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

Makoto MURATSUGU,*[,a] Shigeru KUROSAWA,[b,1)] Yoshihito MORI,[c] and Naoki KAMO[b]

Department of Laboratory Medicine, Asahikawa Medical College,[a] Asahikawa 078, Japan, Faculty of Pharmaceutical Sciences, Hokkaido University,[b] Sapporo 060, Japan, and Institute for Molecular Science,[c] Myodaiji, Okazaki 444, Japan. *Received June 24, 1991*

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 immunogloblin G (referred to as $F_{\bar{a}}$hIgG) 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 (hIgG) 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) (hIgG or Fc fragment of hIg G (hIgG-Fc)) and also of the reaction with protein A (pA). Because direct measurements of binding with ^{125}I-labeled hIgG (hIgG*) or hIgG-Fc (hIgG-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 $F_{\bar{a}}$hIgG to a solid phase). When the concentration of $F_{\bar{a}}$hIgG 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[2)] and immunoassays.[3)] In a previous paper,[3)] adsorption and desorption of $F(ab')_2$ anti-human immunogloblin ($F_{\bar{a}}$hIgG) on a plasma-polymerized allylamine (ALAM) was investigated. $F_{\bar{a}}$hIgG was physically adsorbed to ALAM(GLA): GLA refers to a flat glass plate. The ALAM(GLA) adsorbed much more goat $F_{\bar{a}}$hIgG 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).[4)] In fact, the use of ALAM(GLA) as a solid phase provided better dose response in the two-site IRMA of human IgG (hIgG) 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.[3)]

The adsorption of $F_{\bar{a}}$hIgG 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[5)]; 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 hIgG. However, decreasing the concentration of $F_{\bar{a}}$hIgG 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 ^{125}I-labeled protein A (pA*) from Amersham, UK; ALAM from Wako, Japan; goat $F_{\bar{a}}$hIgG from Cappel, U.S.A.; hIgG from Sigma, U.S.A.; Fc fragment of hIgG (hIgG-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.[3)]

Iodination of $F_{\bar{a}}$hIgG, hIgG, and hIgG-Fc $F_{\bar{a}}$hIgG, hIgG, and hIgG-Fc were iodinated with Na^{125}I by a modification of the chloramine-T method[6)] as described previously.[3)] The reaction buffer was substituted at the end for the sodium phosphate buffer (I=0.15, pH 7.3). The percentage incorporation of radioactivity into protein was 94—98%. Specific radioactivities of labeled $F_{\bar{a}}$hIgG ($F_{\bar{a}}$hIgG*), hIgG*, and hIgG-Fc* were 5.4, 7.7 and 8.0×10^7 cpm·mg^{-1}, respectively. Protein concentration was estimated as described previously.[3)]

Adsorption of $F_{\bar{a}}$hIgG* to ALAM(GLA) or PVC The adsorption experiments were performed by the previous method.[3)] Briefly, the solid phases were incubated with $F_{\bar{a}}$hIgG* (0.3 ml) of different concentrations for 2 h at 25 °C, then the concentration of $F_{\bar{a}}$hIgG* solution and the surface concentration of $F_{\bar{a}}$hIgG* on the solid phases after they were washed four times with 1 ml of PBS-N (Dulbecco's phosphate buffered saline (−), 0.02% NaN_3) were calculated from the radioactivity measured with a gamma counter.

