



Short Communication

**Use of SPR Biosensor for the Study of Proteolytic Action of a Serine
Protease Enzyme**

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Received: 4 May 2011; / Revised: 29 May 2011; / Accepted: 15 July 2011

Abstract

Proteases constitute one of the major classes of biomolecules playing a key role in biological processes. In the present study, we used an SPR biosensor for the study of proteolytic action of a commonly used serine protease enzyme called trypsin against four proteins i.e. lactate dehydrogenase, gelatine, poly l lysine and bovine serum albumin. The system showed the proteolytic action of trypsin in real time and this proof of principle experiment proved to be cost effective. Furthermore, the equipment BI 2000 may also be used to study the inhibitory action of some physical and chemical agents on their proteolytic activity. The results of our study suggested new applications of SPR system for detection and study of proteolytic action of enzymes.

Keywords: Biosensors, SPR, Proteolytic enzyme, Serine Proteases, Proteins

Proteases, also called proteinases are proteolytic enzymes, which constitute one of the most important groups of proteins industrially and academically. Proteases act as molecular knives and are responsible for cutting of proteins into smaller peptides, thus giving them a final shape by

controlling their size, turnover and ultimate destruction. It is estimated that almost 2% of human and 1-5% genomes of infectious agents account for proteases [1-2]. Proteases play a key role in the human physiological processes as well as cell cycle. There is a critical role of proteases in

human and animal diseases such as cancers. Proteases are also important for bacteria, viruses, fungi, insects and parasites for their replication and spread of diseases in humans and other animals [1]. Apart from their medical applications, proteases also have biotechnological applications. Proteases with high activity at different pH, and higher temperatures find great application in detergent, contact lens cleaning agents, effluent treatment, amino acid production and tannery. In depth knowledge of kinetics and catalytic behaviour of proteases is essential for their industrial and medical applications. A rapid, real time and cost effective system for detection and monitoring of their catalytic activity would be of advantage for diagnostics, research and industrial applications [3].

Surface Plasmon Resonance (SPR) based biosensors find increasing applications in the Life Science research and diagnostics for the study of biomolecular interactions and kinetics of their binding as well as detection and identification of entire cells and proteins [4]. In particular, SPR biosensors offer unique opportunity of rapid, label free, real time and cost effective detection and identification of biomolecules [4-6].

SPR is an optical phenomenon in which plasmon waves are excited at the metal dielectric interface. The surface plasmon waves are extremely sensitive to the refractive index changes at the sensor surface and are proportional to the sample mass. So, it is a surface sensitive technique in which small change at the surface due to binding or dissociation of biomolecules brings about the change in SPR signal. In this regard antibodies are the most frequently used biomolecules for the capture of the analyte from the sample [7].

For a reliable quantitative assay of biomolecular interaction in a SPR biosensor, one species needs to be immobilized. The conventional methods of immobilization are physical adsorption, avidin/streptavidin based or covalent attachment. Covalent attachment of biomolecules at the sensor surface is the most frequently used method for reliable assays condition (8-10).

In the present study, a commercial SPR biosensor of Biosensing Instruments Inc (BI 2000)

was used to study the proteolytic action of the trypsin enzyme against a number of proteins in an attempt to investigate the utility of the system as a rapid and convenient approach for studying proteolytic action of enzymes. Trypsin enzyme was purchased from Invitrogen, whereas poly l-lysine, gelatine, bovine serum albumin and lactate dehydrogenase were purchased from Culturex®, Fischer Scientific, Sigma-Aldrich and Roche Diagnostics GmbH respectively. The concentration of protein substrates was 0.01%, whereas varying concentrations of trypsin (0.01 % to 1 ppm) were used to study minimum detection limit of the system. A concentration of 0.01% of trypsin was used to study the proteolytic action of this enzyme against a number of proteins. Chemicals used for self assembled monolayer (SAM) development *i.e.* mercaptopropionic acid and for its activation *i.e.* N hydroxysuccinimide (NHS) and 1-Ethyl-3-[dimethylaminopropyl] carbodiimide hydrochloride (EDC) were obtained from Sigma Aldrich, Fulka and Alfa Aesar respectively. Gold thin films on BK 7 glass with a thickness of 50 nm were provided by the SPR equipment manufacturer.

