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Dissociation constant measurement using combination tapered fiber-optic biosensor (CTFOB) dip-probes

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Abstract

The document reports a novel method of measuring dissociation constant (k_D) of antibody-antigen interaction using evanescent wave based combination tapered fiber-optic biosensor (CTFOB) dip-probes. The method was demonstrated by measuring the dissociation constant of human Interleukin-6 (IL-6) and anti-IL-6 interaction. Sandwich immunoassay was used to generate fluorescence signal proportional to antigen-antibody conjugate pairs. A portable CCD based spectrometer setup was used to record spectral profile of the fluorescence signal. The measured value of dissociation constant k_D for IL-6 and capture anti-IL-6 (clone MQ2-13A5) antibodies at room temperature is 588 ± 19 pM .

Keywords

Cytokine; IL-6; fiber-optic; biosensor; fluorescence; dissociation constant; Combination tapered

1. Introduction

Understanding the kinetics of protein-protein interaction is increasingly important for advancing our understanding of the molecular basis of such events.(1-5) For more than one decade, surface plasmon resonance (SPR) detectors, is widely used for the study of these interactions.(2, 6) We are proposing a new method based on combination tapered fiber-optic biosensor (CTFOB) dip-probe to measure dissociation constant (k_D) of an antigen and antibody interaction. These dip-probes do not require any kind of fluidic system(7). The main advantage of the CTFOB technique over the existing methods like SPR is significantly lower cost and portability. Dissociation constant (k_D) is defined as the ratio of dissociation rate constant (k_d) and association rate constant (k_a).

$$k_D = k_d / k_a$$

The method was demonstrated by measuring dissociation constant (k_D) of human Interleukin 6 (IL-6) and anti-IL-6 antibodies interaction.

2. Materials and Methods

All solvents and chemicals were either of analytical grade or chemically pure. Egg albumin (EA) powder, b-Mercaptoethylamine HCl (MEA) and Dry Acetone and Phosphate Buffered Saline (PBS) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Recombinant Human IL-6, purified anti-human IL-6 capture (Clone MQ2-13A5) antibodies and anti-human IL-6 detection (Clone MQ2-39C3) 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).

2-1 Antibody-dye conjugation

The anti-human IL-6 detection (Clone MQ2-39C3) antibodies solution (0.5 mg/ml) was mixed with 0.02M sodium bicarbonate and 64 μ g/ml Alexa fluor 488 fluorophore. The molar ratio of dye and antibodies was 30 and the reaction environment was at pH8.5. After 1 hour incubation in room temperature, the free dye molecules were separated from antibody-dye conjugate by filtering twice using two spin columns (CS-800).

2-2 CTFOB dip-probe preparation

Each probe was a 7.5 cm long multimode optical silica/silica fiber with a 600 μ m core diameter. 1.5cm long of the tip of each fiber-optic probe covered with polyimide buffer was burned off by a Bunsen burner. The tip of probes was then immersed into 10% hydrofluoric acid for 24 hours to remove the cladding part and reduce the tip diameter to about 300 μ m. Because of capillary action, some hydrofluoric acid will ascend into the space between fiber probe and polyimide buffer. The ascended acid will produce 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 washed by distilled water, NaOH (1M), and acetone.

2-3 Antibody immobilizations

First, the sensing part 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 probe into 4.5 mM Sulfo-SMCC in PBS-EDTA (10mM) 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-6 capture antibody (Clone MQ2-13A5), the antibodies were reduced by mixing 0.5 mg/ml of antibody solution in 10 mM PBS-EDTA and 50 mM MEA and incubate 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 μ g/ml reduced anti-human IL-6 capture antibody for 4 hours at room temperature.

3. Result and Discussion

3-1 Theory

When the number of antibody molecules N_b on the probe surface is very small, then the number of antigen-antibody conjugates N_{bg} are given by the following equation.(4)

$$N_{bg}(t, N_g) = \frac{N_b}{\frac{k_D}{N_g} + 1} (1 - \exp[-k_a t (k_a + N_g)]) \quad (1)$$

Where k_D is the dissociation constant, N_g is the concentrations of antigen, k_a is the association rate constant and t is the incubation time. However for low antigen concentration values ($k_D \gg N_g$), the exponential term in above equation for a constant incubation time can be treated as a constant. Thus the antigen-antibody conjugate pair concentration N_{bg} as a function of antigen concentration N_g , can be given as:

$$N_{bg}(N_g) = \frac{C N_b}{k_D/N_g + 1} \quad (2)$$

Here C is a constant and concentration of immobilized antibodies N_b on all the probes, is also a constant. Therefore any signal S proportional to the antibody-antigen concentration N_{bg} can be written as a function of antigen concentration N_g .

$$S(N_g) = \frac{A}{k_D/N_g + 1} \quad (3)$$

Here A is a constant. If we can plot signals proportional to antibody-antigen conjugate for various values of antigen concentrations, we can obtain the value of dissociation constant k_D by fitting the above function to that curve.

3-2 Signal recording and k_D measurements

Sandwich immunoassay was used to generate fluorescence signal proportion to the antibody-antigen conjugate pairs. The spectra profile of the fluorescence signal was recorded by a charged coupled device (CCD) spectrometer based detection setup reported earlier(7). All the detection procedures were operated at room temperature. The probes immobilized with capture anti-IL-6 antibodies were dipped into various concentrations (5 pM, 20 pM, 50 pM, 100 pM, 200 pM) of human IL-6 sample solutions for 2 hour. This step produced the anti-IL-6 capture antibodies and IL-6 conjugate pairs. Nonspecific signal was avoided by mixing these samples with 1mg/ml egg albumin (EA).(7) Four probes were used for each concentration. Probes were washed for 1 min with washing buffer (PBS with 0.02% Tween-20) and background signal spectral profiles of each probe were recorded. Fluorescence signal proportional to antibody-antigen conjugates on each probe was generated, by incubating each probe for two hours in 1µg/ml of labeled anti-IL6 solution. Fluorescence spectral profile of each probe was recorded. Finally, the signals for each probe were extracted by using least square fitting method described in our earlier work(7). A dose response curve shown in Fig. 1 was plotted from the extracted signal for various concentrations.

Function in Eq. 3 was fitted to the dose response curve in Fig. 1 with $R^2 = 0.995$. The computed value of dissociation constant for human IL-6 and anti-IL-6 antibody (Clone MQ2-13A5) is found to be $k_D = 588 \pm 19 \text{ pM}$.

4. Conclusion

We have demonstrated that the developed CTFOP can successfully measure the dissociation constant k_D for an antibody-antigen interaction. The method was used to compute dissociation constant for human IL-6 and anti-IL-6 antibody (Clone MQ2-13A5). The computed value of dissociation constant is found to be $k_D = 588 \pm 19 \text{ pM}$. For comparison, we could not find any reported value of k_D for human IL-6 and anti-IL-6 antibody measured by any other method. The value reported here corresponds to interaction of human IL-6 and anti-IL-6 antibody in the presence of 1mg/ml of EA.

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Figure 1.
Variation of fluorescence signal proportional to anti-IL-6/IL-6 conjugate pairs with antigen IL-6 concentration.