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Profiling Of Clinically Relevant Proteoforms In Human Tears Using Chip-Based Capillary Electrophoresis Coupled To Mass Spectrometry

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ABSTRACT

Purpose: Demonstrate the potential for profiling in very short times proteoforms in tears using a microfluidic chip capable of highly efficient electrokinetically driven separations hyphenated to mass spectrometry.

Methods: Tear proteins extracted from Schirmer tips (Clement Clarke International, Harlow UK) in an aqueous solution are directly analyzed by capillary electrophoresis (CE) via ZipChip™ source interface (908 Devices Inc., Boston, MA) coupled to mass spectrometry in a Thermo Scientific™ Q Exactive™ HF system without further sample processing.

Results: We demonstrate that very highly reproducible electropherograms can be generated from the very small protein amounts present in the few microliters of tear collected on one Schirmer strip. The peaks in the electropherograms represent over 60 proteoforms in the tear sample of one single donor.

INTRODUCTION

Tears maintain the health of the front of the eye and provide clear vision. Dry eye syndrome is defined as the low volume or low quality production of tears to sufficiently lubricate and nurture the eye, and affects up to 20% of the population above age 60. Currently, the presence of dry eye is determined either by subject self-reported history or by measuring tear flow using Schirmer strips. Here, we explore the use of top-down proteomics as a viable biomarker detection strategy for various eye related conditions / diseases. We propose the combination of Schirmer strips for sample collection, protein extraction, and CE-MS/MS as an accurate and sensitive way to identify proteoform biomarkers in tear fluid for clinical research.

MATERIALS AND METHODS

Sample Preparation

Protein standards to evaluate protein separations in the microfluidic device were purchased from Thermo Fisher Scientific. Tears were purchased from Lee Biosolutions and Schirmer Tear Test strips were purchased from Clement Clarke International.
ESI-MS was performed on a Q Exactive HF hybrid quadrupole-Orbitrap™ instrument. Seven proteins were selected that 1) evenly covered a MW range of 12kD – 66kD, 2) presented mostly clean, modification and adduct-free ESI spectra, and 3) whose ESI charge state distributions covered a wide m/z range from 500-2000.

Test Method(s)

Samples were introduced via autosampler into the microchip attached to a Q Exactive HF MS. Seven proteins were selected that 1) evenly covered a MW range of 12kD – 66kD, 2) presented mostly clean, modification and adduct-free ESI spectra, and 3) whose ESI charge state distributions covered a wide m/z range from 500-2000. .

Data Analysis

Raw files were deconvoluted using Thermo Scientific™ BioPharma Finder™ mass informatics platform and deconvoluted masses were matched to a database populated with tear proteoforms detected using top-down proteomics applying high resolution high mass measurement accuracy LC-MS/MS-based approaches.

PERFORMANCE EVALUATION OF INTACT PROTEIN SEPARATION

FIGURE 1: (A) ZipChip source interface that mounts onto mass spectrometer, and autosampler for automatic injections. (B) Picture of ZipChip device. (C) Picture of chip microfluidic architecture integrating capillary electrophoresis and electrospray mass spectrometry. (D) Picture of electrospray plume illuminated with green laser.

