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Highly specific detection of IL-8 protein using combination tapered fiber-optic biosensor dip-probe

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Abstract

We are reporting detection of IL-8 in a mixed protein solution, using combination tapered fiber-optic biosensor (CTFOB) dip-probe. Sandwich immunoassay was used as the detection technique. The specificity of the sensor was established by using two types of negative control probes. It is demonstrated that with the help of these CTFOB dip-probe we could successfully detect IL-8 with high specificity in protein mixture. The lowest detected concentration of IL-8 was 150 pM.

Keywords

Cytokine; IL-8; fiber-optic; biosensor; fluorescence; Combination tapered

1. Introduction

The most popular fiber-optic biosensors currently in use are tapered fiber-optic biosensors (TFOB).^(1–5) Among the TFOBs, combination tapered fiber-optic biosensors (CTFOB) have highest sensitivity.^(6–8) We are reporting successful detection of cytokine IL-8 using combination tapered fiber-optic biosensor (CTFOB) dip-probe developed in our lab. These dip-probes will not use any special fluidic system. The main advantage of the CTFOB technique over the existing methods like ELISA is significantly lower cost and their use as on-site fast disposable sensing probes. Cytokines are hormone-like polypeptides that are secreted in the course of immunologic and inflammatory responses. They function as intercellular signals, are produced by a variety of different cell types, and regulate both local and systemic inflammatory responses. Cytokines are also immunoregulators in wound healing and immune responses. We could successfully detect as low as 150 pM of IL-8 concentration. Sandwich assay was used for detection. Capture antibodies of IL-8 were immobilized on the probe surface while the detection antibodies of IL-8 were labeled with Alexa 488 dye. Signals were recorded with the help of a CCD based spectrometer. Specificity of the method was established with two types of negative control probes.

2. Material and Method

All solvents and chemicals were either of analytical grade or chemically pure. b-Mercaptoethylamine HCl (MEA) and Dry Acetone and Phosphate Buffered Saline (PBS)

were obtained from Fisher Scientific (Pittsburgh, PA, USA). Recombinant Human IL-8, Recombinant Human IL-6, purified anti-human IL-6 capture (Clone MQ2-13A5) antibodies and purified anti-human IL-8 capture (Clone JK8-1) and detection (Clone JK8-2) antibodies were obtained from Biolegend (San Diego, CA, USA). EDTA, immobilization reagent Sulfo-SMCC, and 3-Aminopropyltriethoxysilane (APTS) were obtained from Pierce (Rockford, IL, USA). Amine reactive Alexa fluor 488 fluorophore was obtained from Molecular Probes (Eugene, OR, USA) and CS-800 spin columns were from Princeton Separations (Adelphia, NJ, USA). Tween-20 was obtained from Sigma Aldrich (St. Louis, MO, USA). The silica/silica Optical Fibers were obtained from Polymicro Technologies (Phoenix, AZ, USA). Lysozyme was obtained from MP Biomedicals (Solon, Ohio, USA).

2-1 Antibody-dye conjugation

Anti-human IL-8 detection (Clone JK8-2) antibodies concentration was 0.5 mg/ml. In order to achieve optimum antigen-dye conjugation, antibodies stock solution was used and mixed with 0.02M sodium bicarbonate and 64 $\mu\text{g/ml}$ Alexa fluor 488 fluorophore. The sodium bicarbonate solution is to create pH8.5 environment. The molar ratio of dye and antibodies was 30. After 1 hour incubation in room temperature, the free dye molecule was filtered out by using two spin columns. Finally, the labeled anti-IL-8 detection antibody stock solution (0.5 mg/ml) was further diluted to 1 $\mu\text{g/ml}$ with PBS mixed with 1 mg/ml Lysozyme.

2-2 CTFOB dip-probe preparation

Each probe was a 7.5 cm long multimode optical silica/silica fiber with a 600 μm core diameter. Approximately 1.5 cm long tip of each fiber-optic probe covered with polyimide buffer was burned off by a Bunsen burner and the probes were decontaminated by sonicating it in soap solution. The tips of probes were then immersed into 10% hydrofluoric acid for 24 hours to remove the cladding part and reduce the tip diameters to 300 μm . Because of capillary action, some hydrofluoric acid will ascend into the space between fiber probe and polyimide buffer. The ascended acid will slowly etch the probe and form the taper part. The average tapered angle for all the probes was around 0.058 ± 0.002 rad. After taken out from hydrofluoric acid, the probes were first sonicated in de-ionized water for 5 min, then in 1M NaOH solution for 10 min, then again in de-ionized water for 5 min, and finally in acetone for 2 min.

2-3 Antibody immobilization

The sensing part (1 cm from tip) of a tapered fiber probe was immersed in 2% APTS solution in dry acetone for 1 min to derivatize primary amines on the probe surface. These amines group then react with hetrobifunctional cross-linker Sulfo-SMCC by incubating the probes into 4.5 mM Sulfo-SMCC in PBS-EDTA (10 mM) solution for 1 hour. Sulfo-SMCC groups could bind to amine groups and create a maleimide-activated probe surface. To expose the sulfhydryl group of the anti-human IL-8 capture antibody (Clone JK8-1), the antibodies were reduced by mixing 0.5 mg/ml of antibody solution in 10 mM PBS-EDTA and 50 mM MEA and incubating at 37°C for 90 minutes. The spin column was then used to filter out the MEA from antibody stock solution. The maleimide-activated probe surface could bind to sulfhydryl group by immersing the probe in 10 $\mu\text{g/ml}$ reduced anti-human IL-8 capture antibody for 4 hours at room temperature.