Dose–Response Curves for hIgG and hIgG-Fc Dose–responses were obtained by the method described previously.[3)] Experiments were performed at 25 °C. The hIgG (0—1 mg·ml^{-1}) or hIgG-Fc (0—0.3 mg·ml^{-1}) were diluted 1 : 200 with 1 : 10 BA-N (BA containing 0.02% NaN_3), and these antigen (Ag)-solutions (0.3 ml) of different concentrations were incubated for 2 h with ALAM(GLA) or PVC pre-coated with $F_{\bar{a}}$hIgG. 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.[3)]

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

Effect of $F_{\bar{a}}$hIgG Concentrations on Dose Response ALAM(GLA) and PVC were coated with $F_{\bar{a}}$hIgG of different concentrations (0.01—1 mg·ml^{-1}). After they were washed, hIgG (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 clear, the present system of two-site IRMA is presented first. It consists of three steps: the first step is adsorption of $F_{\bar{a}}$hIgG 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

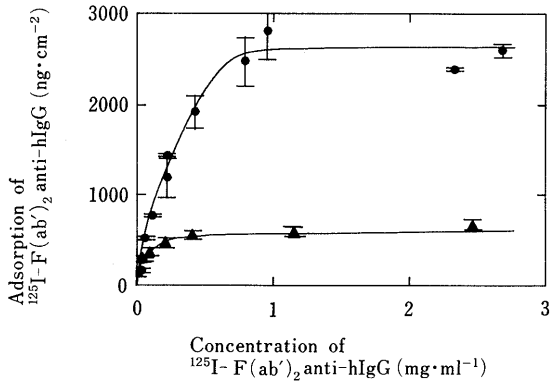

Fig. 1. Adsorption Isotherms of $F_{\bar{a}}$hIgG

●, ALAM (GLA); ▲, PVC. The vertical bars designate the standard deviation for the mean of duplicate.

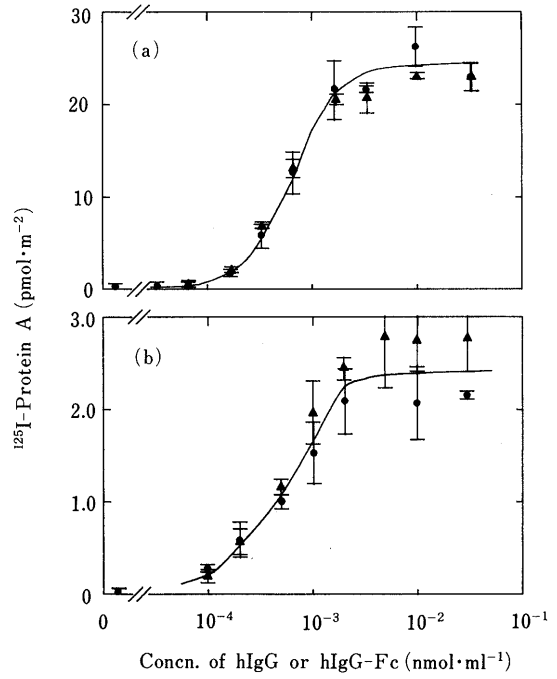

Fig. 2. Dose–Response Curves for hIgG (a) and hIgG-Fc (b) on the Solid Phases Binding a High Amount of $F_{\bar{a}}$hIgG

The solid phases coated with 1 mg·ml^{-1} $F_{\bar{a}}$hIgG. ●, ALAM (GLA); ▲, PVC. The vertical bars designate the standard deviation for the mean of duplicate.

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 $F_{\bar{a}}$hIgG* from PVC similarly to the real two-site IRMA was investigated as described previously.[3] 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 hIgG between PVC and ALAM(GLA) coated with 1 mg·ml^{-1} $F_{\bar{a}}$hIgG. 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 hIgG-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

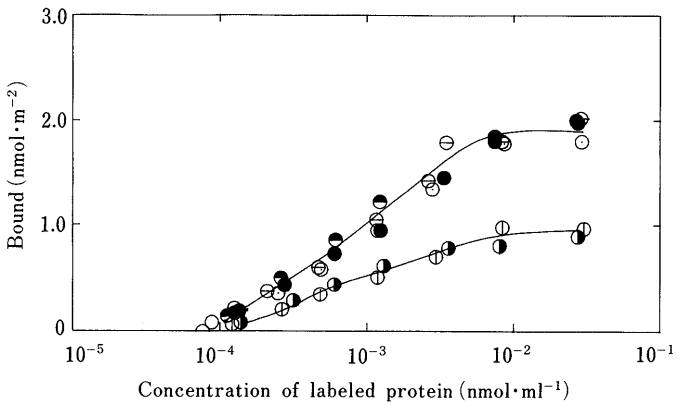

Fig. 3. Binding of ^{125}I-Labeled Protein to Solid-Phase-Supported $F_{\bar{a}}$hIgG

