Real-Time Analysis of Biomolecular Interactions:

Integration of Biomolecular Interaction Analysis and Mass Spectrometric Amino-acid Sequencing

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Introduction

Biomolecular interaction analysis (BIA), using surface plasmon resonance (SPR), is an important technique in the understanding of biomolecular function, providing quantitative information such as kinetic parameters and equilibrium constants for complex formation[1-2]. Additional uses of the technology are discovery of novel protein-protein interactions and screening of small molecules, as potential therapeutics. To accelerate the process, we have now development a novel system to integrate BIA with MS/MS to sequence proteins bound to the sensorchip surface . In the medle experiments, epitope tagged recombinant protein and 1, 4, 5-inositol trisphosphate (IP_3) binding protein present in the total cell lysates were isolated on the sensorchip and sequenced by MS/MS analysis[3].

Methods

Expression of Recombinant Proteins

E.coli BL21(DE3) was transformed with:

1. pRSET-His-xFKBP to express the Xenopus FKBP-binding protein (xFKBP) with a 6 x His-tag.
2. pET3a-T604 to express the ligand binding domain (IP3 binding protein) of 1,4,5-inositol phophate receptor type 1[2]. Detction by Western blotting indicated a basal expression level (50-200 ng/mL) for both proteins.

Biomolecular Interaction Analysis

System: BIACORE X (Biacore AB)

Running buffer: 50 mM HEPES, 150mM NaCl, 50 μ M EDTA, 0.005% noctylglucopyranoside, pH7.4.

Sensor surfaces:

1. Biothinylated (1, 4, 5)-IP_3 analogue bound to Sensor Chip SA (streptavidin).
2. His-tagged xFKBP: Chip NTA (nitrilotriacetic acid) pre-activated with nickel solution.

On-chip-digestion and On-line recovery

An air partition method was used to deliver minute volume enzyme, Achromobacter protease I (**Fig. 1**). Flow was halted to allow digestion at 37C, showing digestion curve. After the response stabilized, the flow was re-started and the digested peptides were

collected directly in an RPC capillary pre-column (1 X 0. mm i. d.).

Mass Spectrometric Analysis.

System: HPLC-ESI/MS/MS consists of Finnigan LCQ ion trap mass spectrometer (ThermoQuest, San Jose, CA) and a HPLC model 140D syringe pump (PE Biosystems) with a flow splitter (**Fig. 2**).

Results

Figure 3 shows the SPR response after injection of cell lysate containing His-tagged xFKBP. The amount of the protein digested on the sensor surface was calculated to be <160 femtomole. The HPLC-ESI/MS/MS study showed three intense ions at m/z=844.0, 914.0 and 1344.6, corresponding to peptides eluted from the capillary column. The peptide ion at m/z=844.3 was fragmented in the ion trap cell and sequenced by MS/MS analysis. **Figure 4** indicated that nine y and seven b series fragment ions showed the peptide sequence, G76QTVVVHYVGSLENGK91 of xFKBP. **Figure 5** shows the response after injection of cell lysate containing IP3 binding protein. The amount of the protein on the sensor surface was calculated to be <58 femtomole. In the ESI/MS/MS analysis , five intense peptide ions were detected and fragmented for sequence analysis. **Figure 6** indicates a doubly charged peptide ions at m/z=701.0 was identified as P78GANSTTDAVLLNK91 of IP3 binding protein. All expected y series were confirmed, except for y1 and y2 that seemed to be too small to be retained in the ion trap and eight b series were also observed to be major peaks. Clear sequence information was obtained from four other peptid ions that were identified as digested fragments of IP3 binding protein. The consequent sequence coverage was 96/604 amino acids.

CONCLUSION

The ability to handle small quantities of biological samples with higher throughput is greatly to be desired. This demand is a major driving force to miniaturize analytical instrumentation. To achieve this, a microfluidics system has been increasingly recognized as a important platform to perform 'nano-scale' manipulation of biological molecules. A commercial biosensor such as BIACORE® can perform molecular interaction analysis with integrated microfluidics which delivers a small volume of sample solutions to defined locations of sensorchip surface. Hence the sensing flow cells on the microfluidics can function as a 'nano-scale' affinity surface on which a specific binding partner (*i.e.*, antibody, receptor, ligand) is immobilized.

