PERFORM FASTER PROTEOMICS with ProtE

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INTRODUCTION

There is a interest in developing the multidimensional micro-devices. The main target is to reduce time of analysis, amount of sample, cost and to integrate to µTAS. These goals are applicable for various separation methods in proteomics, e.g multidimensional liquid chromatography, capillary- and slab gel electrophoresis.

In the present paper we describe results obtained using a **miniaturized ProtE instrument** and its comparison to the mini-PROTEAN 3-cell (Bio-Rad). Our device is able to perform sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) in less than **11 min**. The device consists of a loading compartment and a plate with a gel chamber (**11 x 15 x 0.37 mm**). Our preliminary experiments show that ProtE is able to separate even complex mixtures of protein viz. cell lysates, and the approx. detection limit of ProtE being **2 - 3 ng**. It is very easily applicable into other proteomics methods; such as immunoblotting, PMF-MS or protein sequencing.

MATERIALS and METHODS

Material: polymethyl methacrylate
silicon coated with SiO_2
Gel Chamber Size: 11 x 15 x 0.37 mm
Gel Composition: 68 µl of resolving gel
(10%, 12% or 15% PAA) + 4% PAA
Buffer: 1xTGS
Sample Buffer: Laemmli sample buffer
Sample Volume: from 0.1 µl to 1 µl
PMF: MALDI-ToF-MS and NCBI database

Table 1: The conditions used during proteomic analysis for comparing mini-PROTEAN or ProtE instrument.

		Mini-PROTEAN 3-cell		ProtE	
method		*time*	*size/ volume*	*time*	*size/ volume*
PAGE	gel	~10 min/50V ~1.5 h/150 V	100 x 75 x 0.75 mm	~11 min/40 V	11 x 15 x 0.37 mm
Blotting	PVDF	~1h/45 mA		~20 min/100 V	
Immunostainning	blocking	~1h	50 ml	~1h	10 ml
	1st antibody	~1h	1 ml	~1h	200 µl
	2nd antibody	~1h	25 ml	~1h	1 ml
Coomassie staining	fixative	~30 min	50 ml	~10 min	25 ml
	stained	~30 min	50 ml	~15 min	25 ml
	destained	~1 h	3x 50ml	~30min	3x 25ml

Fabrication and Design of the ProtE

♦ **PMMA** by micro-millimeter scale machining tools
♦ **silicon wafer** by micro-fabrication (lithography, etching)

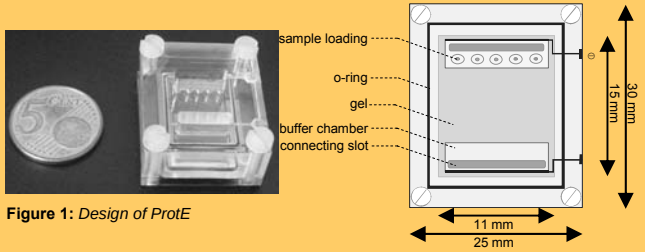

Figure 1: *Design of ProtE*

RESULTS and DISCUSSION

Slab gel electrophoresis and Subsequent proteomics

SDS-PAGE with the ProtE

♦ **5-times more sensitivity** compared to mini-PROTEAN (Figure 2 B & C)
♦ **10-times smaller amount of loaded sample** (0.1 – 1 µl vs. 5 – 10 µl; Figure 3)
♦ **9-times faster separation** (11 min vs. 100 min; Table 1)
♦ limit of detection is **2 - 3 ng** (silver stain)

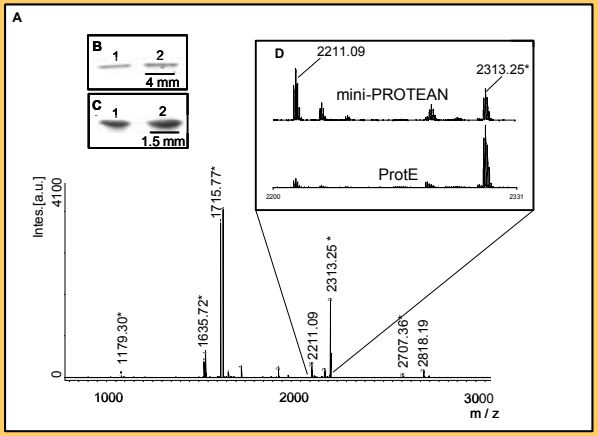

Figure 2: *MALDI analysis and PAGE*. A) MS spectrum of 0.4 µg βLG, B) SDS-PAGE by mini-PROTEAN & C) by ProtE, D) MS spectra comparison. 1) 0.4 µg & 2) 0.6 µg of βLG

Peptide Mass Fingerprinting - Mass Spectrometry

♦ **0.4 µg βLG** band was in-gel alkylated and digested with modified trypsin, analyzed by MALDI-ToF-MS and PMF was identified in NCBI (Figure 2A&D).
♦ Figure 2D shows improved intensity of a peptide fragment using **9-times smaller amount** of enzyme (0.05 -0.1 µg vs. 0.5 µg)

Immunoblotting with the ProtE

♦ **540 ng** of smooth muscle cell lysate was immunoblotted against α-actin (Figure 4)
♦ **5-times smaller amount** of primary antibody (200 µl vs. 1 ml)

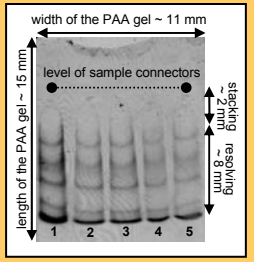

Figure 3: *SDS-PAGE of LMW standards with ProtE.* 1) 1.44 µg, 2)&3) 720 ng, 4)&5) 432 ng. For protocol see Table 1.

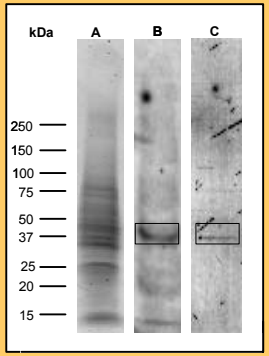

Figure 4: *Separation of 540 ng of smooth muscle cell lysate.* A) SDS-PAGE and B) immunoblotted against α-actin of cell lysate run by ProtE, and C) immunoblotted against α-actin of cell lysate run by mini-PROTEAN. For protocols see Table 1.

CONCLUSIONS

The ProtE instrument for proteomic analysis:
♦ shorter separation time
♦ smaller amount of sample and reagents → cheaper analysis
♦ higher sensitivity
♦ possibility of automation

ACKNOWELGEMENTS

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