RELATIONSHIP BETWEEN ADSORPTION OF IMMUNE ANTIBODIES AND THE CHEMICAL STRUCTURE OF THE ADSORBENTS

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Adsorption methods, widely used in various branches of chemistry, are now beginning to be used in immunological research. The laws governing the selective adsorption of the proteins of immune sera are still, however, inadequately studied. The work of Landsteiner and Stankovic [8], Eisler and Tsuru [6], Friedberger and Putter [7], Bleyer [2], Eisler [3, 4], Eisler and Spiegel-Adolf [5], V. N. Shreder [1], and Olitzki [9] has shown that typhoid and dysentery agglutinins, diphtheria and tetanus antitoxins from immune sera, and also hemagglutinins are adsorbed by activated charcoal, kaolin, kieselguhr, white clay, aluminum hydroxide, ferric hydroxide, and collargol. The adsorption of antibodies by these adsorbents was not selective, and other proteins were adsorbed along with the agglutinins from the immune sera. In Bleyer's [2] experiments, for instance, after adsorption of a typhoid immune serum with charcoal, no agglutinins could be found in it, and tests for proteins with sulfosalicylic acid were negative. According to V. N. Shreder's findings [1], 2 g of freshly prepared aluminum hydroxide completely adsorbed the hemagglutinins from 20 ml of serum, and 91-100% of the other proteins. We found no information on the relationship between the adsorption of normal and immune antibodies in the sera and the chemical structure of the adsorbents in the literature accessible to us.

In the present paper we describe the results of our investigations into the laws governing the adsorption of agglutinins by different adsorbents.

EXPERIMENTAL METHOD AND RESULTS

In the first place we investigated the relationship between the adsorption of antibodies and the chemical structure of the adsorbents. For this purpose we tested the agglutinin-adsorbing power of substances of differing chemical structure, only slightly soluble in water. The following compounds were studied: 1) those containing amino groups: benzidine, α-naphthylamine, thionine, and the anion exchange resins ÉDÉ 10 and ÉDÉ 10P; 2) those incorporating nitro groups: mononitronaphthalene trinitrotoluene, and 2,4-dinitrodiphenylamine; 3) those containing hydroxyl groups: β-naphthol, 8-hydroxyquinoline, phenol red, rosolic acid; 4) those containing quinone groups: naphthaquinone, anthraquinone; 5) those containing aldehyde groups: p-dimethylamidobenzaldehyde; 6) those containing carboxyl groups: naphthalenetetracarbonic acid, methyl orange; 7) those containing sulfo groups: naphthalene-1,6-disulfonic acid and the cation exchange resins KU-1 and KU-2; 8) those containing chlorine: p-dichlorobenzene, polyvinyl chloride, DDT, hexachlorocyclohexane; 9) organic compounds containing metals: dermatol, bismuth salicylate, bismuth citrate, carmine; 10) compounds not containing functional groups: naphthalene, acridine, anthracene; 11) compounds containing certain functional groups: salicylic acid, anthranilic acid, thymol blue, tyrosine, alizarin, α-aminoanthraquinone, α-aminohydroxyanthraquinone, 2,4-dinitrochlorobenzene, m-nitroaniline and 2,4-dinitrophenylhydrazine.

The experimental method was as follows: To 2 ml of crude agglutinating rabbits' sera, diluted 1:100 in physiological saline (pH = 7.2) was added 100 mg of the test substance in powder form, after it had first been sifted through a standard brass mesh of gauge 40μ. The sera with the adsorbents were kept at a temperature of 20° for 20 minutes and periodically stirred with a glass rod. After an exposure of 20 minutes, the mixture of adsorbent and serum was centrifuged to complete translucency, and the naked-eye agglutination test carried out with the supernatant serum in tubes by the usual method with the aid of formol diagnosticums. For control purposes, naked-eye agglutination tests were carried out with sera which had not been adsorbed with test substances. The results of the tests were read by means of a loupe. The difference between the titer of agglutinins in the unadsorbed serum and in the serum adsorbed with the test serum showed the adsorptive activity of this substance. When evaluating the results of adsorption of agglutinins, we took into consideration the fact that fine powders are capable of absorbing small quantities of protein irrespective of their chemical structure. If, therefore, after adsorption of an immune serum with a chemical substance, the intensity of agglutination was

