Chimeric IgG-binding Receptors Engineered from Staphylococcal Protein A and Streptococcal Protein G*

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Chimeric Fc receptors, consisting of the IgG-binding domains of both staphylococcal protein A and streptococcal protein G, were constructed. An efficient bacterial expression system was used to produce the recombinant proteins, which vary in size and number of IgG-binding domains. The purified receptors were analyzed by immunodiffusion and a competitive enzyme-linked immunosorbent assay to establish the relative binding strength to various polyclonal and monoclonal immunoglobulins from different species. The results demonstrate that protein A and protein G have complementary binding patterns and that the chimeric receptors retain the binding capacities of both the parental constituents. This suggests that these novel chimeric receptors might be versatile reagents for immunochemical assays.

Staphylococcal protein A (SPA) is used as a reagent in a variety of immunoassays (Langone, 1982), taking advantage of its affinity to the constant (Fc) part of various immunoglobulins (Forsgren and Sjöquist, 1966). Immobilized SPA has also been valuable for purification of IgG by solid phase affinity chromatography (Hjelm et al., 1972). However, SPA has limited binding to several species and subclasses of IgG, which has hampered its use. This had led to a search for other Fc-binding proteins with broader specificity.

Based on studies of staphylococci and streptococci, Myhre and Kronvall (1981) proposed five different types of Fc receptors. Type I is represented by Staphylococcus aureus protein A, type II by group A streptococci, type III by human group C and G streptococci, type IV by bovine group G streptococci, and finally type V by Streptococcus zoenepidemicus. All five classes of receptors show distinct binding patterns to the constant region of various classes and subclasses of mammalian IgG.

A comparison between one type III receptor, protein G (SPG), and protein A (Björck and Kronvall, 1984 and Åkerström and Björck, 1986) led to the conclusion that SPG, compared to SPA, binds at least equally well and often superior to all tested polyclonal immunoglobulins and human IgG subclasses. Based on these results, it was suggested that protein G could replace protein A in immunoassays, in particular those involving IgG with weak binding to SPA, such as sheep IgG, some mouse monoclonals, and human IgG3.

However, a detailed evaluation of the binding strength of SPG to various immunoglobulins was hampered due to the difficulties of dissolving the Fc receptor from the bacterial cell wall. Treatments using trypsin or pepsin (Myhre and Kronvall, 1981), specific phages (Christensen and Holm, 1976), acid (Havlicek, 1978), alkali (Grubb et al., 1982), and papain (Björck and Kronvall, 1984) gave heterogeneous materials with molecular weights ranging from 30,000 (Grubb et al., 1982) to 100,000 (Havlicek, 1978). This might explain the conflicting binding patterns of SPG reported. A method to obtain large quantities of a more well defined material is, therefore, desired.

Recently, the gene coding for protein G was isolated (Guss et al., 1986 and Fahnestock et al., 1986), which allows production of a recombinant protein. Using this approach, Guss et al. (1986) compared the relative binding of SPA and SPG to different immunoglobulins at physiological conditions. The results suggested, in contrast to earlier reports of protease-released material, that protein G is not superior to protein A in its binding to all immunoglobulins but rather has a complementary binding pattern, i.e. protein G binds stronger than protein A to polyclonal IgG from cow, horse, and sheep, while the reverse was observed for polyclonal IgG from guinea pig and dog.

This paper describes the production of a defined and homogeneous recombinant protein G and reports on its binding characteristics. The results indicate that it might be possible to obtain an optimal IgG-binding receptor by combining the binding activities of the class I and the class III receptors. We have, therefore, by gene fusion techniques constructed also two chimeric Fc receptors consisting of the IgG-binding domains of both SPA and SPG, varying in size and number of IgG-binding domains. Immunological assays demonstrate that these novel receptors retain the binding capacities of both SPA and SPG.

MATERIALS AND METHODS AND RESULTS AND DISCUSSION

Functional Analysis of the Novel IgG-binding Receptors by Immunodiffusion—Different gene fragments encoding parts of staphylococcal protein A and streptococcal protein G were used to assemble the three constructs schematically outlined in Fig. 2, B, C, and D. These are compared to the structure of...
Chimeric IgG-binding Receptors

A

B

C

D

Fig. 2. Schematic drawing of the genes encoding the IgG-binding receptors used in the study. Boxes show the size and relative position for the gene fragments encoding the staphylococcal protein A signal sequence (S), the protein A IgG-binding regions (E, D, A, B, and C), and a part of the cell wall binding region of protein A (X'). The synthetic IgG-binding domain based on region B of protein A (Z). Empty boxes represent coding sequences not relevant for IgG binding. A, protein A obtained from S. aureus; B, protein G as produced by the recombinant host; C, protein AG; and D, protein ZZG.

