ANTIGEN-ANTIBODY REACTION BY MONOLAYER TECHNIQUE EXPERIMENTS WITH RABBIT IgG ANTIBODY AND ITS FRAGMENT

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SUMMARY

With the monolayer technique, values of about 99.7 Å for rabbit anti-egg albumin IgG antibody, and about 58.9 Å for Fab fragment of IgG were obtained as the thickness of the adsorbed antibody layer. As these values determined in the saturated state were confirmed, it may be reasonable to assume that they correspond approximately to the long axis of both molecules.

INTRODUCTION

Langmuir's monolayer technique has been applied by many investigators in observing various biological phenomena, and fundamental knowledge concerning antigen-antibody reaction has been thus obtained (1-8). The thickness of the adsorbed layer on the metal slide was usually determined by the method of Blodgett and Langmuir (9) or the ellipsometer (7).

The present authors have reported that the maximal thickness of the adsorbed antibody layer was 250-270 Å by using atoxyl-azo-proteins and their rabbit antisera, and never exceeded this value even when strong antisera with a high titer were used (6). However, still some ambiguities remained concerning the exact evaluation of the above figure. These points in mind, the authors reinvestigated the antigen-antibody reaction on monolayer film by using the purified rabbit IgG antibody fraction and its fragment.

MATERIALS AND METHODS

Antigens and antisera: Hen egg albumin (Ea) and human hemoglobin A (Hb-A) recrystallized according to the method of Drabkin (10) were used as antigens. One ml of Ea (10 mg/ml) was immunized into albino rabbits with Freund's complete adjuvant technique, and the antisera with high antibody titer were obtained from several rabbits (RAEa-P-1). Sheep anti-rabbit IgG antiserum (SAR IgG) was also prepared in the same manner. IgG and its fragments: Normal and anti-Ea (RAEa) IgG fractions were purified from γ-globulin fractions of both normal sera and antisera precipitated with ammonium sulfate on DEAE-cellulose column chromatography (11). The amount of IgG was assayed by the OD_{280} absorption and also the single radial immunodiffusion method. IgG fragments were prepared by papain treatment according to the method of Porter (12) and separated chromatographically into Fraction (Fr) I, II (Fab) and III (Fc). Complement: Purified Cl prepared from guinea pig complement was supplied by Dr. N. Tamura of the National Cancer Center Research Institute, Tokyo. The preparation showed roughly a 5-fold increase in

Abbreviations: IgG, immunoglobulin G; Ea, hen egg albumin; SAR IgG, sheep anti-rabbit IgG antisera; RAEa-P-1 and RAEa IgG, pooled rabbit anti-Ea antiserum No. 1 and its IgG fraction; gpC and Cl, guinea pig complement and its first component

