IMMUNOLOGIC AND ENZYMATIC REACTIONS CARRIED OUT AT A LIQUID-SOLID INTERFACE

Some eight years ago a new method called immunoelectroadsorption was devised in this laboratory to detect immunologic reactions (1). It consists in adsorbing on a metallized glass slide with the help of a weak current (0.3 mA), a layer of antigen, the slide itself acting as one electrode. From the thickness adsorbed when such an antigen-coated slide is dipped into a homologous antiserum, it can be determined whether or not an immune reaction has taken place. The thicknesses are of the order of 100 Å, determined with an ellipsometer. It is possible to detect immunologic reactions with solutions of antigen as weak as 10^{-13} g/ml, if the nickel-plated slides used have been "activated" by placing them in a magnetic field of a few thousand gauss with the lines of force perpendicular to the slide, the metallized surface facing the south magnetic pole. The "activation" can also be obtained by evaporation of the nickel layer in the magnetic field properly oriented. "Inactivation" of an "active" slide is possible by submitting the slide to a magnetic field with the lines of force parallel to the surface or perpendicular to it with the metallized surface facing the north magnetic pole. Activation is a reversible phenomenon (2).

A specific adsorption of antibodies or tryptic action can be observed when a drop of an antiserum or of a trypsin solution is placed on top of a Formvar membrane 50–200 Å thick protecting a densely packed layer of antigen deposited on an active slide. Tryptic action is detected in the following way: after washing off the trypsin drop and dissolving the blanket, the thickness that can be adsorbed from an antiserum is much smaller on the area of the slide where the trypsin drop was deposited on top of the membrane (see column 6 of Table I). In other words, the antigen layer has been sufficiently hydrolyzed to prevent its interaction with antibodies. No adsorption of antibodies or trypsin action is observable, if the experiment is repeated under the same conditions with an inactive slide instead of an active one. To observe this long-range interaction through a Formvar membrane, the concentration of the antigen solutions used for the adsorption should be $> 10^{-6}$ g/ml, or the antigen should consist of densely packed layers formed on the surface of a Langmuir trough and transferred to the slide. Some of the results obtained have been condensed in Table I. It is apparent that tryptic action can occur on an active slide through a membrane over 200 Å thick since, after the dissolution of the membrane, from 9 to 14 Å could be adsorbed from an antiserum on the area previously occupied by trypsin compared with 33–44 Å on the area not treated by trypsin. The figures of the lower part of the table, show that antibodies can be specifically adsorbed on top of a membrane only if the slides are active.

An important question to answer is the following. Where are the antibodies located after adsorption in the presence of a membrane? Are they immobilized on the outer surface of the membrane or have they diffused through a forced diffusion process? Forces other than those due to a concentration gradient would be involved in both cases, since no adsorption occurs if the slide is inactive. The most recent data are in favor of the view that the antibodies remain on the outer surface of

TABLE I

TRYPTIC ACTION AND IMMUNOLOGIC INTERACTION THROUGH A FORMVAR MEMBRANE

Slide no.	Slide condition	Concn. bovine albumin g/ml 2′ or transferred layers	Formvar thickness	Trypsin treatment	Antibody thickness adsorbed after removal of membrane	Antibody thickness adsorbed before removal of membrane
			Å		Å	Å
1	Active	10^{-3}	240	Yes	9	—
				No	45	—
2	Inactive ⊥N2′	10^{-3}	130	Yes	33	—
				No	33	—
3	Active	10^{-4}	170	Yes	13	—
				No	39	—
4	Active	$(\downarrow\uparrow)_3$	198	Yes	14	—
				No	65	—
5	Inactive ‖2′	$(\downarrow\uparrow)_3$	205	Yes	75	—
				No	65	—
6	Inactive ⊥N2′	$(\downarrow\uparrow)_4$	130	Yes	58	—
				No	61	—
7	Active	$(\downarrow\uparrow)_4$	52	—	—	50
8	Inactive ‖2′	$(\downarrow\uparrow)_4$	63	—	—	15
9	Active	None	61	—	—	21
10	Active	$(\downarrow\uparrow)_4$	88	—	—	52
11	Inactive ‖2′	$(\downarrow\uparrow)_4$	57	—	—	21
12	Active	$(\downarrow\uparrow)_4$	211	—	—	44
13	Inactive ‖2′	$(\downarrow\uparrow)_4$	145	—	—	0

‖2′ means that the magnetic field was applied for 2′ with its lines of force parallel to the surface of the slide. ⊥N2′ indicates that the field was applied for 2′ with the lines of force perpendicular to the slide, the metallized surface facing the north magnetic pole.
$(\downarrow\uparrow)_3$ or $(\downarrow\uparrow)_4$ indicates that 3 or 4 double layers of bovine albumin were transferred to the slide by successive immersions and emersions.

the membrane for the following reason: an active antigen-coated slide protected by a membrane, after adsorption of antibodies, is brought down to −40°C and treated with $C_2H_4Cl_2$. Not only is the membrane dissolved but the antibodies are also washed away. Antibodies adsorbed directly on an antigen-coated slide cannot be washed away with $C_2H_4Cl_2$ at any temperature. Once the membrane and antibodies have been removed, another membrane can be deposited followed by another specific adsorption from an antiserum. This cycle consisting in deposition of a membrane, adsorption of antibodies, and removal of membrane and antibodies could be repeated five times, with a total of 230 Å of antibodies adsorbed compared with 98 Å if the slide was treated directly with the antiserum. The antigen-antibody combination would be a sandwich-like complex, antigen and antibody being separated by the membrane. The fact that a long-range order of the nickel surface pro-

motes a long-range interaction indicates that cooperative action among the antigen molecules must occur and that one should consider the reaction of an assembly of molecules reacting as a whole. The best explanation for these interactions is to assume they involve specific Lifshitz-van der Waals forces.

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