Analysis of Proteins Bound to Contact Lenses

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Abstract

The purpose of this study was to determine whether there are any proteins that attach to contact lenses that can be removed by cleaning solutions and if so, whether it is possible to quantify them by Bradford assay and identify them by nanoliquid chromatography-tandem mass spectrometry (nanoLC-MS/MS). Our results indicate that the contact lenses indeed contain equal amounts of proteins, as determined by quantitative Bradford assay. NanoLC-MS/MS analysis led to the identification of several proteins, including lipocalin-1, lysozyme and tryptophan 5-hydroxylase. The significance of our findings is discussed.

Keywords: Biochemistry; Mass spectrometry; Proteomics

Introduction

A major problem for people who wear contact lenses is the accumulation of debris on the lenses, such as proteins. This can often lead to discomfort or damage of the lenses. According to one study there is a significant amount of protein accumulation on contact lenses after extended wear [1]. Proteins found to accumulate on lenses are albumin, PMFA, IgG, IgA, lactoferrin and subunits of protein G in all samples [2]. These proteins are known as "tear film proteins" and they are found within the aqueous layer of the eye. The main functions of these proteins are to cleave cell wall constituents to kill bacteria (lysozymes), scavenge lipids from the cornea surface (tear lipocalin), and to inhibit microbe action by competing with them for iron (lactoferrin) [3]. However, when these proteins accumulate extensively on the lenses, people begin to feel more discomfort [2].

The purpose of this study was to determine whether there are proteins on the contact lenses and if so, to also determine the relative concentration of these proteins in the contact lens cleaning solution using the Bradford assay [4]. We also sought to identify the most abundant proteins on the contact lenses using nanoLC-MS/MS. NanoLC-MS/MS is a mass spectrometry-based method known to determine the amino acid sequence of peptides and proteins [5-16]. We found that when we use different cleaning solutions, the concentration of proteins removed from the contact lenses is very similar. NanoLC-MS/MS analysis led to identification of several proteins, including lipocalin-1, lysozyme, tryptophan 5-hydroxylase and human keratins. The significance of this study is discussed.

Methods

Materials

Three types of contact lenses solutions were used (solution C, solution E and solution B) to clean two different types of contact lenses (K and L). The resulting letter combination shown in Table 1 (i.e. LC, LE, LB) represent contact lenses L washed with solution C (LC), E (LE) or B (LB). The initial volume of pooled samples were measured (Table 1), dried using a SpeedVac concentrator and then re-suspended in 500 μL of water.

Protein Quantification was performed according to Bradford method [4].

Protein fractionation and identification

SDS-PAGE was performed according to Sokolowska I, et al. [17,18]. For LC-MS/MS analysis and protein identification, the gels were stained with Coomassie Blue dye. The protein bands that were observed on the gel were excised and digested by trypsin according to Petrareanu C, et al. [19-21]. The peptides mixtures were then extracted, concentrated and then analyzed by nanoLC-MS/MS, according to Roy U, Sokolowska I, et al. [19-22]. The MS raw data files were processed by Protein Lynx Global Server (PLGS 2.4, Waters Corp) and then the resulting pkl files were submitted to Mascot database search. The PLGS processing parameters and database search parameters are described in Petrareanu C, et al. [19-21]. The MS/MS spectra that led to identification of proteins were verified in the raw files by Mass Lynx software (version 4.1; Waters Corporation).

Results and Discussion

Protein quantification

Initially, we determined the concentrations of proteins present in each solution sample using the Bradford assay. Since each sample had different volumes, the total amount of protein in the sample was determined (Table 1). As observed, the highest amount of protein was detected in sample C (LC and KC), with an average of 1.26 mg protein, followed by sample E (LE and KE), with an average of 0.579 mg protein, and sample B (LB and KB), with an average of 0.439 mg protein. However, when protein concentration was investigated, no great difference was observed (Table 1) and the protein concentration ranged from 0.020-0.032 mg/mL in all samples. These data suggest that regardless of the solution used, the protein concentration in the solution that was used to clean the lenses is similar and consistent.

Protein fractionation and identification: SDS-PAGE and nanoLC-MS/MS analysis

To verify whether we can identify proteins in our samples, we

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Sample	Volume (ml)	Absorbance at 595 nm	Protein Concentration (mg/ml)	Total protein present in sample (mg)
LC	46	0.032	0.032543003	1.49697815
LE	23	0.028	0.030993336	0.712846738
LB	15	0.002	0.020920502	0.313807531
KC	44	0.008	0.023245002	1.022780102
KE	16	0.02	0.027894003	0.446304045
KB	18	0.029	0.031380753	0.564853556

