Plasma-Polymerized Allylamine Film Used as a New Solid Phase in Immunoradiometric Assay (IRMA): Effect of Antibody (F(ab′)_2 Fragment) Concentration on Dose Response in Two-Site IRMA

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Allylamine (ALAM) film was plasma-polymerized on a flat glass (referred to as ALAM(GLA); GLA refers to a flat glass plate), for use as a solid phase in two-site immunoradiometric assay (two-site IRMA). Adsorption of F(ab′)_2, anti-human immunoglobulin G (referred to as F3-hlgG) to ALAM(GLA) was larger than adsorption on a polyvinyl chloride plate (referred to as PVC). Contrary to the expectation that the dose response for human IgG (hlgG) on ALAM (GLA) was better than that on PVC, the dose responses on both solid phases were the same. This phenomenon was independent of molecular size of the antigen (Ag) (hlgG or Fc fragment of hlgG (hlgG-Fc)) and also of the reaction with protein A (pA). Because direct measurements of binding with 125I-labeled hlgG (hlgG* or hlgG-Fc* (hlgG-Fc*)) showed no difference between ALAM(GLA) and PVC, the phenomenon was not due to the second step in the system of two-site IRMA (an Ag–Ab reaction (Ab refers to antibody)). These results indicated that the phenomenon was due to the first step (the adsorption of F3-hlgG to a solid phase). When the concentration of F3-hlgG immobilized on the solid phases was lowered, a significant increase in the dose response was observed for ALAM(GLA).

Keywords plasma-polymerization; allylamine film; adsorption; polyvinyl chloride; immunoradiometric assay; antibody; F(ab′)_2 fragment

Introduction

Recently, the plasma-polymerization technique has been used to provide new substrata for a variety of sensors and immunoassays. In a previous paper, adsorption and desorption of F(ab′)_2 anti-human immunoglobulin (F3-hlgG) on a plasma-polymerized allylamine (ALAM) was investigated. F3-hlgG was physically adsorbed to ALAM(GLA); GLA refers to a flat glass plate. The ALAM(GLA) adsorbed much more goat F3-hlgG and desorbed less protein than the GLA did. It is well known that the assay sensitivity increases with increasing amounts of antibody (Ab) on a solid phase in two-site immunoradiometric assay (IRMA). In fact, the use of ALAM(GLA) as a solid phase provided better dose response in the two-site IRMA of human IgG (hlgG) in comparison with GLA, and the ALAM(GLA) was practical for use as a solid phase in the two-site IRMA of human serum IgG.

The adsorption of F3-hlgG to ALAM(GLA) was much more than to polyvinyl chloride plate (PVC), which is one of the plastics often used as a solid phase in various immunoassays; hence, it was expected that ALAM(GLA) was a better solid phase than PVC in two-site IRMA. Contrary to this expectation, no significant difference was observed between ALAM(GLA) and PVC in the magnitude of dose response for hlgG. However, decreasing the concentration of F3-hlgG led to a significant difference in dose response between these solid phases.

Experimental

Materials The following materials were obtained as indicated: Na[125]I and 125I-labeled protein A (pA*) from Amersham, UK; ALAM from Wako, Japan; goat F3-hlgG from Cappel, U.S.A.; hlgG from Sigma, U.S.A.; Fc fragment of hlgG (hlgG-Fc) from Jackson, U.S.A.; Block ace® (BA), from Dainippon, Japan; GLA (7 × 5 × 0.15 mm) from Matsunami, Japan; PVC (7 × 5 × 1 mm) from Kasai, Japan.

Plasma Polymerization ALAM film was plasma-polymerized on a flat glass plate as described previously. Iodination of F3-hlgG, hlgG, and hlgG-Fc F3-hlgG, hlgG, and hlgG-Fc were iodinated with Na[125]I by modification of the chloramine-T method as described previously. The reaction buffer was substituted at the end for the sodium phosphate buffer (pH 7.3). The percentage incorporation of radioactivity into protein was 94–98%. Specific radioactivities of labeled F3-hlgG (F3-hlgG*), hlgG*, and hlgG-Fc* were 5.4, 7.7 and 8.0 × 10⁶ cpm mg⁻¹, respectively. Protein concentration was estimated as described previously.

Adsorption of F3-hlgG* to ALAM(GLA) or PVC The adsorption experiments were performed by the previous method. Briefly, the solid phases were incubated with F3-hlgG* (0.3 ml) of different concentrations for 2 h at 25°C, then the concentration of F3-hlgG* solution and the surface concentration of F3-hlgG* on the solid phases after they were washed four times with 1 ml of PBS-N (Dulbecco’s phosphate buffered saline (−), 0.02% NaN₃) were calculated from the radioactivity measured with a gamma counter.

Dose–Response Curves for hlgG and hlgG-Fc Dose–responses were obtained by the method described previously. Experiments were performed at 25°C. The hlgG (0–1 mg ml⁻¹) or hlgG-Fc (0–0.3 mg ml⁻¹) were diluted 1:200 with 1:10 BA-N (BA containing 0.02% NaN₃), and these antigen (Ag)-solutions (0.3 ml) of different concentrations were incubated for 2 h with ALAM(GLA) or PVC pre-coated with F3-hlgG. Then, the Ag-solution was removed and diluted pA* (0.3 ml) was added to the washed pieces. After further incubation (19 h), the radioactive solution was removed and the bound pA* on the washed pieces was determined by the radioactivity. The pieces were washed as above. The molecular weight of pA was assumed to be 42 kilodaltons (kDa). BA was used as a blocking agent for nonspecific binding.

