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IMMUNE COMPLEX FORMATION ENHANCES THE BINDING OF STAPHYLOCOCCAL PROTEIN A TO IMMUNOGLOBULIN G

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SUMMARY: Staphylococcal protein A binds efficiently to the Fc region of goat immunoglobulin G antibodies only after they are immune complexed to immobilized, but not fluid-phase, polyvalent antigen (human myeloma immunoglobulin E protein) or monovalent hapten (methotrexate). Compared to fluid-phase or immobilized free immunoglobulin G, the reactivity of anti-immunoglobulin antibodies bound to solid-phase antigen was enhanced at least 300-fold. Results with immobilized methotrexate indicated that two molecules of immunoglobulin G must be bound in proximity to bind one molecule of protein A. Thus, aggregation appears to be a necessary condition for protein A binding.

INTRODUCTION

Protein A isolated from Staphylococcus aureus binds specifically to the Fc region of IgG[1] from several species (1-4). Complexes between protein A and IgG can induce potentially harmful immunological reactions including activation of the complement system (5, 6) and hypersensitivity reactions in experimental animals and man (7, 8). However, some species and subclasses of IgG react poorly or not at all with protein A. For example, goat IgG reacts only 0.1% as efficiently as human or rabbit IgG (4, 9). There is some indication that immune complex formation may enhance the reactivity between protein A and certain species of IgG (9, 10). This enhancement observed with goat IgG (9) has not been studied in detail, although it may be a useful approach to determine if there is a conformational change in the Fc region in response to antigen binding at the Fab sites on the antibody molecule. Also, this reac-

[1] Ig, immunoglobulin

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tivity may serve as a model for the analogous binding and activation of C1, the first component of complement, by immune or heat aggregated IgG. For these reasons, we used [^{125}I]labeled protein A to quantify the effect of immune binding on the reactivity between protein A and goat IgG antibodies directed against a polyvalent antigen (a human myeloma IgE protein) and a monovalent hapten (methotrexate).

MATERIALS AND METHODS

Protein A was purchased from Pharmacia Fine Chemicals, Piscataway, NJ. Methotrexate was obtained from Sigma Chemical Co., St. Louis, MO. Human myeloma IgE (14 mg/ml) was given by Dr. T. Ishizaka, Johns Hopkins University, Baltimore, MD, and was at least 95% immunochemically pure based on radioimmunoprecipitation and immunoelectrophoresis.

IgG fractions. The IgG fraction of goat anti-human IgE (ε-chain specific) was purchased from Cappel Laboratories, Downington, PA. Goat anti-methotrexate serum was supplied by Dr. Lawrence Levine, Department of Biochemistry, Brandeis University, Waltham, MA (4). The IgG fraction was isolated by chromatography on DEAE cellulose (11) and concentrated by filtration using a Diaflo PM10 membrane from Amicon Corp., Lexington, MA. Anti-IgE and anti-methotrexate were shown to be specific for the respective antigens (4, 9).

IgG determinations. The concentrations of goat IgG protein and antibody IgG were determined as reported earlier (9). Anti-IgE contained 12.6 mg IgG/ml and 0.4 mg antibody IgG/ml. Anti-methotrexate contained 8.8 mg IgG/ml and 0.026 mg antibody/ml.

[^{125}I]protein A. One hundred micrograms [^{125}I]protein A was prepared as described previously (12) and had specific activity of 100 Ci/mmole. The functional activity was 80%.

Immobilized ligands. Polyacrylamide beads were used to prepare solid phase adsorbents. IgE was bound covalently through amino groups to the free carboxyl groups of Immunobeads, and methotrexate via its carboxyl group(s) to the free amino groups of Affi-gel 701 (Bio-Rad Laboratories, Richmond, CA) as described previously (4).

General assay procedure. The binding of fluid phase IgG antibody to the appropriate solid phase adsorbent was carried out under optimal conditions (4) in barbital buffered 0.01 M EDTA, containing 0.1% gelatin, pH 7.4. In the two-step procedure, aliquots (0.1 ml) of diluted beads and fluid-phase components were incubated at 30°C for 1 hr, then washed with two 3-ml portions of buffer. Excess [^{125}I]protein A (0.1 ml; approximately 40,000 cpm) was added and the bead-[^{125}I]protein A mixture carried through a similar incubation and washing procedure. In some experiments (see below), the reactions were carried out in a single step. [^{125}I] bound to the beads was counted in a Packard Model 5360 auto-gamma spectrometer. The counter efficiency was 70%.