Fig. 3. Double immunodiffusion of immunoglobulins and the Fc receptors. Purified polyclonal IgG and IgG-binding receptors were diluted in phosphate-buffered saline, pH 7.0, and applied to the wells. The plates were stained with Coomassie Brilliant Blue. A, protein A; B, protein G; C, protein AG; and D, protein ZZG.

TABLE I

| Species      | A   | G   | AG  | ZZG |
|--------------|-----|-----|-----|-----|
| Rabbit       | 1.0 | 1.0 | 1.0 | 1.0 |
| Human        | 0.7 | 1.0 | 1.4 | 1.7 |
| Cow          | 5.0 | 1.5 | 1.9 | 1.7 |
| Horse        | >30 | 0.5 | 7.1 | 2.7 |
| Goat         | 3.3 | 3.0 | 2.9 | 2.3 |
| Guinea pig   | 0.7 | 1.3 | 1.7 | 2.7 |
| Sheep        | >30 | 1.5 | 11  | 5.3 |
| Dog          | 0.3 | 6.0 | 0.9 | 2.3 |
| Pig          | 0.3 | 3.6 | 1.0 | 1.3 |
| Rat          | >30 | >30 | >30 | >30 |
| Mouse        | 12  | >30 | 12  | 15  |
| Chicken      |     |     |     |     |

The immunodiffusion studies of the chimeric receptors (Fig. 3) indicated that the novel receptors consisting of both the class I and the class III receptors might have a combined binding capacity of the parental proteins. The results of the inhibition studies presented in Table I support this conclusion, although quantitative differences were observed for protein AG and protein ZZG. An interesting feature is that in all cases where protein A shows high affinity, the binding capacity of protein AG exceeds protein ZZG and vice versa. This probably reflects the fact that protein AG has five IgG-binding domains of protein A, while protein ZZG only contains two protein A-like domains.

The relative amount of the various immunoglobulins needed to inhibit the binding of rabbit IgG to the chimeric receptors is usually slightly higher than the values for the parents. The reduction in binding may depend on differences in molecular weight or the molar amounts of the relevant IgG-binding domains. The binding of the four receptors (A, G, AG, and ZZG) to the microtiter well may also be influenced by factors such as size and overall structure of the proteins. However, despite the semi-quantitative nature of the assay, G, demonstrating that the novel receptors are also functionally chimeric.

Comparative Binding to Polyclonal IgG—A competitive enzyme-linked immunosorbent assay was used to investigate the ability of native and recombinant Fc receptors to bind to polyclonal IgG from different species. The results presented in Table I demonstrate differences in the relative binding pattern between protein A and protein G. Protein A has a significantly higher affinity to polyclonal IgG from dog, pig, mouse, and guinea pig, while the opposite is true for IgG from cow, horse, and sheep. Protein A binds slightly better to human IgG, while protein G binds somewhat stronger to goat IgG. None of the receptors interacts with chicken IgG, and the binding to rat IgG is in both cases weak. The two proteins, therefore, exhibit complementary binding patterns for mammalian IgG. A similar pattern has been reported earlier for protein A and an Fc receptor from a group C streptococcus (Reis et al., 1984). Reis et al. (1986) reported on different binding patterns and concluded that the streptococcal class III Fc receptor reacts both stronger and with a wider range of IgG species than staphylococcal protein A. However, the differences in relative affinity between their results and the data presented here (Table I) are probably due to differences in assays, binding conditions, and origin of the protein G molecule. The remarkable difference in pH dependence of the binding of various IgG by protein G and protein A, noted by Åkerström and Björck (1986), emphasizes that direct comparisons of binding assays performed at different conditions might be hazardous.

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it is possible to conclude from Table I that the novel chimeric receptors AG and ZZG have a wider range of IgG species reactivity than either of the two parental protein A or protein G receptors.

Comparative Binding to Human IgG Subclasses—The relative binding of the four receptors to human myeloma immunoglobulins is presented in Table II. As shown earlier (Guss et al., 1986; Åkerström and Björck, 1986; Reis et al., 1986), protein G binds strongly to human IgG3 in contrast to protein A, which shows low affinity to this subclass. Both the chimeric receptors bind IgG3, but the relative affinity is considerably lower than for protein G, especially for protein AG. It is possible that protein ZZG, which to 60% consists of protein G domains, has an IgG3-binding activity which is closer to that of protein G than protein AG with only 38% of protein G domains.