2-4 Signal generation and recording

Sandwich immunoassay was used to generate the fluorescence signal which was proportional to the concentration of the sample. All the detection procedures were operated at room temperature. The probes immobilized with capture anti-IL-8 (Clone JK8-1) antibodies were dipped into various concentrations of human IL-8 sample solutions for 1 hour; four probes were used to detect each concentration. All the IL-8 sample solutions were diluted with 1 mg/ml non-specific protein (Lysozyme) to simulate serum sample. The probes were washed for 1 min by washing buffer which is PBS mixed with 0.02% Tween-20. Immediately after washing, the background signal spectral profiles were recorded for each probe. During recording of signal, probes were immersed in PBS to keep them wet. After background recording, probes were incubated in 1 µg/ml labeled anti-IL8 detection antibody solution for 1 hour. After washing, the fluorescence spectral profile of each of the probes was recorded while keeping it immersed in PBS. All the fluorescence signal profiles were recorded using experimental set up reported earlier(8).

2-5 Signal extraction by least square fitting method

The fluorescence spectral profile ($f_s(\lambda)$), is the sum of two spectral profiles—the auto-fluorescence from any non specific protein bindings (background) to the probe surface ($f_B(\lambda)$), and the characteristic spectral profile of labeled detection antibodies($f_{FI}(\lambda)$). Thus the fluorescence spectral profile can be express as a linear combination of those two spectral profiles.

$$f_s(\lambda) = a f_b(\lambda) + b f_{FI}(\lambda) \quad (1)$$

Fluorescence signal was extracted from the total signal by least square fitting method using equation 1.(8)

3. Results and Discussion

3-1 The detection of IL-8

Solutions of several concentrations of IL-8 (25pM, 60pM, 150pM, 400pM, 700pM, 1000pM, 1500pM, 2000pM, and 2500pM) were prepared using commercially available recombinant IL-8. Our ultimate goal is to use these probes for quantitative estimation of IL-8 in serum samples. Serum samples always contain high concentration of several other proteins. To simulate serum solution condition, we mixed high concentration of (1 mg/ml) Lysozyme in each solution. For detection of each concentration, four probes were used. Capture anti-IL-8 antibodies were immobilized on each probe and each of these probes was incubated in respective concentration of sample for 1 hour. After taking out of the sample each probe was washed and incubated for one hour in 1 µM concentration of labeled anti-IL-8 detection antibodies. Fluorescence signal for each probe was recorded and actual signal was separated from non specific signal using least square method(8). A typical recorded fluorescence signal (at 150 pM) with background signal and extracted signal is shown in figure 1. Variation of fluorescence signal with IL-8 concentration is plotted in figure 2. Each plotted point in the graph is average of the signals from four probes. Small error bars

indicate reproducibility of the signal in each concentration. The dose response curve fits nicely to an exponential growth function with $R^2 = 0.992$. The curve is almost linear below 1.5 nM. Saturation tendency above 1.5 nM is because of limited amount of immobilized capture antibodies on the probe surface.

3-2 The specificity and sensitivity of IL-8 detection

The specificity of CTFOB probes for IL-8 detection is evaluated with the help of two types of negative control probes. Sandwich immunoassay technique requires two kinds of antibodies (capture and detection) to make a sandwich of antigen. Ideally, lack of any layer should not show any signal. The negative control 1 measures the non specific signal in the absence of antigen IL-8 while negative control II demonstrates that the signal produced is only due to specific interaction of capture antibodies and antigen IL-8.

For negative control 1, four probes with immobilized capture anti-IL-8 antibodies were incubated in 1 mg/ml Lysozyme solution without any IL-8 sample for 1 hour. After washing, the probes were incubated in labeled anti-IL-8 detection antibodies.

For negative control 2, instead of capture anti-IL-8, four probes were immobilized with anti-IL-6 antibodies. All these probes were incubated in very high concentration (1 nM) of IL-8 solution for 1 hour. Subsequently these probes were incubated in labeled anti-IL-8 detection antibodies solution for 1 hour.

Signals from these probes were extracted using least square fitting method(8). Figure 3 shows the average intensity and standard deviation of two negative control signals compared with low concentrations of IL-8 signals. From the graph, the 60pM and 25pM data points are hard to distinguish from two negative control signals, but the 150pM data point is quite above the negative control ranges. From this observation we concluded that the developed CTFOB probes can detect as low as 150 pM of IL-8 in the presence of a high concentration (1 mg/ml) of another protein (Lysozyme).

4. Conclusion

We have demonstrated that the developed CTFOB dip-probe can successfully detect cytokine IL-8 down to 150pM (1.26 ng/ml) even in the presence of high concentration of another interfering protein (1 mg/ml Lysozyme). Furthermore, two negative controls demonstrate the specificity and sensitivity of IL-8 detection result. The CCD based experimental setup shows its superiority to discriminate the auto-fluorescence from the original spectral profiles. The method presented here reduce the need of washing process and eliminate the need of probe pre-treatment with any kind of blocking buffer. The calibration curve indicates that the developed CTFOB can be used to quantitatively estimate the concentration of IL-8 in serum sample.

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Figure 1. Recorded spectral profile of the fluorescence signal from a probe incubated in a 150 pM of IL-8 sample solution. The signal spectral profile was separated from the background profile by use of least square fitting with $R^2=0.998$. Overlapping dashed line with extracted signal is the actual spectral profile of labeled antibodies.

Figure 2.
Dose response curve of IL-8 fluorescence signal. Each data point is an average of signals from four probes.

Figure 3.
Average signals of lower concentrations of IL-8 with negative control signals.