We focused on the microfluidics of BIA as a effective platform to detect, capture, digest and deliver the mid- to low-femtomole of proteins for mass spectrometry. The sample consumption, including the dead volume for microfluidics, was 15 μL of cell lysate. These results show that

integration of BIA-MS/MS should prove to be a general and highly versatile system for discovering novel interactions or novel binding partners from a limited quantity of biological fluid. Since MS/MS analysis can identify proteins based on single-peptide fragmentation spectra, the system should allow for analysis of not only simple one to one protein-protein interaction, but also protein complexes and molecular components can be identified.

References

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(2) Natsume, T.; Hirota, J; et.al. *Biochem Biophys Res Commun* **1999**, 260, 527-533.
(3) Natsume, T.; Nakayama, H. et.al. *Anal Chem* **2000**, in press

Fig. 1

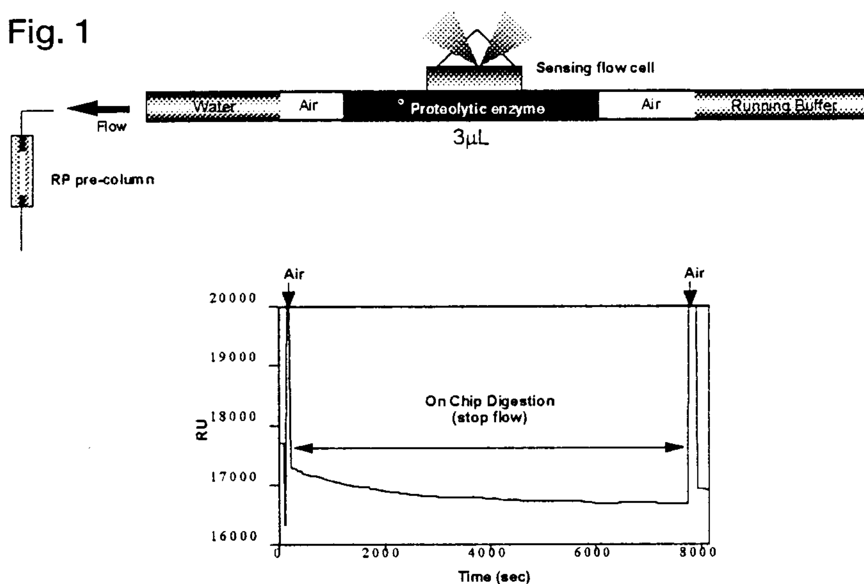

Fig. 2

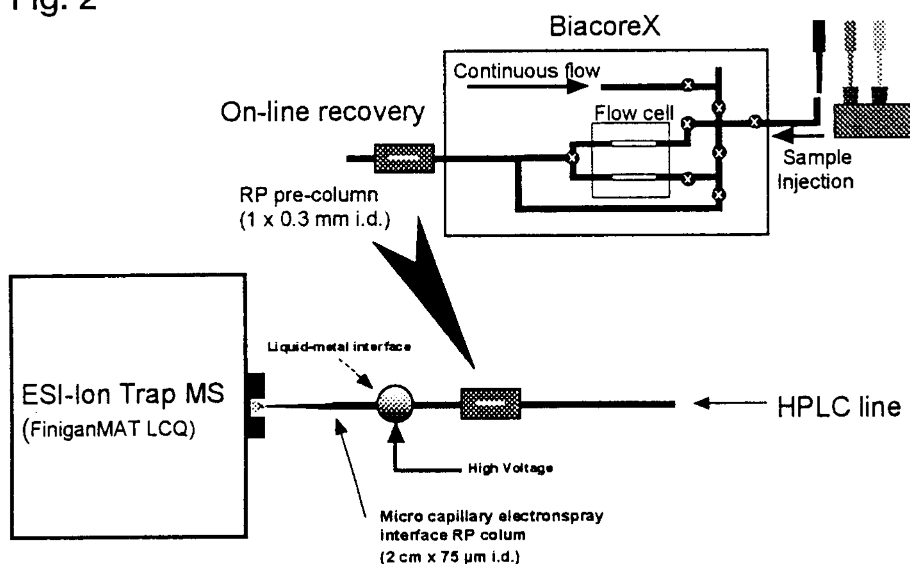

Fig. 3

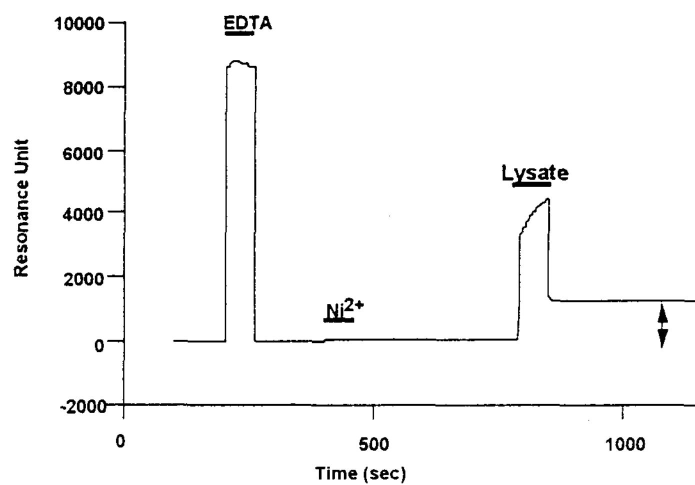

Fig. 4

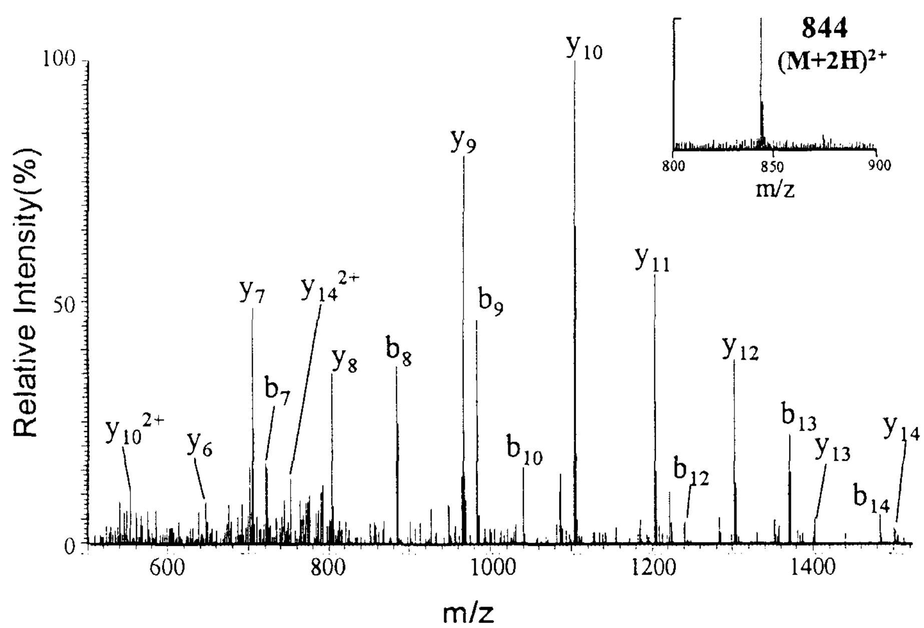

Fig. 5

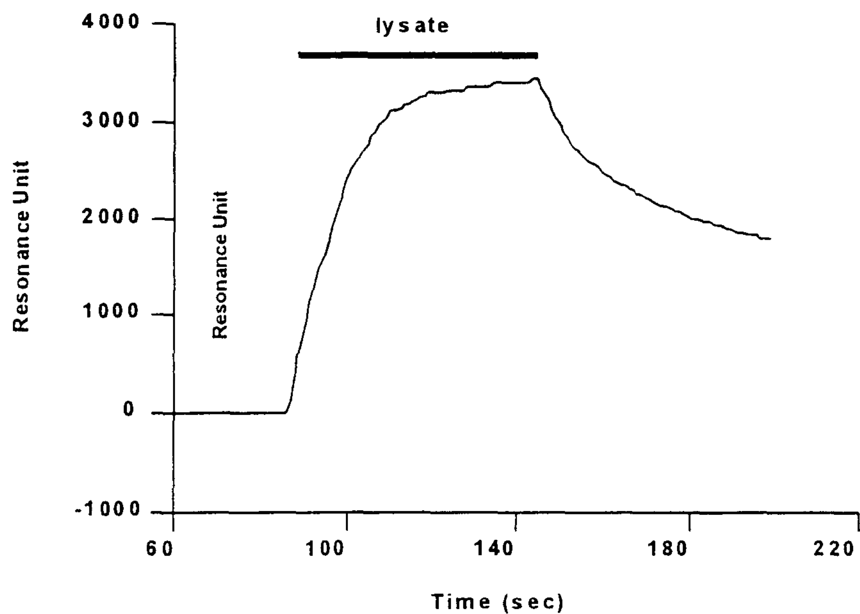

Fig. 6