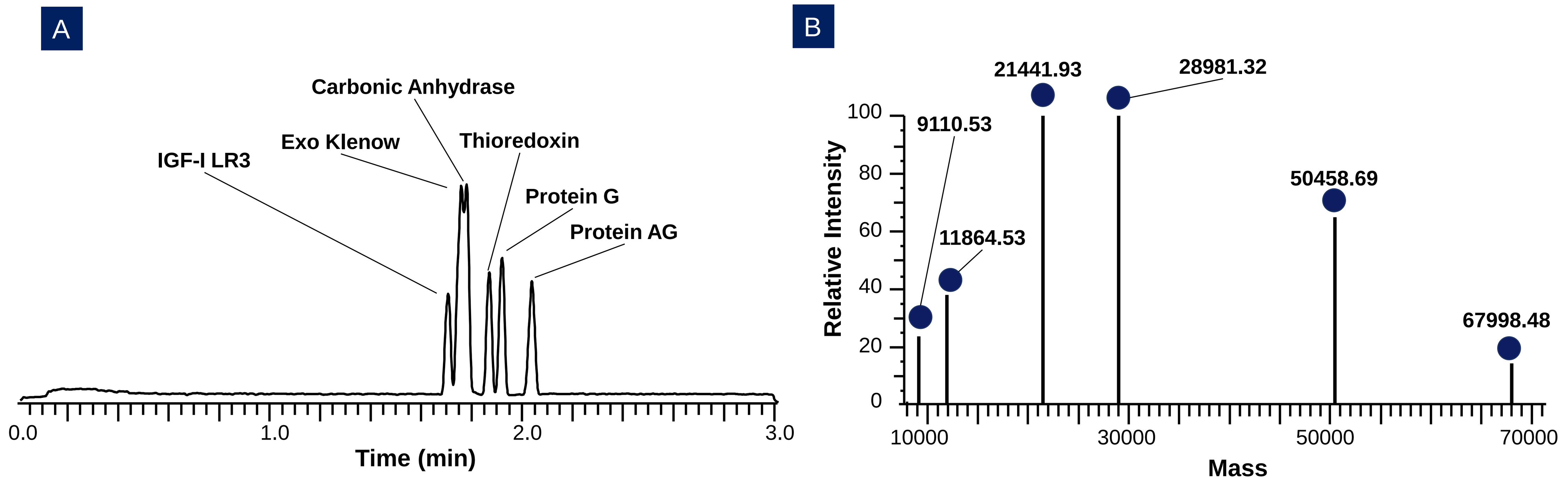

FIGURE 2: (A) Electropherogram of the Thermo Scientific™ Pierce™ Intact Protein Standard Mix in a 3 min analysis. (B) Deconvoluted masses using Biopharma Finder software for each of the six proteins using sliding window deconvolution feature.

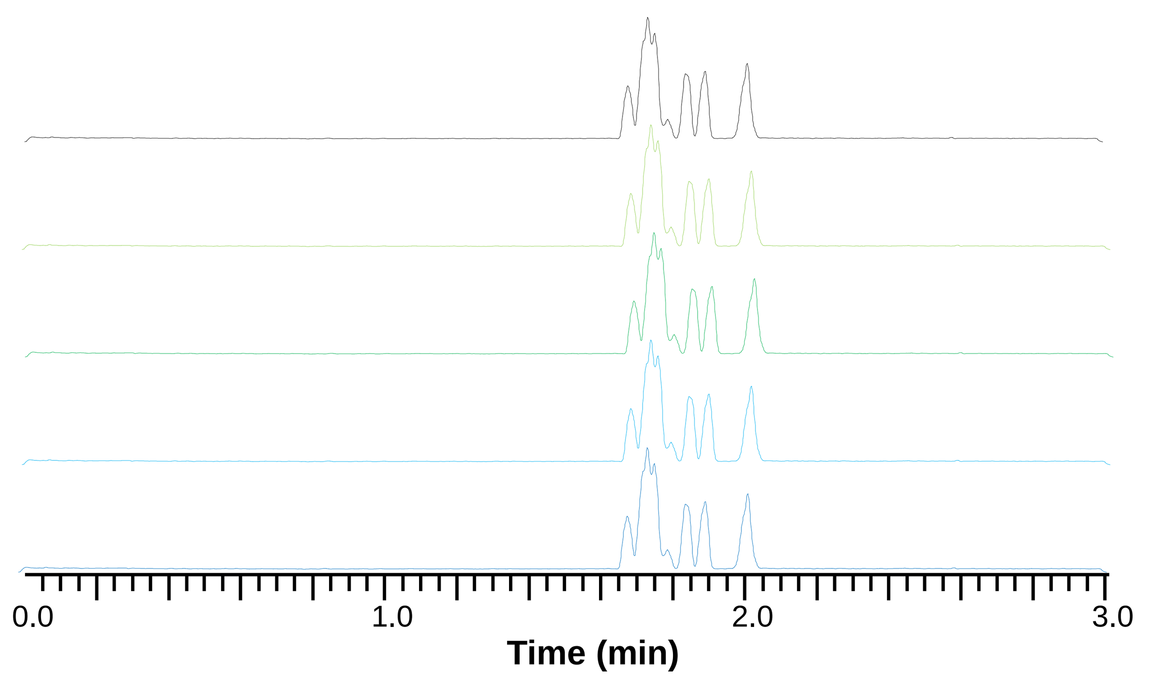

FIGURE 3: (A) Five representative electropherograms from 10 replicate injections of the Pierce Intact Protein Standard Mix.

