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Direct Assessment of HBsAb-HBsAg Interaction by Interferometry

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In this paper we report attempts to directly detect the different binding property between monoclonal hepatitis B virus surface antibody (Mab) and multiclonal hepatitis B virus surface antibody (Multiab) with hepatitis B virus surface antigen, which was coated on a pretreated glass slide. In the procedure, an optical transducer based transmittance interferometry was used to detect this interaction in real time. At the end of the interaction, an increase in protein layer thickness of about 7nm and 4.1nm for Mab as well as 6nm and 2nm for Multiab were obtained at the concentrations of 10μg/ml and 1μg/ml, respectively. The dynamic data derived from the film thickness change were evaluated with bimolecular model. Compared with the interaction between Multiab and antigen, higher kinetic constant between Mab and its corresponded antigen was obtained.

Keywords: biosensor; antigen; antibody; bimolecular model

INTRODUCTION

The strong and specific binding of target molecules to the binding sites of an antibody plays an important role in several fields. The assessment of affinity constants is of fundamental importance, in particular for the characterization of monoclonal antibodies (Mabs) with respect to their analytical performance and

cross reactivity [1]. Methods for this task are usually based on the determination of the equilibrium concentrations of the reactions. Equilibrium concentrations are classically determined by dialysis, by quenching or transfer of fluorescence, by immuno-precipitation of radio-labeled analyses, or by centrifugation and filtration techniques [2]. All these methods required specific properties of the analyses and are not practical when fast and efficient screening is demanded. Recently, a new concept for characterizing affinity interactions by real-time monitoring of the binding event at a transducer surface has become popular since Pharmacia introduced a commercial system for this technique in surface plasmon resonance (SPR). The association and dissociation rate constants of the interaction can be derived from the binding curve. And the affinity constant is determined from the ratio of the rate constants or the equilibrium coverage. In the procedure, immobilization of one of the interacting compounds is one of the most important requirements.

In this paper, the interaction between immobilized antigen and antibodies was performed and a transmittance interferometry was utilized to monitor the event on line [3, 4]. The changes of optical constants of protein layers on the slide were monitored by the instrument. Neither immobilized ligand nor analyte was labeled. To compare the different binding character between Mab and multiclonal antibody (Multiab), simple bimolecular model was used according to the film thickness change.

EXPERIMENT

Purified hepatitis B virus surface antigen (HBsAg) was diluted to 100μg/ml with buffer and immobilized on a cleaned glass slide through self-assembled thin film of silane coupling agent. After coating with the HBsAg film, the chip was washed several times with PBS. Both Mab and Multiab were diluted to 10μg/ml, 1μg/ml, respectively and antibody was added. Thirty minutes after

addition of protein, the chip was rinsed three times in 1 M PBS. Goat anti-human IgG was applied as control. For dissociation steps, PBS-Tween was added. The procedure was monitored by a transmittance interferometry, where grating CCD camera recorded the spectra information transmitted from transducer surface. To computer the binding/dissociation constants, a simple bimolecular model was used.

RESULTS AND DISCUSSION

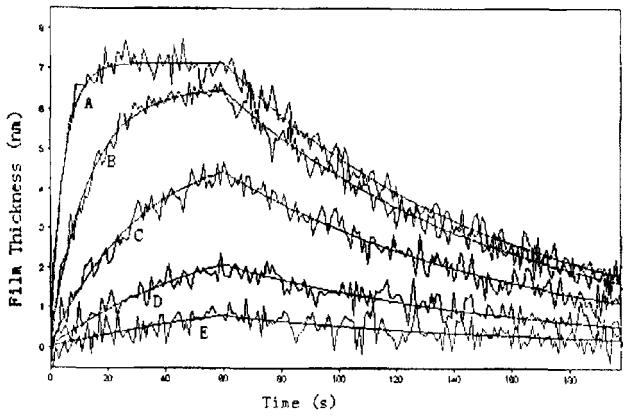

FIGURE 1 The thickness change of the antigen-antibody components film. Curve a, c and curve b, d represented the dynamic interaction procedure of Mab and Multiab respectively. And curve a, b and curve c, d derive from different antibody concentration of 10μg/ml and 1μg/ml, respectively.

Figure 1 present our experimental results for antibody binding on the antigen film. At the incubation end, the addition of HBsAb resulted an increasing in the film thickness by about 7nm for Mab and 6nm for Multiab at the concentration of 10 μg/ml, respectively. When the 1 μg/ml antibody were added, the thickness

increased 4.1nm for Mab and 2nm for Multiab, respectively. For the control, there was 0.5nm film thickness increasing at the end. Simple bimolecular model was used to study the kinetic analysis according to the in real time detected thickness change of the film. Data analysis indicates that the binding constants are $9.93e+05M^{-1}s^{-1}$ for the interaction between Mab and antigen as well as $6.81e+05M^{-1}s^{-1}$ between Multiab and antigen. The dissociation constants are $0.01s^{-1}$ for both Mab and Multiab. In this figure, it is visible that the thickness of bound protein layers is different even the concentrations of Mab and Multiab are the same. Several factors effect the measured result: the concentration of analyte, the adsorption capacity of ligand, and incubation time. It is generally agreed that there is positive relationship between protein concentration and their corresponded film thickness [3, 4]. In this paper, the concentration is measured based on the solid state sensitive layer and the measured concentrations of ligand represent the effect concentration of bound antibody. This solid state based method is fairly different with the traditional methods and its measured concentration is generally less than that derived from the traditional methods. Compared with Multiab, the component of Mab is more homogenous and it shows higher solid state based concentration on the same concentration. Therefore, the thickness of bound Mab is higher than the Multiab on the same concentration. The different concentration based on the solid state film can also be utilized to explain why the binding constant between Mab and antigen is larger.

References

[1] Steward, M.W. and Steengaard, J. *J. Immunol, Methods,* **Vol. 78,** 173–190 (1985).
[2] Price.Ch P., Newman, D. J., Eds. *Principles and practice of immunoassay,* Stockton Press, New York, 1991.
[3] G. Gauglitz, A. Brecht, G. Krous, *Sensor and Actuators B* **Vol. 11,** 21–27 (1993).
[4] V. Lin, K. Motesharei, K.S. Dancil et al. *Science* **Vol. 278**, 31, (1997).