Binding of 125I-Labeled Protein to Solid-Phase-Supported F3-hlgG ALAM(GLA) and PVC coated with F3-hlgG (1 mg ml⁻¹) were incubated for 2 h at 25°C, with hlgG* or hlgG-Fc* (0.3 ml) at different concentrations in test tubes pre-coated with BA. The bound hlgG* and hlgG-Fc* were determined by counting bound radioactivity on the solid phases. The molecular weight of hlgG and hlgG-Fc were assumed to be 150 and 50 kDa, respectively.

Effect of F3-hlgG Concentrations on Dose Response ALAM(GLA) and PVC were coated with F3-hlgG of different concentrations (0.01–1 mg ml⁻¹). After they were washed, hlgG (0.05, 0.1 mg ml⁻¹) diluted 1:200 with 1:10 BA-N was added to the test tubes. Other experimental conditions and procedures were similar to those described above.

Results and Discussion

In order to make our explanation clearer, the present system of two-site IRMA is presented first. It consists of three steps: the first step is adsorption of F3-hlgG to a solid phase, the second step is an Ag–Ab reaction and the final step is the reaction between pA* and the Ag.

Figure 1 shows that ALAM(GLA) adsorbed the protein more than PVC. This result was independent of the increase
of surface area of ALAM(GLA), since the observation of a scanning electron microscope indicated that ALAM(GLA) was flatter and smoother than PVC (data not shown). The desorption of $F_2$hlG from PVC similarly to the real two-site IRMA was investigated as described previously. There was no significant difference of desorption between ALAM(GLA) and PVC (data not shown). These results led to the expectation that ALAM(GLA) was a better solid phase than PVC.

However, Fig. 2a shows no difference in the dose response for hlG between PVC and ALAM(GLA) coated with 1 mg·mL$^{-1}$ $F_2$hlG. No difference was observed, either, when the concentration of the protein was lowered to 0.3 mg·mL$^{-1}$ (data not shown).

The dose–response curve for hlG-Fc on ALAM(GLA) was also identical with that on PVC (Fig. 2b). This implies the independence of molecular size of the second layer. It was suspected that if the size of the Ag was large, the availability of the Ag to the Ab and/or pA would become limited. This, however, was not the case.

Another possibility is that the binding of pA* with the Ag would be limited, although the levels of the bound Ag differed between ALAM(GLA) and PVC. In order to find out how many Ags were bound to the solid-phase-supported $F_2$hlG, the binding of hlG* or hlG-Fc* to $F_2$hlG on ALAM(GLA), PVC, and GLA were measured directly. hlG* and hlG-Fc* displayed similar binding behavior (Fig. 3). The bound hlG* to $F_2$hlG on ALAM(GLA) was identical with that on PVC. The same result was obtained for hlG-Fc. In addition, the binding of hlG* was the same as that of hlG-Fc* on each solid phase coated with $F_2$hlG. However, the binding of pA* to hlG was larger than to hlG-Fc (Fig. 2). These results suggest that the affinity of pA* for hlG-Fc is smaller than that for hlG. It is known that the $K_d$ between rabbit IgG-Fc fragment and pA (fragment B) is $3.3 \times 10^{-9}$ mol·L$^{-1}$, which is larger than the value ($1 \times 10^{-9}$ mol·L$^{-1}$ $7.9 \times 10^{-9}$ mol·L$^{-1}$) for rabbit IgG.

These results indicate that the phenomenon does not depend on the second step in this assay nor the molecular weight of the Ag. Therefore, it was considered that the first step, adsorption of $F_2$hlG, influenced this phenomenon. To investigate this influence, the solid phases were coated with $F_2$hlG of varying concentrations. Figure 4 shows...
that the dose response on ALAM (GLA) increased as the concentration of $F_2\text{hIgG}$ increased, and reached a constant level at ca. 0.1 mg·ml$^{-1}$ $F_2\text{hIgG}$ or tended to decrease slightly in the more concentrated region. Figure 1 shows that the adsorption of $F_2\text{hIgG}^*$ to this solid phase was saturable at more than 0.9 mg·ml$^{-1}$. On the other hand, the dose response on PVC increased as the concentration of $F_2\text{hIgG}$ increased and reached a plateau at 0.3–0.4 mg·ml$^{-1}$ $F_2\text{hIgG}$. For PVC, a plateau was observed at more than 0.4 mg·ml$^{-1}$. These results indicate the presence of a peculiar interaction between ALAM(GLA) and (labeled) $F_2\text{hIgG}$, but the cause of this peculiar interaction is unknown at present.

When the concentration of $F_2\text{hIgG}$ was more than 0.3–0.4 mg·ml$^{-1}$, there was no significant difference of dose response between on ALAM(GLA) and on PVC. The difference of dose response between these solid phases increased as the concentration of coating protein decreased (Fig. 4). Figure 5 shows the clear difference in dose response on ALAM(GLA) and PVC when they were coated with 0.01 mg·ml$^{-1}$ $F_2\text{hIgG}$. Through Figs. 2a and 5, the bound pA* did not diminish extremely, even if the concentration of $F_2\text{hIgG}$ was low. For PVC, this binding decreased to about 1/4, when the concentration of hIgG was $10^{-2}$ nmol·l$^{-1}$. This result indicates that the consumption of expensive $F_2\text{hIgG}$ was repressed.

Molecular interpretation of the finding reported here requires further investigations. In addition, the question of whether a similar finding will be observed for other solid phases and for other Ag–Ab reactions should be examined.

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