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ADSORPTION OF F(AB')$_2$ANTI-HUMAN IMMUNOGLOBULIN G TO PLASMA-POLYMERIZED ALLYLAMINE FILM COVERED ON A SILVER PLATE

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Abstract To increase the sensitivity of immunoassay or immunosensor, many amounts of antibody should be immobilized. Allylamine thin film was formed on a flat silver plate by plasma-polymerization and this plate was referred to as Ag(ALAM). Adsorption of F(ab')$_2$anti-human IgG onto Ag and Ag(ALAM) was investigated. The following results were obtained: (1) The adsorption isotherm of F(ab')$_2$anti-hIgG onto Ag(ALAM) or Ag was a Langmuir type. The saturation binding for this antibody onto Ag(ALAM) was approximately 2-fold than that on Ag. (2) Ag(ALAM) and Ag were used as solid phases in two-site immunoradiometric assay (IRMA) of human serum IgG, and dose responses were compared. The dose response on Ag(ALAM) occurred at lower concentration than that on Ag and the magnitude of the dose response on Ag(ALAM) was larger than that on Ag.

INTRODUCTION

Immunoassay is an important technique for detection of micro biocomponents, and needs high selectivity, sensitivity, and rapidity.[1] The two-site immunoradiometric assay (IRMA) is a well-known high sensitivity and selective method.[2] Figure 1 shows the concept of IRMA. This process consists of three steps. First step is adsorption of the first antibody onto a solid phase, the second step is an antigen-antibody reaction, and the final step is the reaction between radioactive second antibody and the antigen. In general, it is considered that the dose response of IRMA increases with an increase in immobilized amount of the first antibody, but it is not confirmed mathematically so far.

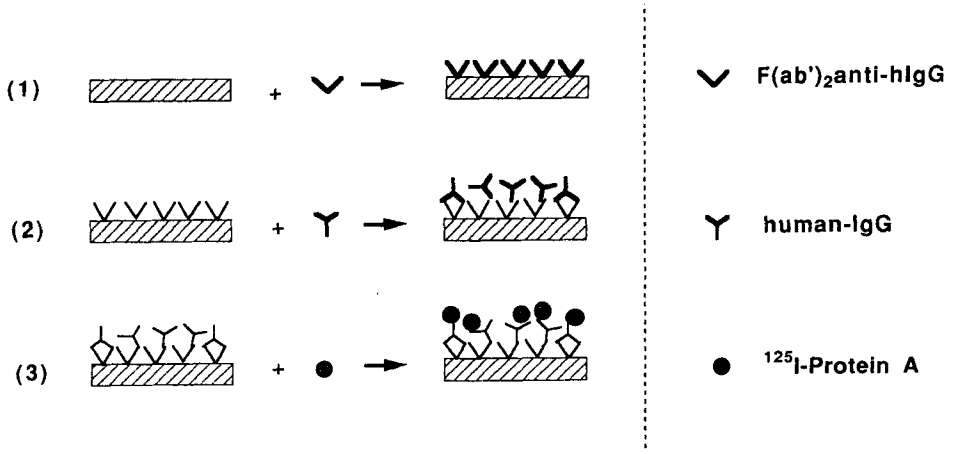

FIGURE 1 Concept of the two-site immunoradiometric assay (IRMA).
This assay consists of three steps: the first step is adsorption of the first antibody onto a solid phase, the second step is an antigen-antibody reaction, and the final step is the reaction between radioactive second antibody and the antigen.

We examined the computational simulation according to Rodbard model.[3] Figure 2 shows results of IRMA under the ideal condition. It is apparent that the dose response of IRMA increase with q1 (see legend of Fig. 2) increasing. In other word, increasing the immobilization amount of first antibody is important to increase the dose response of IRMA. We must fix antibody onto the solid phase surface without inactivation as much as possible. One of the immobilization method is to fix them chemically, but this process is somewhat troublesome. Another method is physical process: Antibody is adsorbed spontaneously to the surface. This process is easy to handle, but we must choose the surface-coating material so that as much as antibody should be adsorbed.

Plasma-polymerization has been recently used to make an ultra thin film on variety of substrata. The coating film adheres strongly to substratum, and is highly resistant to chemical and physical treatment.[4] Since the plasma-polymerization technique can easily modify characteristics of the surface of materials, we expect that the modification by this technique enables to increase the adsorption of the antibody. Various kinds of material (pure metals, metal oxides, alloys, polymers and ceramics) were dipped into various protein solutions to examine biocompatibility of the materials. Williams and Williams found that the adsorption of human serum albumin onto Ag demonstrated very different characteristics from other metals.[5] We choose Ag plate as the model solid phase, since

Ag should be expected a good adsorbent of protein.[5] We choose F(ab')$_2$anti-human IgG as a model protein, since F(ab')$_2$ fragment was adsorbed much more than whole IgG antibody. In addition, it is well known that use of F(ab')$_2$ fragment instead of whole IgG antibody for antigen-binding sites eliminates positive false due to the presence of the rheumatoid factor, when labeled anti-antibody is used as the final reagent, and that labeled protein A binds to the Fc portion of whole IgG antibody.

