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Peptide-based biosensor for real-time monitoring of protease biomarkers

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Summary:

Here we propose a peptide-based assay using a specific combination of linker and peptides to design a robust biosensor for proteases. The implementation of a cleavage-based mechanism results in the sensitive and specific detection of a clinically relevant biomarker (MMP-9). The developed assay is characterized using real-time electrical and optical techniques and demonstrates its potential as a tool for the detection of MMP-9.

Keywords: peptide interface, protease biosensor, matrix metalloproteinase (MMP-9), optical technique, electrochemical technique

Background, Motivation and Objectives

Biosensors provide a sensitive and highly selective means of detecting biomarkers. The biosensor is typically based on the measurement of signal changes resulting from the biomolecular interactions that occur at the interface between the electrode and biomolecular layers. Among the various disease biomarkers, enzyme-based biomarkers such as proteases show significant potential in the design of a sensitive biosensor due to their robust cleavage mechanism. Peptides are cleaved by protease at specific sites (Fig. 1), and can therefore be used as one of the most promising candidates for monitoring protease activity [2, 3]. Among the different classes of proteases, overexpression of MMP-9 (matrix metalloproteinase - 9) is associated with diseases such as cancer [2], and neurodegenerative or inflammatory diseases [1]. Therefore, the early clinical detection of MMP-9 is essential to intervene in disease progression and would lead to new treatment options for patients. In recent years, there has been significant development in the field of protease biosensors using peptides as substrates [2, 3]. However, the problem of false signal contributions due to non-specific binding of cleaved peptide fragments or proteases themselves on the sensing electrode reduces the detection sensitivity [3]. A further challenge is the cross-reactivity of peptides with other proteases or proteins present in the biological samples [3]. In this paper, we report the development of a specific peptide interface on a gold surface, modified with a PEG-based linker molecule, for the detection of MMP-9. The assay was optically monitored in situ by non-invasive multi-parametric surface plasmon resonance (MP-SPR) spectroscopy. The results obtained were further validated by electrochemical measurements. The concentration-dependent activity of MMP-9 on the sensing platform is analyzed and compared.

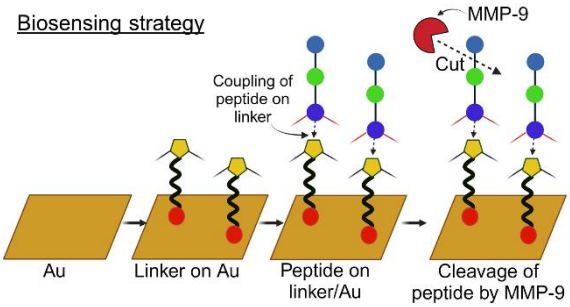

Fig. 1. Biosensing strategy of the proposed peptide-based MMP-9 assay.

Research novelty

The combined optical and electrical monitoring of MMP-9 using the peptide cleavage mechanism has not been extensively explored. Our work contributes a simple, rapid, and sensitive MMP-9 assay that has been validated in real time by optical (MP-SPR) and electrical (potentiometric) detection methods. The label-free assay using PEG-based linker molecules reduces the false signal contribution due to non-

specific binding of MMP-9 or the cleaved peptide fragments on the sensor surface. The electrical characterization of the surface by comparing the change in the potential difference is performed at a very low current (1 nA). To the best of our knowledge, this is the first report on the potentiometric study of peptides for MMP-9 detection and shows potential application in the design of point-of-care devices.

Results

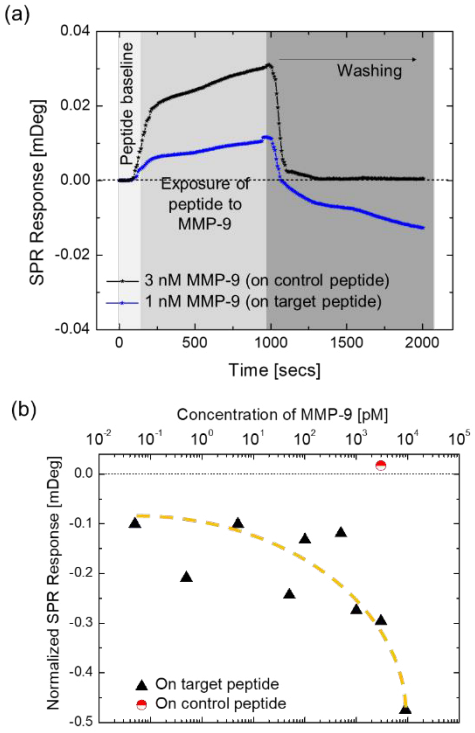

Fig. 2. a) SPR sensogram showing the peptide baseline, its subsequent exposure to MMP-9, followed by the buffer washing step. The SPR response of the target peptide to 3 nM MMP-9 goes below the baseline during the washing step. By contrast, the signal response of the control peptide to 3 nM MMP-9 goes back to the baseline. b) Normalized SPR response at different concentrations of MMP-9 in buffer (PBS, pH 7.4). The dashed line is a guide to the eye.

By modifying the surface of Au with SAMs of PEG-based linker molecule (450 µM), we first validated the significant reduction in the non-specific binding of control peptides (195 µM) and MMP-9 using MP-SPR. This proved that the assay is suitable to develop a specific and sensitive peptide biosensor. Figure 2a, b shows the proteolytic hydrolysis of the target peptide (195 µM) upon exposure to various concentrations of MMP-9. As expected, the control peptide, which does not contain any MMP-9 cleavage sites, was not digested. Using this assay, we were able to qualitatively detect MMP-9 at concentrations as low as 0.05 pM. In the next step, the proposed assay was tested in the electrical measurement setup where we observed a linear dependence of the signal response on the MMP-9 concentration up to 1 nM (Fig. 3b). Future experiments are planned to test the MMP-9 activity in the electrical setup in buffer as well as in complex samples at different MMP-9 concentrations.

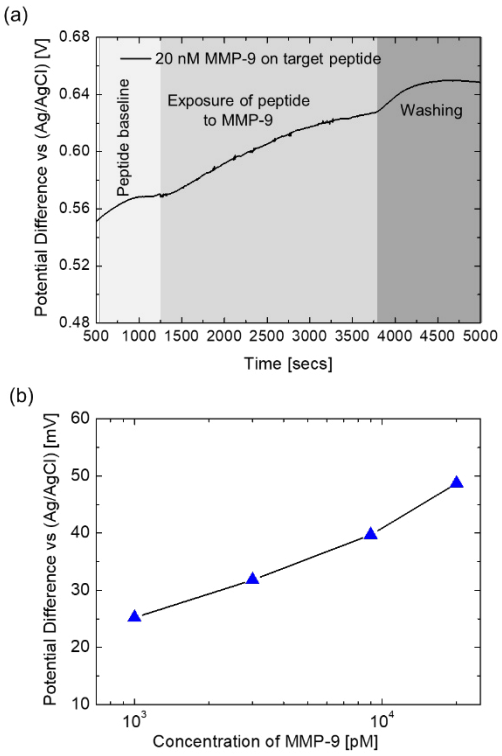

Fig. 3. a) Time profile of the change in the potential difference of the peptide/linker modified Au surface versus the Ag/AgCl reference electrode upon exposure to 20 nM MMP-9 in culture medium (RPMI-1640). b) Dependence of the signal response upon exposure to different concentrations of MMP-9 ranging between 1 – 20 nM.

References

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