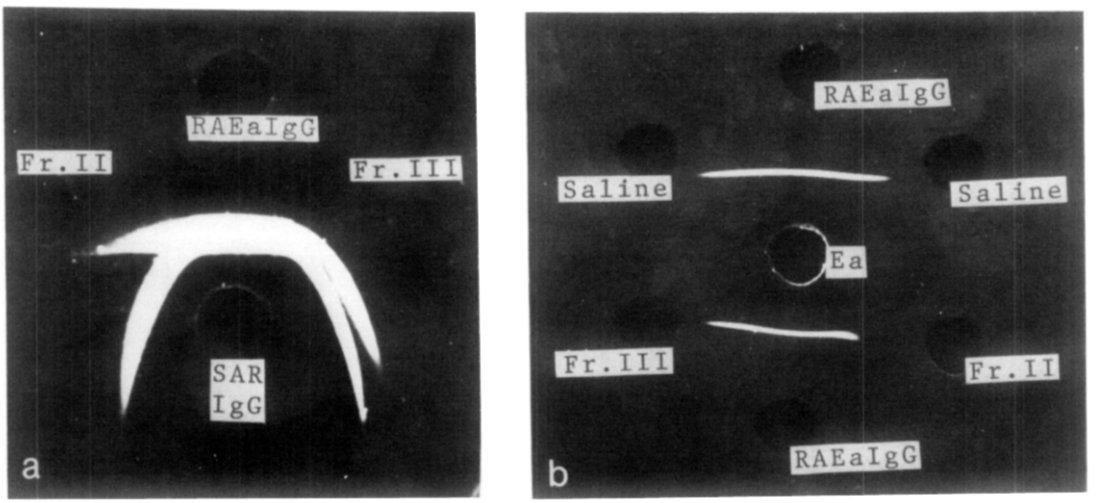

Fig. I Ouchterlony tests with the materials employed

activity over that in the original complement and it was preserved at −70° C until use. Immunodiffusion and quantitative precipitin tests: For a check of the serological nature of the materials and determination of their amount, double diffusion (Ouchterlony) and single radial immunodiffusion tests were done according to the conventional methods (13). Quantitative precipitin test was also carried out and the concentration of proteins was determined by OD at 280 nm. Ultra centrifugation: Analytical ultracentrifugation was performed by UCA-1A (Hitachi) through the kind assistance of Dr. T. Kameyama, Faculty of Medicine, Juntendo University, Tokyo. Monolayer technique: The metal slide coated with a built-up film of barium stearate and conditioned with a 10^{-3} molar solution of thorium nitrate was dipped into 1:10 dilution of antigen stock solution. Thirty minutes later, the slide was taken out and the thickness of the antigen layer was measured optically using the method of Blodgett and Langmuir (9). Then the slide was again dipped into various concentrations of antibody solution. After 20 hours, the increase of thickness by the adsorption of the antibody was measured. The whole procedure was carried out at 5° C except for optical measurements.

RESULTS

1. The nature of the serological systems employed The protein concentration of RAEa IgG was 36.1 mg/ml by OD_{280} ($E^{1\%}_{1cm}$ =13.8 in standard value), and this value was almost the equivalent of twice the IgG or antibody content in the original antiserum when compared with the single radial immunodiffusion test. The antibody content of RAEa IgG was 2.4 mg protein/ml by the quantitative precipitin test. The protein concentration of Fr I, II and III were 0.17, 6.0 and 3.3 mg/ml, respectively. In our IgG preparation, the absence of aggregated IgG was confirmed by the analytical ultracentrifuge pattern and $S_{20,w}$=6.4 was obtained. By the Ouchterlony test, RAEa IgG and its Fr II showed only one precipitin line against SAR IgG and a spur was observed from RAEa IgG side (Fig. Ia). Further, only one precipitin line was seen between RAEa IgG and

Table 1 The thickness of antigen and antibody layers (Å)

Antigen		Further increase of thickness with			
		normal IgG	RAEa IgG	Fr II	RAEa-P-1
Ea	41.4 ± 1.9	5.5 ± 1.0	99.7 ± 1.9	58.9 ± 2.3	248.7 ± 1.5
Hb-A	45.1 ± 3.1		3.3 ± 1.6		

Table 2 The effect of dilution of antibody reactants and of the addition of complement reagents on the thickness of antibody layer (Å)

Treatment		RAEa IgG	Fr II
Undiluted		99.7 ± 1.9	58.9 ± 2.3
Diluted into	1:2	97.6	57.6 ± 1.8
"	1:4	62.8	
"	1:8	53.5 ± 1.1	
Antibody reactants alone		99.7	58.9
"	+ gpC*	" +91.2	" +0
"	+ Cl**	" +92.0	" +0

* gpC was applied after the reaction of antibody on antigen layer.

**One fourth aliquots of complement reagents were added into undiluted antibody reactants.

Ea, but the line was inhibited at the side of Fr II (Fig. Ib). This suggests the presence of the non-precipitating antibody in Fr II, but not in the other fragment tested. For this reason and because of the higher protein concentration, Fr II was mainly used in subsequent experiments as the source of Fab fragment.

2. Reaction of RAEa IgG to the adsorbed film of antigens As shown in Table I, the adsorption of Ea on the conditioned surface of the metal slide gave a 41.4 Å increase in thickness. The adsorption of RAEa IgG on this antigen film caused a further increase of 99.7 Å. When original antiserum, instead of RAEa IgG, was adsorbed on the Ea antigen film, the total thickness

was 248.7 Å. The average thickness of Hb-A film was 45.1 Å, but RAEa IgG scarcely reacted on this heterologous antigen layer and the increase of thickness was as little as 3.3 Å. Furthermore, the thickness of non-specific adherence of normal rabbit IgG on Ea film was 5.5 Å and it appears to be negligibly small.