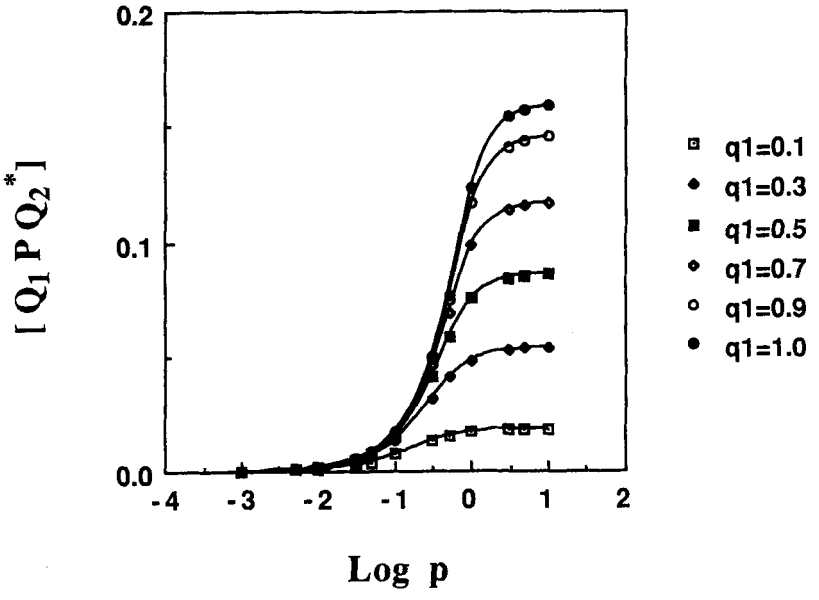

FIGURE 2 Computational calculation of IRMA under ideal condition.
The notation is according to reference 3: first antibody (Q_1), unknown antigen (P), radioactive second antibody (Q_2), and p (the 'dose' of antigen). q1 is a variation parameter. $p=[P]+[Q_1P]+[PQ_2^*]+[Q_1PQ_2^*]$, $q1=[Q_1]+[Q_1P]+[Q_1PQ_2^*]$

EXPERIMENTAL

The following materials were obtained as indicated: Na^{125}I and [^{125}I]-protein A from Amersham, UK; allylamine from Wako Pure Chemical Ind., Ltd., Japan; goat F(ab')$_2$anti-human IgG (F(ab')$_2$anti-hIgG) from Cappel, USA ; human IgG (hIgG) from Sigma Chemical Co., USA; Block Ace® (BA, BA was used as a blocking agent for non-specific binding in immunoassay), from Dainippon Pharmaceutical Co., Ltd., Japan; Silver plates from Nilaco Co., Ltd., Japan (purity 99.98%, 5×5×0.2 mm). Reagents used were of analytical or guaranteed grade, and were purchased from Wako

Pure Chemicals (Osaka). Water was prepared with Milli-Q (Millipore, Ltd.) and had a specific resistance of more than 18 MΩ/cm.

The apparatus and procedure for the plasma-polymerization of allylamine were essentially the same as described previously.[6] A film of about 50 nm thickness was synthesized on both Ag surfaces. The Ag plate covered with allylamine thus prepared is referred to hereafter as Ag(ALAM). The adsorption of labeled $F(ab')_2$anti-hIgG on Ag(ALAM) and Ag was measured by the same method described previously.[7] Dose-responses were obtained by the same method described previously.[6] The hIgG (0 - 1000 $\mu g \cdot ml^{-1}$) was diluted 1:200 with 1:10 BA and these antigen-solutions of different concentrations were incubated with Ag(ALAM) or Ag pre-coated with $F(ab')_2$anti-hIgG. The same procedure was carried out except that pre-coated with $F(ab')_2$anti-hIgG were absent (reference experiment for non-specific binding). After incubation, the antigen-solution was removed and the diluted ^{125}I-protein A was added to the washed pieces. After further incubation, the radioactive solution was removed and the amount of labeled protein A bound on the washed pieces was calculated from the radioactivity measured by the gamma counter.

RESULTS AND DISCUSSION

A film obtained was not dissolved into water, methylalcohol, ethylalcohol or acetone, which are good solvents for the monomer. The film was slightly yellowish and glossy. It was very stable and resistant to chemical and physical treatment. Characterization of the plasma-polymerized allylamine film was described previously.[6] The adsorption isotherms for labeled $F(ab')_2$anti-hIgG on Ag(ALAM) and Ag are shown in Figure 3. The protein on Ag(ALAM) and Ag appeared saturable at protein concentrations of about 1000 $\mu g \cdot ml^{-1}$, indicating that the adsorption of $F(ab')_2$anti-hIgG was a Langmuir type. The binding constant and saturation binding were determined from the Hofstee plot .[6] They were 8.93 $l \cdot mol^{-1}$ and 181.8 $nmol \cdot m^{-2}$ for Ag(ALAM). For Ag, they were 7.71 $l \cdot mole^{-1}$ and 87.9 $nmol \cdot m^{-2}$. The saturated level on Ag(ALAM) was about 2 times larger than that on Ag, whereas no significant change of the binding constant was observed.