RESULTS AND DISCUSSION

Experiments with immobilized antigens. The ability of [^{125}I]protein A to bind goat IgG antibody that was complexed to immobilized antigen is illustrated

by the curves in Figure 1. These were two-step experiments in which increasing amounts of immobilized ligand were incubated with antibody, and the beads were washed free of unbound reagents before [^{125}I]protein A was added. The binding curves obtained with the macromolecular and presumably polyvalent antigen IgE (Fig. 1A) reached plateau values. There was no significant binding of [^{125}I] protein A to the IgE beads without added antibody. Similar results were obtained with immobilized IgM and goat anti-IgM (9). The amount of goat anti-

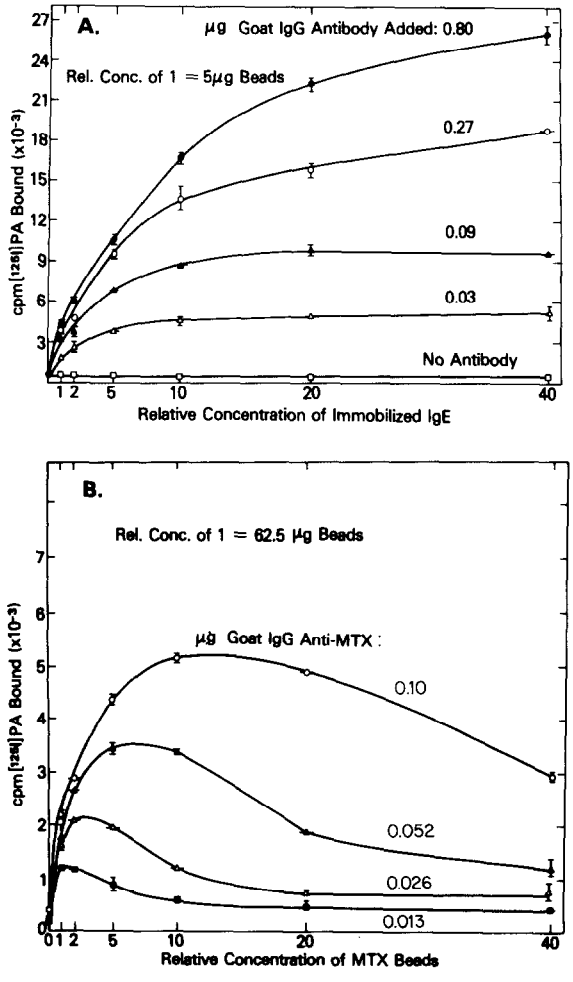

Fig. 1. Binding of [^{125}I]protein A (0.1 ml; 40,000 cpm added) to increasing amounts of A. immobilized IgG pretreated with differing amounts of the IgG fraction of goat anti-IgE serum or B. immobilized methotrexate pretreated with the IgG fraction of goat anti-methotrexate serum. Other details of the binding assay are given in Materials and Methods.

body present in the dilutions of IgG tested ranged between 0.03-0.80 μg. In contrast, even 4-10 μg immobilized or fluid phase goat IgG failed to react with [^{125}I]protein A (4, 9), and 3 μg [^{125}I]labeled goat IgG failed to bind to immobilized protein A (9). If the ability of fluid-phase or immobilized goat IgG to react with [^{125}I]protein A is compared to the reactivity of this antibody when complexed to immobilized IgE (Fig. 1A), the enhancement is at least 300-fold (10 μg vs. 0.03 μg). The binding curves shown in Figure 1B were obtained with the immobilized monovalent hapten methotrexate and IgG goat anti-methotrexate containing between 0.013 and 0.26 μg specific antibody. In this case for each level of antibody there was a maximum in the binding curve that depended on the amount of immobilized methotrexate added.

Quantitation of bound antibody. Based on the binding of [^{125}I]protein A at limiting levels of antibody in Figure 1A, the dilution of IgG, and the specific activity of [^{125}I]protein A, the concentration of specific anti-IgE antibody was calculated. For this purpose, results obtained with the highest dilution of antibody (1/1,350) were used since these conditions would favor the predominance of 1:1 complexes between [^{125}I]protein A and IgG. The value of 0.39 mg specific IgG/ml was essentially the same as the concentration obtained from the direct binding of [^{125}I]labeled IgG fraction to immobilized IgE [0.40 mg/ml (9)].

This agreement may imply that once IgG is bound to immobilized antigen, all the antibody is able to bind [^{125}I]protein A regardless of subclass, and the ratio of IgG antibody to protein A is 1:1. Although this ratio may actually reflect the binding of one molecule of protein A per molecule of IgG, it could also result from the bivalency of protein A for IgG and a sufficiently high density of antigen molecules on the bead surface. Thus an arrangement of closely bound IgG antibody molecules bridged by bivalent protein A molecules would give the appearance of a 1:1 ratio, although each protein A would require two IgG molecules for binding.