The mercaptopropionic acid SAM was developed by submerging the gold sensor into the mercaptopropionic acid solution (10mM) in ethanol for overnight at 4°C. The sensor was washed with ethanol and then ultra-pure water followed by activation with NHS (100 mM) and EDC (100 mM) prior to the attachment of the proteins on the surface. The binding event of the proteins on the sensor surface was monitored in real time as a rise in the SPR dip position (mDeg). Trypsin was injected after covalent binding of these proteins to study the proteolytic action of the enzyme on the immobilized proteins. The minimum detection limit of the system for the trypsin enzyme was determined by making different dilutions of trypsin in PBS (0.01%-1.0 ppm). Moreover, action of heat inactivated trypsin (65°C for 30 min) on these proteins was also studied to monitor action of enzyme inhibitors on their function in real time.

The results of our study showed positive shift in dip position after the injection of proteins (Figs. 1 & 2), which indicated covalent binding of these protein species on the gold sensor surface. As can

be seen in Figure 1, the SPR biosensor produces rapid results with higher signal to noise ratio. Injection of trypsin enzyme caused a short event of an increase in the dip position (indicating enzyme substrate binding) followed by a fall as a result of enzymatic degradation of proteins by it. However, there has been no fall in SPR dip position observed in the case of heat inactivated trypsin due to its inability to enzymatic degradation of proteins. The maximum time required by the system for protein immobilization on the SAM and the study of proteolytic action of enzyme was 15 minutes. The results were reproducible and the values given in the figure 3 are mean of three readings. This system is

extremely sensitive and could detect as low as 10 ppm enzyme in PBS.

Biosensor research has experienced significant growth in last decade as evident from ever increasing number of research publications on the subject. SPR biosensors are one of the major types of optical biosensors used in Life Sciences. SPR biosensors have already reached commercialization and many companies are offering state of the art SPR equipments for rapid and real-time study of bimolecular interactions. However, applications of SPR biosensors are still limited to research only and further work on sensitivity enhancement and cost-effectiveness has been debated in the past [11- 14].