Protein Accession	Time	CV	Intensity	CV
P05019	1.68	1%	1.83E9	25%
Q99757	1.83	0%	2.5E7	16%
P00921	1.88	0%	2.4E9	18%
P02976	1.75	0%	3.4E9	10%
P0921	1.90	0%	3.3E9	24%
P00582	2.02	0%	1E9	39%

TABLE 1: Table summarizing the migration time and raw intensity variation for each of the proteins

PERFORMANCE EVALUATION OF EXTRACTION AND ANALYSIS OF TEAR PROTEINS FROM SCHRIMER STRIPS

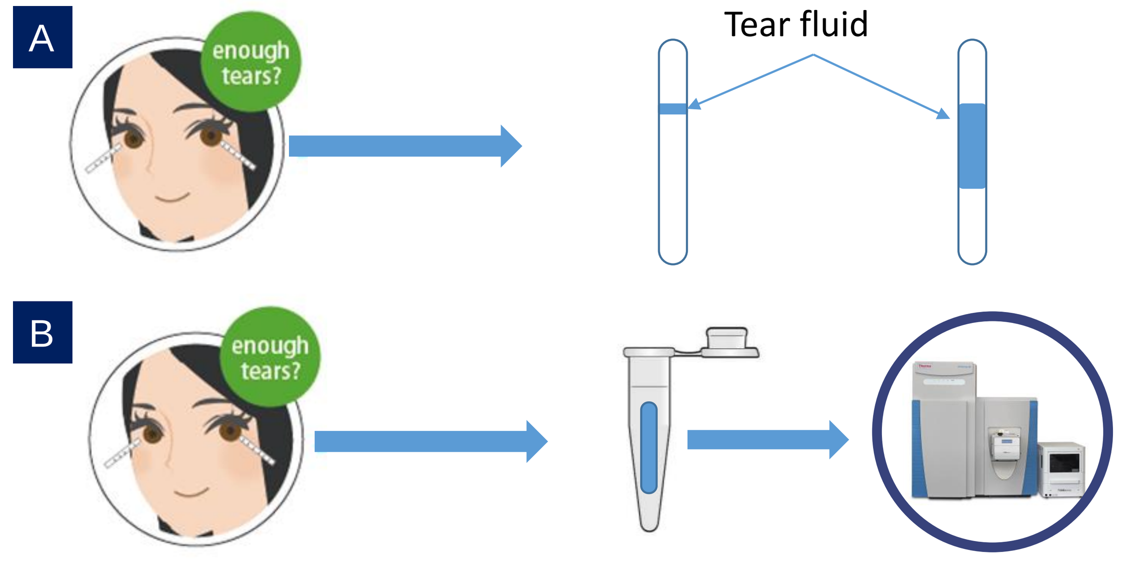

FIGURE 4: (A) Current strategy for extraction and study of tears. (B) Proposed strategy using water:acetonitrile extraction and analysis using ZipChip-Orbitrap MS.