ALAM (GLA): ^{125}I-hIgG (○), ^{125}I-hIgG-Fc (●); PVC: ^{125}I-hIgG (⊖), ^{125}I-hIgG-Fc (◓); GLA: ^{125}I-hIgG (◍), ^{125}I-hIgG-Fc (◑).

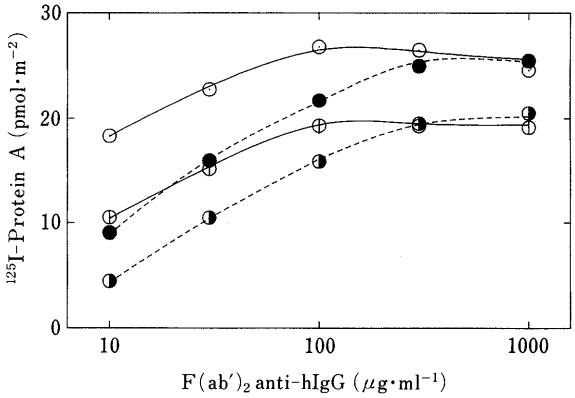

Fig. 4. Effect of $F_{\bar{a}}$hIgG Concentrations on Dose Response

Solid and broken curves are for ALAM (GLA) and PVC, respectively. ○, ●, 3.33×10^{-3} nmol·ml^{-1}hIgG; ◍, ◑, 1.67×10^{-3} nmol·ml^{-1} hIgG.

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 $F_{\bar{a}}$hIgG, the binding of hIgG* or hIgG-Fc* to $F_{\bar{a}}$hIgG on ALAM(GLA) , PVC, and GLA were measured directly. hIgG* and hIgG-Fc* displayed similar binding behavior (Fig. 3). The bound hIgG* to $F_{\bar{a}}$hIgG on ALAM(GLA) was identical with that on PVC. The same result was obtained for hIgG-Fc. In addition, the binding of hIgG* was the same as that of hIgG-Fc* on each solid phase coated with $F_{\bar{a}}$hIgG. However, the binding of pA* to hIgG was larger than to hIgG-Fc (Fig. 2). These results suggest that the affinity of pA* for hIgG-Fc is smaller than that for hIgG. It is known that the K_D between rabbit IgG-Fc fragment and pA (fragment B) is 3.3×10^{-7} mol·l^{-1},[7] which is larger than the value (1×10^{-10} mol·l^{-1},[8] 7.9×10^{-9} mol·l^{-1}[9]) 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 $F_{\bar{a}}$hIgG, influenced this phenomenon. To investigate this influence, the solid phases were coated with $F_{\bar{a}}$hIgG of varying concentrations. Figure 4 shows

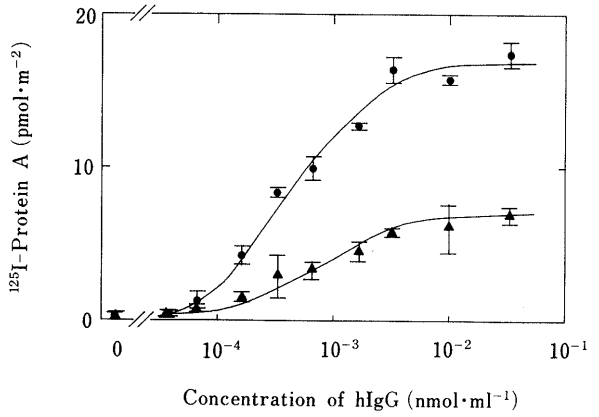

Fig. 5. Dose–Response Curves for hIgG on the Solid Phases Binding a Low Amount of $F_{\bar{a}}$hIgG