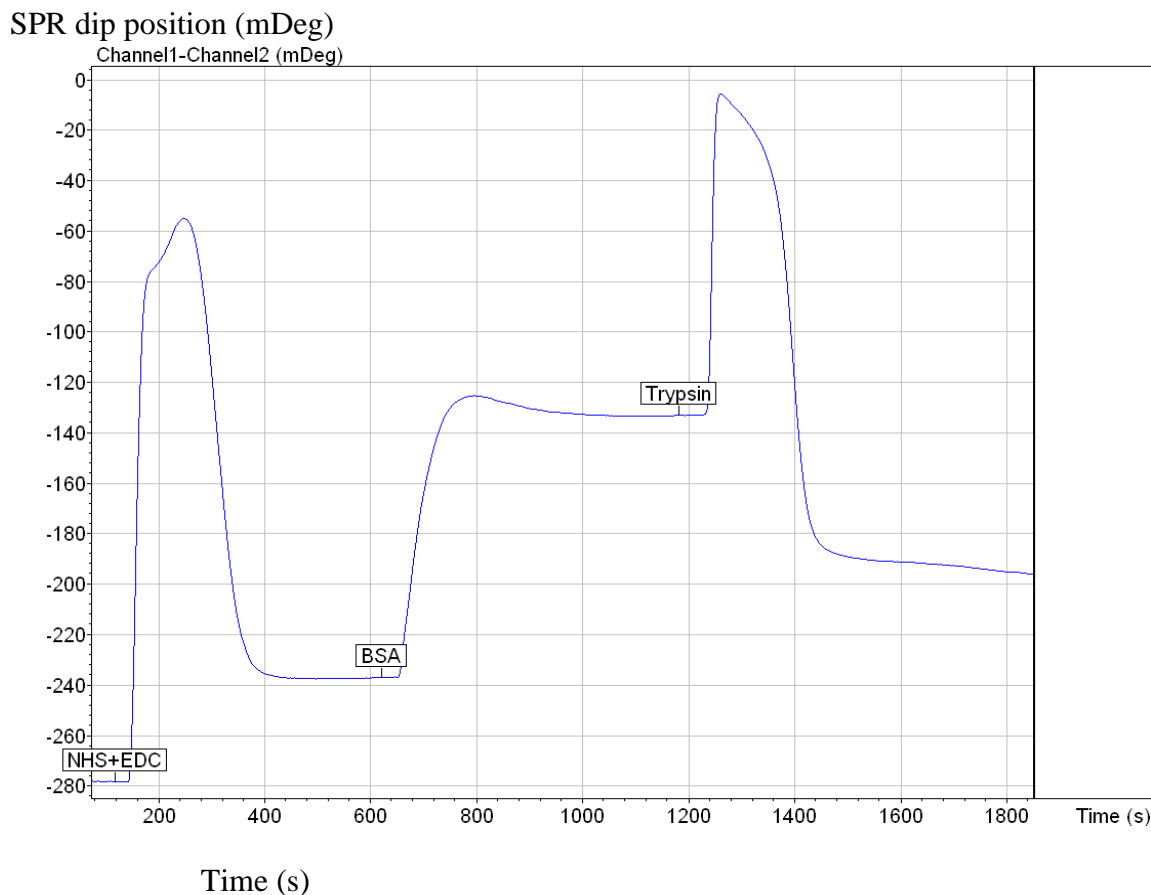


Figure 1. SPR sensogram showing change in SPR dip position (y axis) as a result of injection of N-hydroxysuccinimide (NHS) and 1-Ethyl-3-[dimethylaminopropyl] carbodiimide hydrochloride (EDC), substrate protein (BSA) and enzyme (trypsin). The time (s) in X axis shows change in dip position after injection of NHS/EDC and proteins. There is a rise in SPR curve position after injecting NHS, EDC and BSA, whereas it drops after trypsin injection.

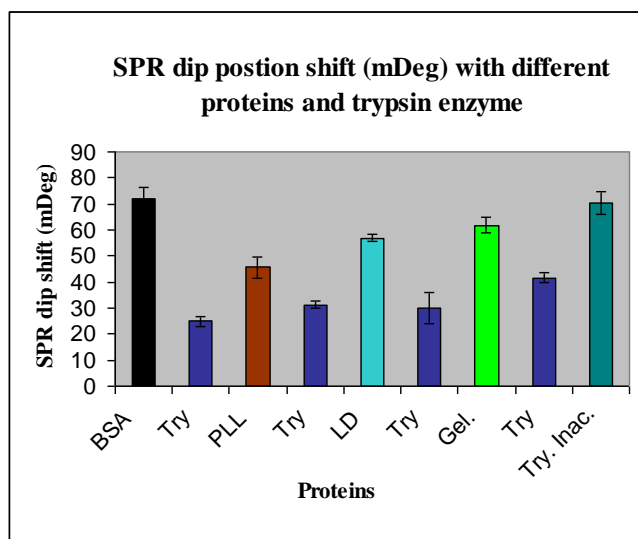


Figure 2. SPR dip position shift in milli degrees (m Deg) with a number of proteins and digestion with trypsin enzyme. The SPR dip position rises after injection of substrate proteins, but drops with trypsin enzyme due to enzymatic digestion. The last bar shows SPR dip shift upon injection of the heat inactivated trypsin enzyme. Abbreviations: Try= trypsin; Gel: gelatin; PLL= poly L lysine; LD= lactate dehydrogenase

Trypsin is a serine protease enzyme with substrate affinity for positively charged amino acids arginine and lysine [5-6]. Digestion of four proteins by trypsin was investigated after covalently immobilizing them on the gold sensor surface. Physical binding of proteins on the gold thin film is weak and dissociates gradually by the passage of time through continues flow of running buffer (PBS) before, proteins (substrates) need to be covalently immobilized on the gold film to get a steady curve showing permanent attachment and therefore reproducible results. The BI 2000 SPR used in this study showed reproducible results. The results of our study clearly indicated that SPR biosensor may be used for the screening of proteolytic action of enzymes as well as their quantitative detection. Furthermore, kinetics of enzymatic digestion can also be studied using with this SPR. Therefore, this system may also be a promising technique in protein chemistry and enzymology due to its sensitivity, cost-effectiveness and its ability to produce results in real time. Inhibitory action of certain physical and chemical agents on enzyme activity may also be

studied using this SPR biosensor. In our case, heat inactivated trypsin did not produce any negative shift in SPR angle position, showing our controls were working well in this proof of principle experiment.

Therefore, we concluded that SPR biosensor of Biosensing Instrument, USA may well be used for detection and monitoring of proteases activity in real time as well as action of certain physical and chemical agents on their catalytic activity both quantitatively and qualitatively

Acknowledgements

This work was sponsored by Higher Education Commission of Pakistan through grant *No.20-760/R&D/06*, COMSATS Institute of Information Technology Pakistan and Department of Biomedical Engineering of Florida International University, Miami, USA.

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