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Electrochemical Proteolytic Beacon for Detection of Matrix Metalloproteinase Activities

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Matrix metalloproteinases (MMPs) are a family of extracellular, zinc-dependent proteinases that are capable of degrading all components of the extracellular matrix.[1] MMPs have been implicated in a variety of disease states, including arthritis, periodontal disease, and tumor cell invasion and metastasis.[2] They also have been proposed as important biomarkers of infection and inflammation.[3] Detecting the specific activity of MMPs could play a central role in the diagnosis and treatment of diseases and in the detection of infectious agents and their toxins.

Current methods for detecting MMP activity involve enzyme-linked immunoassay (ELISA), Western blotting, polyacrylamide gel electrophoresis,[4] and the use of a wide variety of probes[5] (e.g., fluoregenic peptides,[5a,b] radiolabeling,[5c] and dyes[5d]). The fluorescent labeled peptide substrates in particular have been used extensively.[5a,b] These substrates act as "bait" for the protease and report once cleavage occurs. The assay can be quite sensitive because each protease can cleave multiple substrates. Furthermore, these assays demonstrate not only biomarker presence, but also the activity of MMPs. However, such fluorescence-linked assays are often complicated by the requirement of an elaborate excitation and detection scheme and by the broad emission bands. This communication describes an alternative method, which uses an electrochemical proteolytic beacon (EPB) for detecting MMP activity. Using the EPB offers the capability of "on−off" voltammetric signaling for the specific MMPs.

The electron-transfer properties of helix peptide have been well documented.[6a,b] Direct transduction of a signal corresponding to peptide cleavage events into an electronic signal provides a simple, sensitive route to detect MMP activity. The new approach uses an electrode-attached helix peptide with a specific sequence labeled with an electroactive reporter, ferrocene (FC), as the cleavage-sensing element ("signal-on," Figure 1A). Upon sensing cleavage in the presence of MMP, the EPB is cut and separates from the electrode surface, leading to a large, readily measurable signal decrease ("signal-off," Figure 1B).

In the current study, a helix peptide with sequence RPLALWRSC labeled with a FC reporter was used to detect matrix metalloproteinase-7 (MMP-7) activities. Previous studies have shown that a similar sequence peptide had discriminatory activities for MMP-7 in the MMP family.[5a] The peptide was synthesized by a conventional stepwise solid-phase peptide synthesis method using the Fmoc protection. The amino group at N-terminal was used to conjugate with FC. The synthetic FC-peptide EPB was purified using C_{18} reverse-phase high-performance liquid chromatography. The molecular weight (1328.3) of the purified FC-peptide was verified by mass spectrometry analysis (See Support Information (SI), Figure S1).

The EPB was prepared by self-assembling a FC-peptide monolayer on a gold electrode surface (see details in SI). The molecular orientation of the FC-peptides was studied by Fourier transfer infrared reflection−absorption spectroscopy (FTIR−RAS). The tilt angle of the helix axis was 34° based on the amide I/amide II absorbance[6b,c] (see SI Figure S2), indicating that the helix axis in each SAM was oriented. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used to investigate the on−off signal of the FC-peptide−Au electrode during MMP-7 cleavage. Figure 2 A presents the typical cyclic voltammograms of the FC-peptide−Au electrode before (red curve) and after (blue curve) cleavage with 2 ng mL^{-1} MMP-7 and then analyzed in 0.6 M $NaClO_4$ solution. Prior to cleavage, a pair of well-defined redox peaks (red line, $E_{pa} = 0.26$ V; $E_{pc} = 0.22$ V) related to the reduction and oxidation of FC were observed. The redox potentials of FC shift around 100 mV comparing with that of free FC (see SI, Figure S3) which represents a slow electron transfer of helix peptide owing to the long-range electron transfer reaction.[6a] The peak currents were directly proportional to scan rates, implying electrochemical reactions of surface-bound redox species[7] (see SI, Figure S4). The surface coverage of FC-peptide was estimated from the area of the cyclic voltammetric peaks corresponding to the oxidation of ferrocene centers and found to be 1.03×10^{-10} mol/cm^2 (see details

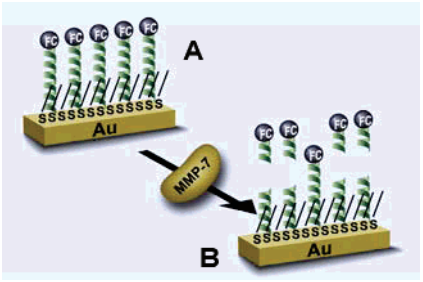

Figure 1. Schematic of EPB for detection of matrix metalloproteinase: (A) self-assembling electrochemical FC-peptide conjugate on the gold substrate; (B) cleavage of EPB in the presence of MMP-7.

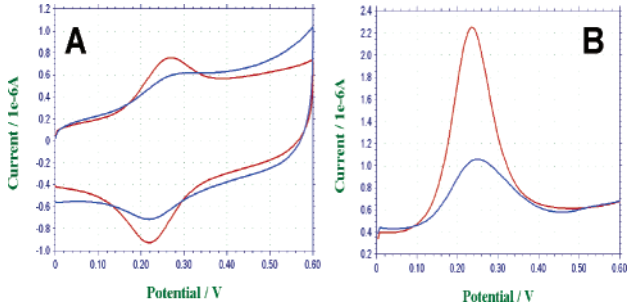

Figure 2. (A) Cyclic voltammograms of the FC-peptide−Au electrode in 0.6 M $NaClO_4$ solution before (red curve) and after (blue curve) cleavage with 2 ng mL^{-1} MMP-7. The cleavage was performed for 30 min by dipping the electrode in tricine buffer (50 mM tricine, pH 7.4/0.2 M NaCl/10 mM $CaCl_2$/50 uM $ZnCl_2$/0.005% Brij 35): potential scanning rate, 100 mV/s. Panel B shows corresponding square wave voltammograms. SWV scanning was performed from 0 to 0.6 V with a step potential of 4 mV, amplitude of 20 mV, and a frequency of 25 Hz. All potentials are referred to an Ag/AgCl reference electrode.