The solid phases were coated with 0.01 mg·ml^{-1} $F_{\bar{a}}$hIgG. ●, ALAM (GLA); ▲, PVC. The vertical bars designate the standard deviation for the mean of duplicate.

that the dose response on ALAM (GLA) increased as the concentration of $F_{\bar{a}}$hIgG increased, and reached a constant level at *ca.* 0.1 mg·ml^{-1} $F_{\bar{a}}$hIgG or tended to decrease slightly in the more concentrated region. Figure 1 shows that the adsorption of $F_{\bar{a}}$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 $F_{\bar{a}}$hIgG increased and reached a plateau at 0.3—0.4 mg·ml^{-1} $F_{\bar{a}}$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) $F_{\bar{a}}$hIgG, but the cause of this peculiar interaction is unknown at present.

When the concentration of $F_{\bar{a}}$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} $F_{\bar{a}}$hIgG. Through Figs. 2a and 5, the bound pA* did not diminish extremely, even if the concentration of $F_{\bar{a}}$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 $F_{\bar{a}}$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.

Acknowledgment We are grateful to Ibaraki Research Laboratory, Hitachi Chemical Co., Ltd. for financial support; to Mr. T. Nagahara, Laboratory for Radio-Active Isotope Research, Asahikawa Medical College for his expert technical help. We are also obliged to Mr. Marc N. Weedon-Newstead for correcting the English in the manuscript. This work was partially supported by the Shimadzu Science Foundation.

References

1) Present address: *National Chemical Laboratory for Industry, 1–1 Higashi, Tsukuba, Ibaraki 305, Japan.*
2) H. Nakajima, M. Esashi, and T. Matsuo, *Nippon Kagaku Kaishi*, **1980**, 1499; S. Tahara, M. Yoshii, and S. Oka, *Chem. Lett.*, **1982**, 307; N. Yamamoto, S. Nagaoka, T. Tanaka, T. Shiro, K. Honma, and H. Tsubomura, "Proceedings of the International Meeting on Chemical Sensors," Kodansha and Elsevier, Tokyo, New York, and Amsterdam, 1983, pp. 699—704; G. Kamprath and R. Hintsche, *Anal. Lett.*, **22**, 2423 (1989); N. Hazemoto, S. Isizaka, M. Haga, Y. Kato, S. Kurosawa, N. Kamo, and Y. Kobatake, *Chem. Pharm. Bull.*, **37**, 2153 (1989); S. Kurosawa, N. Kamo, Y. Kobatake, and I. Toyoshima, *J. Membr. Sci.*, **46**, 157 (1989).
3) M. Muratsugu, S. Kurosawa, and N. Kamo, *J. Colloid Interface Sci.*, **147**, 378 (1991).
4) S. Dakubu, R. Ekins, T. Jackson, and N. J. Marshall, "Practical Immunoassay," ed. by W. R. Butt, Marcel Dekker, New York, 1984, p. 71.
5) S. Jackson, J. A. Sogn, and T. J. Kindt, *J. Immunol. Methods*, **48**, 299 (1982); P. Lems-Van Kan, H. W. Verspaget, and A. S. Peña, *ibid.*, **57**, 51 (1983).
6) A. E. Bolton and W. M. Hunter, "Handbook of Experimental Immunology," Vol. 1, ed. by D. M. Weir, L. A. Herzenberg, C. Blackwell, and L. A. Herzenberg, Blackwell, Oxford, 1986, p. 26.1.
7) D. Lancet, D. Isenman, J. Sjödahl, and I. Pecht, *Biochem. Biophys. Res. Commun.*, **85**, 608 (1978).
8) E. O'Keefe and V. Bennett, *J. Biol. Chem.*, **255**, 561 (1980).
9) M. Sandor and J. J. Langone, *Biochem. Biophys. Res. Commun.*, **100**, 1326 (1981).