Two-site IRMA using Ag(ALAM) was performed because of two purposes; One is to examine the amounts of adsorbed $F(ab')_2$anti-human IgG by IRMA. It should be stressed that this method enables to check the amounts of the active antigen adsorbed on the surface, while the adsorption experiment with use of ^{125}I-labeled antigen gives the total amounts including inactivated antigen if present. Second purpose is to consider whether this coating can be used as a solid phase for two-site IRMA or not.

Figure 4 shows that if the concentration of $F(ab')_2$anti-hIgG is *ca.* 1000 $\mu g \cdot ml^{-1}$, the adsorption of protein on both Ag(ALAM) and Ag is saturated. On Ag(ALAM), the dose-response occurred at more than 6.67×10^{-4} $nmol \cdot ml^{-1}$ hIgG. The binding of labeled protein A increased with increasing hIgG concentration and reached a plateau (210 $pico\text{-}g \cdot cm^{-2}$). On the other hand, the dose-response on Ag occurred at more than 1.67×10^{-3} $nmol \cdot ml^{-1}$ of hIgG, which is somewhat concentrated than that for Ag(ALAM). The binding of labeled protein A increased with increasing amount of

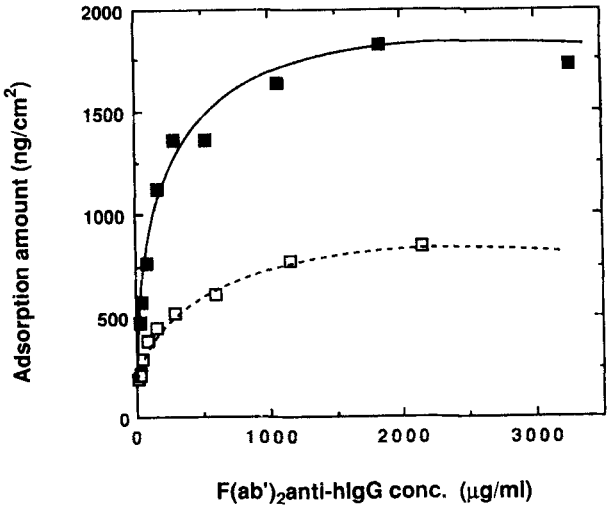

FIGURE 3 Adsorption isotherms of $F(ab')_2$anti-hIgG for Ag(ALAM) and Ag. ■, Ag(ALAM); □, Ag. The experiment was performed in duplicate. Experiments were performed at 25 °C.

hIgG and reached a plateau. The plateau levels are about 120 $pg \cdot cm^{-2}$ for Ag plate. When BA instead of $F(ab')_2$anti-hIgG was coated on Ag(ALAM) and Ag, little radioactivity of labeled protein A was observed on these solid phases, compared with the $F(ab')_2$anti-hIgG-coated substrata (data not shown). The non-specific binding of labeled protein A, however, was only seen when the concentration of hIgG was very high (over 1×10^{-2} $nmol \cdot ml^{-1}$). This figure is consistent with the amounts of absorbed $F(ab')_2$anti-hIgG at 1000 $mg \cdot ml^{-1}$. This fact implies that all $F(ab')_2$anti-hIgG molecules may be adsorbed as a reactive form.

As a summary of this paper, adsorption of $F(ab')_2$anti-human IgG onto Ag and Ag(ALAM) was investigated. In addition, Ag(ALAM) was used as a solid phase in two-site immunoradiometric assay (IRMA) of human serum IgG. We conclude that plasma-polymerized allylamine film may be used to advantage the fixing antibody.

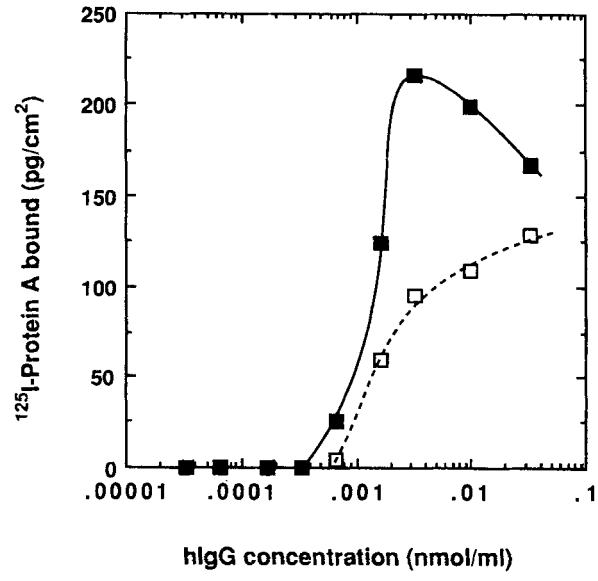

FIGURE 4 Dose-response curve for hIgG in two-site immunoradiometric assay. Solid and broken curves are for the solid phases coated with $F(ab')_2$-hIgG and with BA, respectively. ■, Ag(ALAM); □, Ag. The experiment was performed in duplicate. Experiments were performed at 25 °C.

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