TABLE 1. Relationship Between Adsorption of Typhoid H-Agglutinins and Chemical Structure of Adsorbents

Organic compounds	Functional groups	Agglutinin titers after adsorption					Diagnosticum control
		1:10	1:20	1:40	1:80	1:160	
β-naphthol, phenol red	Hydroxyl	++++ / ++++	++ / ++	± / ±	− / −	− / −	− / −
Naphthalene-1,6-disulfonic acid	Sulfo groups	++++	+++	−	−	−	−
Cation exchange resin KU-2		+++	±	−	−	−	−
Naphthalenetetracarbonic acid	Carboxyl	++++	+++	±	−	−	−
p-Dimethylamidobenzaldehyde	Aldehyde	++++	+++	+	−	−	−
Naphthoquinone	Quinone	++++	+++	−	−	−	−
Anthraquinone	"	++++	+++	−	−	−	−
Alizarin	Quinone and hydroxyl	++	+	−	−	−	−
α-aminoanthraquinone	Quinone and amino	++++	++++	+	−	−	−
α-aminohydroxyanthraquinone	Quinone, amino and hydroxyl	++++	+++	−	−	−	−
Mononitronaphthalene	Nitro groups	++++	+++	+	−	−	−
Trinitrotoluene		++++	+++	+	−	−	−
α-naphthylamine	Amino groups	++++	+++	++	±	−	−
Thionine		++++	+++	++	±	−	−
Anion exchange resin ÉDÉ 10P		++++	+++	++	±	−	−
Hexachlorocyclohexane	Chlorine	++++	++++	++	±	−	−
Naphthalene		++++	++++	++	±	−	−
Anthracene		++++	++++	++	±	−	−
Control serum (unadsorbed)		++++	++++	++	+	−	−

Note. In diluting the sera, both control and adsorbed, the initial dilutions (1:100) were taken as unity. Serum controls (dilution 1:100) were negative.

TABLE 2. Intensity of Adsorption of Agglutinins by Certain Substances from a "Flexner C" Rabbit Agglutinating Serum

Adsorbents	Weighed samples of adsorbent in mg	Serum agglutinin titers after adsorption					Weight of protein adsorbed in mg	Protein adsorbed as % of total serum protein
		1:32	1:64	1:1:8	1:256	1:512		
Alizarin	500	++++	++	–	–	–	8.30	24.48
β-naphthol	The same	++++	++++	++	–	–	7.03	20.65
Naphthalene-1,6-disulfonic acid	"	++++	++	–	–	–	6.10	17.997
Anion exchange resin EDE 10P	"	++++	++++	+++	+	–	11.4	33.62
Agglutinin titer of unadsorbed serum (control)	"	++++	++++	+++	++	–	–	–

Note. Adsorption was carried out on 2.5 ml of serum, diluted 1:5 with physiological saline; + designates the intensity of agglutination; – absence of agglutination. In the dilution of the control and adsorbed sera, the initial dilutions (1:5) were taken as unity. Controls of sera and diagnosticum negative.

reduced only by +, this adsorption was considered to be nonspecific, i.e., independent of the chemical structure of the substance. If, on the other hand, after adsorption of the serum the agglutinin titer fell by one dilution or more, then in this case the adsorption was regarded as specific for the particular chemical substance (the titer of a serum was taken to be agglutination with an intensity of + +).

Investigation of the adsorptive power of the substances listed showed that organic compounds in whose molecules there are no functional groups (naphthalene, anthracene), did not adsorb agglutinins. Similarly, substances in whose molecules there are functional chlorine and amino groups did not adsorb agglutinins. Substances containing hydroxyl, aldehyde, carboxyl, quinone, sulfo, and nitro groups possessed the property of adsorbing agglutinins (compounds listed in the second, third, fourth, fifth, sixth, seventh, ninth, and eleventh groups). The magnitude of adsorption of agglutinins from typhoid agglutinating sera by some organic compounds is shown in Table 1.