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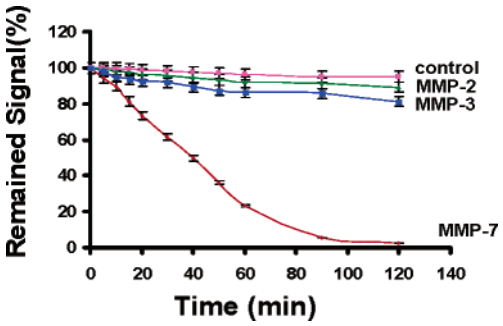

Figure 3. The relationship plot between cleavage time and remaining SWV signals of the FC-peptide−Au electrodes in the flow-injection system. Assays were performed at room temperature and pH 7.4 by injecting 40 μL of 10 ng/mL MMPs and following a different cleavage time (stop flow) and SWV measurement.

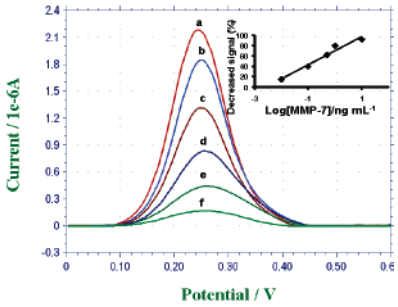

Figure 4. Typical square wave voltammograms of FC-peptide−Au electrodes incubated with different concentrations of MMP-7 (from a to f: 0, 0.1, 0.5, 1, 5, and 10 ng mL^{-1}). Freshly prepared FC-peptide−Au electrode was used in each measurement. Incubation time was 30 min; supporting electrolyte was 0.6 M $NaClO_4$.. SWV scanning was performed from 0 to 0.6 V with a step potential of 4 mV, amplitude of 20 mV, and a frequency of 25 Hz. All potentials are referred to an Ag/AgCl reference electrode.

in SI). After cleavage with MMP-7, the redox peak currents of FC significantly decreased (blue line, signal-off). The amplitude change of the signal is dependent on the density of FC-peptide; a high density of FC-peptide gives a small amplitude change. A signal change was negligible with a bovine serum albumin (BSA) protein under the same conditions (control experiment, results not shown). Figure 2B presents the corresponding square wave voltammograms, which are more sensitive than CV to quantify the MMP-7 activity.

The FC-peptide−Au electrode was thus integrated into a flow-injection system in connection with the SWV measurement to quantify the MMP-7 activity. The specificity of the electrode was first tested by comparing the efficiency of the cleavage of the peptide by MMP-7 with the proteolysis by MMP-2 (gelatinase A) or MMP-3 (stromelysin 1). Figure 3 shows the relationship plot between cleavage time and the remaining SWV signal (percentage) of the FC-peptide−Au electrodes in the presence of different MMPs. One can see that EPB was readily cleaved by the MMP-7, but more slowly by MMP-2 and MMP-3 (approximately 10−15% of the rate of cleavage by MMP-7). Control experiments with BSA show only a 5% signal decrease, which may be attributed to the fouling of protein on the electrode. The results show that the designed EPB can be selectively cleaved and the decrease in amplitude of the voltammogram is increasing with the increase of the cleavage content of peptide. The catalytic constant (K_{cat}) of MMP-7 to attached FC-peptide on the gold electrode surface was estimated as 0.078 s^{-1}.

Figure 4 shows the typical SWVs of the FC-peptide−Au electrodes after 30 min cleavage with different concentrations of MMP-7. One can see the voltammetric peaks of the electrodes are well defined and the peak currents decrease with the increased concentration of MMP-7, indicating that the amount of EPB on the gold electrode surface decreased after cleavage. We found the percentage decrease in signal is dependent on the log of the amount of MMP-7 injected (Figure 4, inset), rather than the amount of MMP-7. It may be attributed to the slow electron transfer of the helix peptide chain and steric hindrance effect of self-assembled FC-peptides and mercaptohexanol on the access of MMP-7. The detection limit of MMP-7 is 3.4 pM (signal-to-noise = 3) which corresponds to 1.35×10^{-16} moles in the 40 μL sample solution. This is comparable to that of immunoassay.[5b] A series of six repetitive measurements of the 5 ng mL^{-1} MMP-7 with six electrodes yielded reproducible results with a relative standard deviation (RSD) of 3.2% (not shown). The day-to-day prepared electrodes show good reproducibility with RSD of 2.1%.

In conclusion, we developed an electrochemical proteolytic beacon from a helical peptide labeled with a ferrocene reporter for detection of MMP-7 activities. Direct transduction of a signal corresponding to peptide cleavage events into an electronic signal provides simple, sensitive route for detecting the MMP activity. The concept can be extended to design multiple peptide substrates labeled with different electroactive reporters, such as metal ions (without potential overlap) for assay of multiple MMPs activities. This approach can be applied to the detection of infectious agents and specific protease activity used in pathogenesis.

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Supporting Information Available: Related experimental conditions (instrumentation, reagents, and procedures) along with additional data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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