18 4711086; fax: +46 18 4713572.
E-mail address: andreas.dahlin@angstrom.uu.se (A.P. Dahlin).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.12.011

microdialysis fluid balance (FR) more sensitive to pressure changes, a common phenomenon in the injured brain, requiring use of colloid osmotic agents in the perfusate and surface modifications (Dahlin et al., 2010; Helmy et al., 2011b, Supplemental Fig. 2). Finally, biological samples often contain immense numbers of different proteins present in low amounts that inherently may vary by orders of magnitude in protein concentration. This is especially true for cerebrospinal fluid (CSF) (Schutzer et al., 2010).

CSF is a transparent fluid that is in equilibrium with the brain and spinal cord since it surrounds the central nervous system (CNS). CSF is composed of 99% water with few cells (0–4 cells/μl), low protein concentration (0.05–0.8 μg/μl) and salt concentration similar to blood (Huhmer et al., 2006). It is still a very complex sample matrix with regard to the vast number of proteins present in a dynamic range spanning at least 12 orders of magnitude (Rozek et al., 2007). CSF reflects the activity of the chemical transport systems of the CNS and its dynamic neurochemistry therefore has great clinical and basic scientific significance.

As always when an artificial material is inserted into a biologically active system such as CSF, a response will occur. Protein adsorption is considered to be the first step in the acute biological response to foreign material. This response initiates a cascade of processes which eventually lead to biofouling and finally encapsulation (Anderson et al., 2008). The protein adsorption mechanism is highly complex, dynamic and is dependent on the properties of the proteins, solid surface and the surrounding liquid. The adsorption process has mostly been studied in systems with one, two or three proteins, and is then fairly well understood (Rabe et al., 2011). However, when more complex protein samples are used, such as CSF or blood plasma, the adsorption mechanism is still unclear and different theories are lively debated in the literature (Vogler, 2012).

To study proteins adsorbed to surfaces is dictated by the common analytical chemistry compromise, where large sample volumes or amounts are needed in order to detect proteins of low concentrations. The field of protein adsorption analysis is a frequent topic for review articles (Nakanishi et al., 2001; Gray, 2004; Rabe et al., 2011). A vast number of different detection techniques, including radiolabelling, immunoassays, spectroscopy and mass spectrometry (MS) have been used to determine protein-surface interactions and structurally characterize the surface during biofouling.

The use of MS for these studies is very promising, since it is a versatile and sensitive detection method well suited for the analysis of peptides and proteins in complex biological samples. The technique can readily be used to simultaneously identify and quantify a vast number of proteins, or it can be adjusted to monitor a particular protein of interest (Oleschuk et al., 2000; Michel and Castner, 2006). Recently, our group presented the on-surface enzymatic digestion (oSED) protocol to determine protein adsorption on microdialysis membranes (Dahlin et al., 2012). The oSED method can be regarded as the opposite of immobilized trypsin digestion protocol (Monzo et al., 2009), where the proteins are immobilized (adsorbed to the surface) and the enzyme (trypsin) is in solution. Proteins are reduced, alkylated and enzymatically cleaved while still adsorbed to the membrane surfaces, rendering tryptic peptides that are desorbed from the surface and its vicinity into aqueous solution. Thereafter, the identification of the proteins in the samples is performed by nanoliquid chromatography (nanoLC) in combination with tandem mass spectrometry (MS/MS).

In this study, a qualitative protein analysis was performed to determine how the protein composition differs between the starting CSF sample, the proteins adsorbed to the membrane, the dialysate and the CSF sample after 24 h of in vitro microdialysis. The questions we sought to answer were: How similar are the protein contents in the CSF sample and the dialysate? What kinds of proteins are lost due to adsorption onto the Polyarylethersulphone (PAES) microdialysis membrane?

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN) and acetic acid (HAc) were obtained from Merck (Darmstadt, Germany). Iodoacetamide (IAA), urea, ammonium hydrogen carbonate (NH_4HCO_3), dithiothreitol (DTT) and trifluoroacetic acid (TFA) were obtained from Sigma Aldrich (St. Louis, MO, USA). Trypsin was of sequence-grade from Roche Diagnostics (Basel, Switzerland). Dextran T250 (Molecular weight 250 kDa, batch No. HE104) was purchased from Pharmacosmos (Holbaek, Denmark). Perfusion fluid composed of aqueous solution of 147 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$ and 0.85 mM $MgCl_2$, was obtained from CMA Microdialysis (Solna, Sweden). Ultrapure water was produced by a Milli-Q+system, Millipore Corp. (Marlborough, MA, USA).

2.2. Cerebrospinal fluid collection

A pool CSF from 10 patients (4 males, 6 females) was obtained by mixing 1 ml of each CSF sample. Before pooling, the samples were centrifuged at 2000 rpm for 10 min. Permission to use human CSF has been granted by the Regional Ethics Committee (Dnr UPS 02-536, 2003-11-25; Dnr 2004; M-228). Samples were frozen and stored at −80 °C immediately after sample acquisition.

2.3. Microdialysis

A syringe pump, PHD 2000, (Harvard Apparatus, Holliston, MA) Programmable was used to deliver a continuous flow of 0.3 μl/min of perfusate, to the CMA 71 microdialysis catheters. Two catheters, 10 mm membrane length, 100,000 Da nominal molecular weight cut-off, Polyarylethersulphone (PAES) – membranes, were used in the study. The sampling was performed during 24 h in 10 ml human CSF held at 37 °C during continuous stirring, resulting in two 432 μl dialysate fractions, one for each probe. After the sample collection, the microdialysis catheters were washed externally and internally by placing them in Milli-Q-water and rinsing with water at a flow rate of 15 μl/min in 5 min followed by 1 μl/min during 25 min. Afterwards, the membranes were removed from the catheters, transferred to 0.5 ml protein low binding vials (Eppendorf, Hamburg, Germany) and dried using a Speedvac system ISS110 (Savant Holbrook, N.Y., USA).

In total, 8 samples were generated, two CSF start samples, two membrane samples, two dialysate samples and two CSF samples taken after the microdialysis experiment.

2.4. Tryptic digestion and sample desalting for protein identification

Protein identification by MS was performed on 300 μl aliquots of CSF collected before and after microdialysis, 300 μl of the collected fractions of the microdialysates and the washed and dried microdialysis membranes. The liquid samples were first dried under vacuum. All samples were redissolved in 200 μl 0.1 M NH_4HCO_3. The disulfide bridges in the proteins were reduced by adding 10 μl of 45 mM DTT and incubating for 15 min at 50 °C. This was followed by alkylation of the cysteines by adding 10 μl of 100 mM IAA and keeping the samples at room temperature for 15 min in darkness. The samples were enzymatically digested by adding 3 μg trypsin to the CSF samples and 1 μg trypsin to the microdialysates and membrane samples. The tryptic digestion was carried out over night at 37 °C. After tryptic digestion, the