Unlike the results observed with the macromolecular antigen, for each concentration of goat IgG antibody to methotrexate there was an optimal con-

TABLE 1
Ratio of Antibody Molecules per Protein A Molecule in Complexes[a]

IgG antibody added (pmol)	Maximum [^{125}I]protein A bound (pmol)[b]	IgG/protein A[c]
0.65	0.223	2.9
0.33	0.147	2.2
0.17	0.087	2.0
0.088	0.047	1.9

[a]Molecular weight of IgG and protein A taken to be 160,000 and 42,000 daltons, respectively.
[b][^{125}I]protein A bound at the peak of each curve shown in Figure 1B.
[c]Ratio of IgG molecules per protein A molecule at the peak of each curve shown in Figure 1B.

centration of beads that gave maximum binding of [^{125}I]protein A. For each curve the maximum amount of [^{125}I]protein A bound was calculated from the specific activity of the [^{125}I]protein A preparation, and the ratio of the average number of IgG molecules per protein A molecule was derived for each of these points. The data summarized in Table 1 suggest that the ratio approaches a limiting value of 2.0 molecules IgG/protein A molecule. This represents the minimum number of IgG molecules in a complex containing [^{125}I] protein A and may represent the only protein A-containing complex since protein A is bivalent in its reaction with IgG.

Single-step incubation. The ability of goat IgG antibody to bind [^{125}I] protein A was greatly enhanced when antibody was bound to immobilized antigen. Thus it appeared likely that binding of [^{125}I]protein A to immune complexed IgG could occur even in the presence of a large excess of non-antibody IgG and that the reactions could be carried out in one step. In this experiment, dilutions of goat anti-IgE ranging between 0.70-45 μg IgG corresponding to 0.02-1.43 μg specific anti-IgE antibody were incubated with different amounts of IgE beads in the presence of [^{125}I]protein A. The series of binding curves shown in Figure 2 demonstrate that [^{125}I]protein A will bind to antibody com-

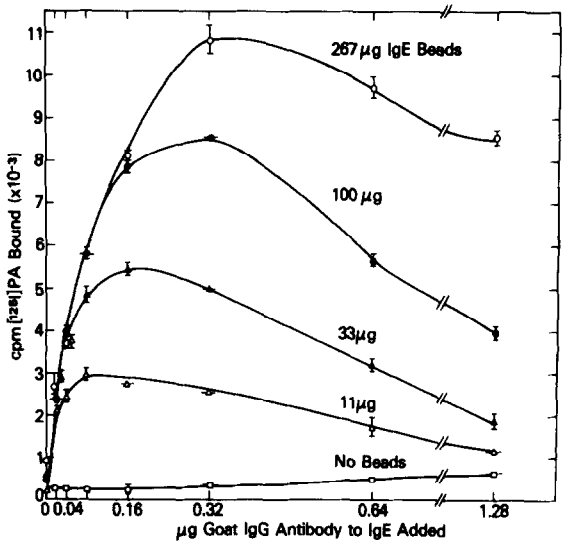

Fig. 2. Binding of [^{125}I]protein A (0.1 ml; 40,000 cpm added) to differing amounts of immobilized IgE carried out in a single step in the presence of increasing amounts of the IgG fraction of goat anti-IgE serum. Other details of the assay are given in Materials and Methods.

plexed to immobilized IgE even in the presence of more than 30-fold the amount of non-antibody IgG. For each amount of beads tested, there was an optimal antibody concentration that gave maximum binding of [^{125}I]protein A since addition of $\geq$ 10 µg IgG should react significantly with fluid-phase [^{125}I]protein A based on previous results (4, 9). Under similar conditions using rabbit anti-IgE, no significant binding of [^{125}I]protein A to IgG beads was detected, since excess non-antibody IgG protein apparently blocked the binding of [^{125}I]protein A to antibodies that were complexed to immobilized IgE (data not shown).

Taken together, our results are consistent with a model in which two molecules of IgG bound in proximity to immobilized antigen or hapten are necessary for protein A binding. A second question is whether aggregation of IgG is sufficient or whether antigen-induced conformational changes in the Fc region also are important for protein A binding.

Barkas and Watson (13) have tested immune complexes formed in solution between chicken antibodies and acetylcholine receptors. Based on indirect

evidence, they associated enhanced binding to protein A-containing Staphylococcus aureus or protein A-Sepharose to conformational changes in the immunoglobulin molecule. They did not consider aggregation of antibody molecules. Also, Wright et al. (14) have shown by spectrofluorometric methods that the binding constant of monovalent fragments of protein A to the Fc region of rabbit IgG in solution was not affected by antigen or hapten binding. They concluded that there was no significant change in the Fc region. The system of Wright et al. was different from ours since they used enzymatically derived fragments of protein A and a species of IgG which normally binds protein A efficiently. Even then, their results are consistent with our findings using goat IgG since we found earlier that fluid-phase antigen- (or hapten)-antibody complexes failed to react significantly with [^{125}I] protein A (9).

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