The application of Fr. II (Fab) on the antigen layer caused a 58.9 Å increase in thickness.

3. The relationship between the concentration of antibody solution and the thickness of the antibody layer. The reactivity of Cl on Ea-RAEa IgG layer Observations were made on the relationship between the serial 2-fold dilution of RAEa IgG and the thickness of the antibody layer obtained. The value observed by the 1:2 dilution of RAEa IgG was 97.6 Å and almost the same as that obtained by the undiluted one. A marked decrease in thickness was observed when IgG was diluted into 1:4 and 1:8 (Table II). The thickness of the Fr II-Ea layer was not too different between the 2-fold dilution of Fr II and the undiluted one.

When the slide covered with antigen film was dipped into 4:1 mixtures of RAEa IgG and Cl, the increase in the adsorbed layer was about 92.0 Å greater than that obtained by the IgG antibody alone. When the slide with antigen was dipped into a mixture of Fr II and Cl, the total thickness of the adsorbed layer was about 59.0 Å which was the same as that obtained by Fr II alone. On the other hand, the separated application of undiluted gpC on the Ea-RAEa IgG layer gave a further increase of about 91.2 Å to the thickness, but no increase was observed in the Ea-Fr II layer.

DISCUSSION

In the present experiment, the adsorbed antigen layer was used in an effort to avoid the influence of the conformational change of the antigen, though monomolecular and adsorbed films are usually used in the study of antigen-antibody reaction by the monolayer technique.

The properties of Fr II resembled those of the non-precipitating antibody, since it did not show any precipitin line against Ea with the Ouchterlony test, but it inhibited the formation of the precipitin line between RAEa IgG and Ea. For this reason, Fr II may consist of Fab but does not contain intact IgG or even if it does the amount is negligibly small. In a previous paper (6), it was reported that the thickness of the adsorbed layer is proportional to the average density of the adsorbed molecule and this also corresponds to one of the dimensions of the adsorbed molecule when measured in the state of saturation.

The thickness of the antibody layer obtained by RAEa IgG was about 99.7 Å and this was almost the same as that obtained by 1:2 dilution. Therefore, this value appears to be obtained in the saturated state and indicates one dimension of the IgG antibody. In this case, the influence of the aggregated IgG antibody on the value need not be considered, since its absence in the employed antibody preparation was confirmed by analytical ultracentrifugation. In the case of the IgG antibody, even though the Fab portions are closely packed together, it is not clear whether the corresponding Fc portions also are packed closely enough to reach its saturated density or not, because in the IgG molecule one Fc portion binds with a pair of the Fab portions. In addition, as the Fab portions bind Fc having some angle at the hinge portion, there is a possibility that the angle and flexibility of the hinge portion may also prescribe the thickness of the antibody layer of the upright standing Fab or the inclined Fab. So, it may safely be said that the saturated value observed with the IgG antibody corresponds roughly to the long axis of the IgG molecule.

By electron microscopy, Valentine et al. (14) reported that the dimensions of the Fab and Fc molecules from rabbit IgG antibody are 60x35 Å and 45x40 Å, respectively. The value in the present study coincided almost with the sum of the long axis of Fab and Fc values of electron microscopy. But, there are other reports relating to the dimension of these molecules, according to which the overall dimensions of the Fab' molecule from purified human myeloma IgGI have been calculated at 80x50x40 Å by crystallographic analysis (15) and the values of 98x42x42 and 99x47x47 Å for human Fab and Fc were reported by low angle X-ray scattering (16).

Difference between the values in Ea-RAEa IgG and Ea-original antiserum combinations (99.7 and 248.7 Å, respectively) will be discussed precisely elsewhere, though from the present study, a part of the difference can be attributed to the fixation of the complement components (Cl) remaining in the antiserum (even after the heat inactivation).

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