Table 1: Volume, absorbance, protein concentration and total protein content of each sample.
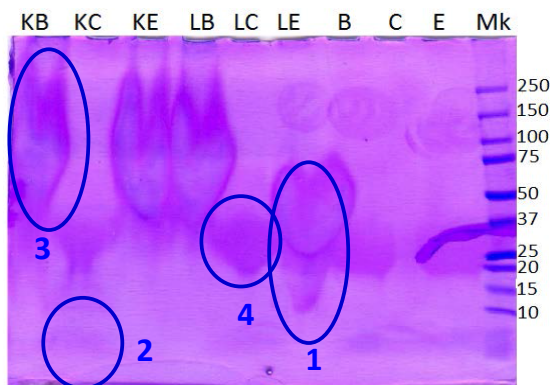
Figure 1: Coomassie blue stained SDS-PAGE gel. The washing solutions that were used to clean contact lenses were separated by SDS-PAGE and stained by Coomassie blue. Samples B, C and E were separated as samples set 1 (KB, KC & KE) or as samples L (LB, LC, & LE), or as protein-free samples (B, C & E). The gel with the bands that were cut shown (right)
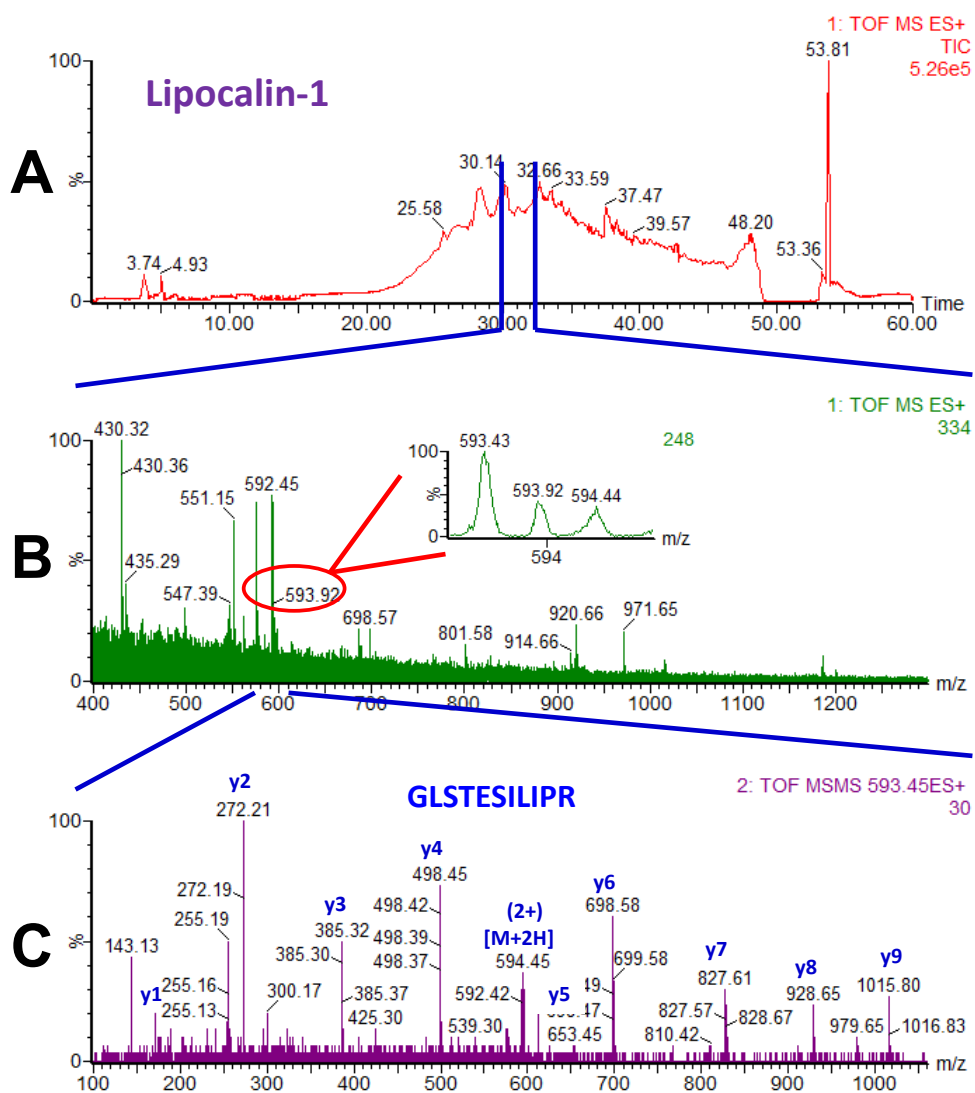

Figure 2: NanoLC-MS/MS analysis. (A) Total Ion Chromatogram (TIC) of a 60 minutes HPLC gradient. (B) At elution time point ~31 minutes, a MS spectrum was recorded. One of the doubly charged peaks (circled) with m/z of 593.43 (insert), was selected for fragmentation and produced a MS/MS spectrum (C). Analysis of the fragment b and y ions led to identification of the peptide GLSTESILIPR, which is part of human lipocalin-1 protein.

fractionated the samples by SDS-PAGE, stained the gel by Coomassie blue staining, and excised the gel pieces labeled 1-4, digested them by trypsin and subjected the resulting peptides mixtures to nanoLC-MS/MS analysis. We chose those bands based on the intensity of the protein bands observed on the gel (all bands were very weakly stained). The SDS-PAGE is shown in Figure 1 (Figure 1).

NanoLC-MS/MS analysis of the peptide mixtures from the gel bands #1-4 led to identification of several proteins, including lipocalin-1, lysozyme and tryptophan 5-hydroxylase. In addition, human keratin was also identified. Identification of lipocalin-1 and lysozyme is significant as they are either found in the eye or on the skin. Lipocalin and lysozymes are both part of the immune response and are found to be in the tear film of the eye. Keratin and tryptophan 5-hydroxylase are both found in the skin and could have been caused by touching the lenses to put them into the eye or possibly from being in contact with the sin or eyelashes near the eye. While it is known that lipocalin-1 and lysozyme are present in the tears, identification of keratins was also a good indication that trypsin digestion was successful, reason for which many times keratin is used as a positive control. The Total Ion Chromatogram (TIC) and the MS and MS/MS spectra that corresponded to a peptide which was part of lipocalin-1 are shown in Figure 2 (Figure 2).

Conclusion

In conclusion, contact lenses contain proteins that are removed by the cleaning solutions. NanoLC-MS/MS analysis led to identification of both the positive control proteins (keratins) and additional proteins, known to be in the tears (i.e. Lipocalin-1) or hands/gloves (keratins). Overall, the project was successful in both protein identification and quantification.

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