It will be seen from the data in Table 1 that the intensity of adsorption of agglutinins by the majority of the tested substances was insignificant, and the titer of the agglutinating serum after adsorption by these substances fell by one or two dilutions. After adsorption of the agglutinating sera by substances not containing functional groups (anthracene, naphthalene) and also by substances having chlorine and amino groups in their composition, the intensity of agglutination fell by only +. Organic compounds containing nitro, aldehyde, and carboxyl groups adsorbed agglutinins only insignificantly. After adsorption of agglutinating sera by these substances the intensity of agglutination fell by + +. Of all the substances which we investigated, those which adsorbed agglutinins most intensively were compounds containing several hydroxyl groups – alizarin and phenol red. The latter completely adsorbed the agglutinins, and after adsorption of the immune sera with alizarin the agglutinin titer fell by two dilutions.

Comparison of the adsorptive activity of anthraquinone, α-aminoanthraquinone, and hydroxyaminoanthraquinone revealed the reciprocal influence of different groups on the intensity of adsorption of agglutinins. For instance, in a serum adsorbed by anthraquinone, the agglutinin titer fell by one dilution; after adsorption by α-aminoanthraquinone only the intensity of agglutination was reduced, but in a serum adsorbed with hydroxyaminoanthraquinone the agglutinin titer was lowered by one dilution. The introduction of an amino group into the anthraquinone ring thus weakens its adsorptive activity, but the introduction of hydroxyl groups considerably increases the adsorption of agglutinins by this compound.

In order to determine the extent to which the adsorption of agglutinins by organic compounds is selective, we carried out a comparative estimation of the fall in the agglutinin titer and the protein content of im-

mune sera after adsorption with certain organic compounds. Adsorption of the agglutinins was carried out by the method described above, but the sera used were diluted 1 : 5 with physiological saline; weighed samples of the adsorbents contained 500 mg. The protein content of the sera was determined by Kjeldahl's micromethod. The results of these experiments are given in Table 2.

We see that alizarin adsorbs 8.3 mg of protein and naphthalene-1,6-disulfonic acid, 7.03 mg of protein, but the agglutinin titers under these circumstances fell by only one or two dilutions. Consequently, the adsorption of agglutinins was not strictly selective, and nonimmune serum proteins were adsorbed, besides agglutinins, onto these particular substances. During the adsorption of the immune serum with anion exchange resin ÉDÉ 10P, containing amino groups, despite the considerable adsorption of serum protein, the agglutinins were not adsorbed.

During the investigation of the effect of certain conditions on the adsorption of agglutinins from immune sera by alizarin, it was found that changes in the pH of the medium within the limits of 6.0-7.4, and in the temperature between 6° and 37°, had no effect on the intensity of adsorption of agglutinins. Similarly, no difference was observed in the degree of adsorption of O and H agglutinins.

Experiments on the desorption of the agglutinins adsorbed on alizarin and cationite KU-2 showed that, in the process of adsorption, the agglutinins are firmly bound to the surface of the adsorbents.

Agglutinins adsorbed on the adsorbents listed above were not desorbed after prolonged agitation in dilute solutions of acids or alkalies, in physiological saline or in hypertonic saline solution.

SUMMARY

Adsorption of immune serum agglutinins by organic compounds was investigated. Compounds with hydroxyl, aldehyde, carboxyl, and quinone groups, as well as sulfo and nitro groups, were capable of adsorbing these agglutinins. Organic substances containing no functional groups, and substances devoid of chlorine and amino-groups did not adsorb agglutinins. Being firmly bound to the surface of adsorbents, the adsorbed antibodies are not desorbed by weak acid or alkaline solutions or by physiological or hypertonic saline.

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