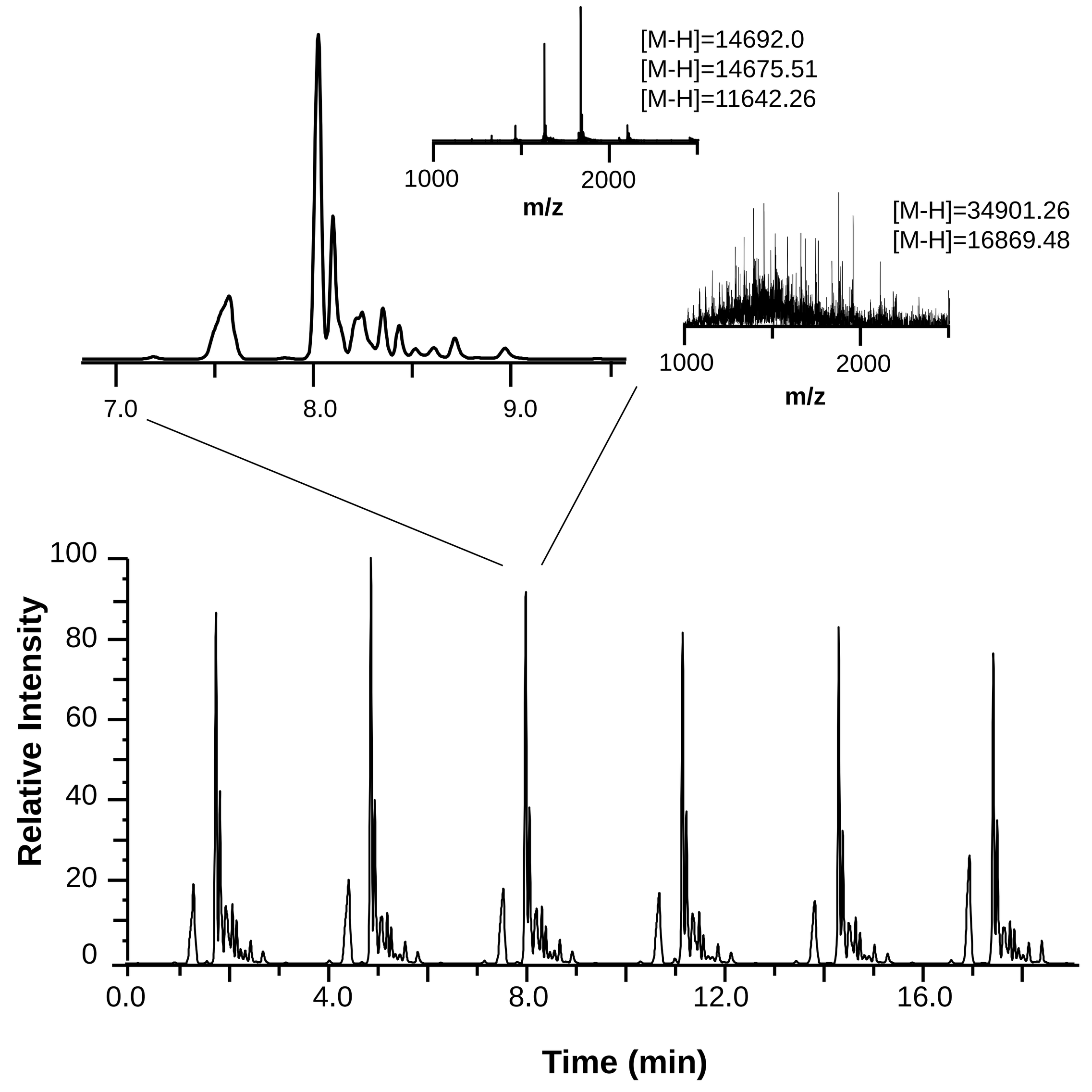

FIGURE 5: Six consecutive replicate injections from 5 µL of human tears as starting material. The data were collected on a Q Exactive HF MS. The inset shows a detailed electrophenogram, as well as, some representative full scan mass spectra and the deconvolution results from Thermo Scientific™ Protein Deconvolution™ 3.0 software.

UNIPROT	NAME	FUNCTION
O75368	SH3 domain binding glutamic acid-rich protein like	Related to glutaredoxins and expressed in retina
P01037	Cystatin SN	Cysteine proteinase inhibitor
P05109	S100 calcium binding protein A8	Proinflammatory proteins. They can have antimicrobial activity
P06702	S100 calcium binding protein A9	Proinflammatory proteins. They can have antimicrobial activity
P12273	Prolactin-induced protein	Potent antiangiogenic, proapoptotic effects.
P31025	Lipocalin 1-like 1; lipocalin 1 (tear prealbumin)	Primary lipid binding protein in tears and is overproduced in response to multiple stimuli including infection and stress.
Q9GZZ8	Lacritin	Modulates secretion by lacrimal acinar cells.
Q9Y5E8	Protocadherin beta 15	Plays a crucial role in the development of the retina
P01009-1	Serpina1	Help control several types of chemical reactions by blocking (inhibiting) the activity of certain enzymes
Q02505-1	Mucin-3a	Maintaining the homeostasis of the wet ocular surface

TABLE 1: Table summarizing some of the most relevant proteins in eye health, proteoforms of which are monitored by our ZipChip-Orbitrap MS platform.

CONCLUSIONS

Capillary electrophoresis coupled to mass spectrometry using microfluidic devices represents a very easy and versatile combination for rapid screening of potential disease biomarkers.

- CE-MS is very reproducible and sensitive for intact (top-down) protein clinical research methods.
- Combining ZipChip and Q Exactive HF instrumentation allows for extremely sensitive clinical research methods using minute amounts of sample.
- ZipChip MS allows for robust profiling and monitoring of several tens of proteoforms in tears in barely 5 min and opens up this technology for single donor tear analysis in ophthalmological applications.

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REFERENCES

1. Erin A. Redman, J. Scott Mellors, Jason A. Starkey, and Michael Ramsey, "Characterization of Intact Antibody Drug Conjugate Variants Using Microfluidic Capillary Electrophoresis – Mass Spectrometry" Anal. Chem. 88, 2220-2226

TRADEMARKS/LICENSING