Table II also shows that proteins A and G have approximately the same binding activity to the other human subclasses, although protein A binds somewhat better to IgG1 (A, G), IgG2 (A, G), and IgG4 (A). Since both parental proteins have similar affinities to subclasses 1, 2, and 4, identical binding of the chimeric receptors is also observed. Table II, therefore, suggests that compared to protein G, the chimeric receptors exhibit no advantage in binding to human IgG subclasses. However, the chimeric receptors still maintain the ability to recognize all the human subclasses of IgG.

Comparative Binding to Mouse Monoclonal IgG—Although the binding pattern of the Fc receptor from streptococci to various immunoglobulins has been determined (Guss et al., 1986; Reis et al., 1986), no quantitative study of the affinity to mouse monoclonals of all subclasses has been reported. Åkerström et al. (1985) determined the binding of three subclasses of radiolabeled mouse monoclonal antibodies to protein A and protein G coupled to Sepharose and nitrocellulose, respectively. They demonstrated higher affinity of two immunoglobulins of subclass 1 and one each of IgG2a and IgG3 to protein G as compared to protein A. It was later confirmed by Åkerström and Björck (1986) that at least two of these monoclonals (of subclass IgG1 and IgG2a) show higher binding to protein G than to protein A at pH between 4 and 8, while protein A binds equally well or stronger at higher pH.

Since subclass IgG2b was not included in these studies and only four monoclonals in total were used, we decided to determine the binding of protein A, protein G, and the two chimeric receptors to two independent monoclonal antibodies of each subclass. The results presented in Table III suggest that protein A has a relatively high affinity to IgG2a, while protein G binds stronger to IgG2b. The affinity to IgG3 is strong for both proteins but is weak to subclass IgG1. These results differ dramatically from those of Åkerström et al. (1985), and the question arises if this is due to differences in binding conditions or individual variability of the monoclonal antibodies. It is interesting to note that the chimeric receptors (AG and ZZG) bind well to IgG2a, IgG2b, and IgG3. This further supports the conclusion that these novel receptors have gained the binding capacity of both parental receptors.

Concluding Remarks—A new concept to obtain novel receptors has been introduced in this paper. With the aid of gene fusions, the gene fragments encoding IgG-binding domains of staphylococcal protein A and streptococcal protein G were assembled, and the resulting proteins were shown to be structurally and functionally chimeric. The advantages of these novel chimeric receptors are several. First, the binding spectra toward polyclonal immunoglobulins from different species are broader than the individual parent proteins. Second, individual subclasses of mouse monoclonals are more likely to have strong affinity to the chimeras than to protein A or protein G itself. Third, the dramatic dependence on pH for binding to both protein A and G might be reduced. The fact that protein G has a low pH optimum (around pH 6), while the pH optimum for protein A is basic (pH 8), obviously enhances this positive effect. Finally, a multivalent receptor containing, in the case of protein AG, as many as eight IgG-binding domains might prove to enhance binding in certain applications, in which availability of the receptor is crucial.

Nilsson et al. (1985) has earlier reported on a similar strategy to construct protein A-enzyme conjugates using genetic approaches. Gene fusions between protein A, β-galactosidase, or alkaline phosphatase were produced and shown to be functional both in IgG binding and enzymatic activity. This approach provided well defined and homogeneous in vivo coupled materials. Recently, such a β-galactosidase conjugate was used to detect specific antibodies in Western blots (Valerie et al., 1987). Using similar techniques an enzyme conjugate coupled to the protein AG molecule can be obtained. Such a tripartite molecule has recently been constructed and shown to be functional in immunoassays.

In conclusion, the results presented here demonstrate that gene fusion techniques are a very powerful tool to obtain novel proteins with improved functional properties. It is likely that chimeric receptors, such as the two described here, might in the near future prove to be useful for various immunological and biochemical applications.

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M. Eliasson, unpublished data.
Supplementary Material to

Chimeric IgG-binding Receptors ENGINEERED FROM STAPHYLOCOCCAL PROTEIN A AND STREPTOCOCCAL PROTEIN G

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MATERIALS AND METHODS

Bacterial strains and immunoglobulins. E. coli strain 115 (Inganas et al. 1982) was used as host for all plasmid constructions and for production of the proteins. Human polyclonal IgG was obtained from Kabi-Vitrum, Sweden. The other polyclonal immunoglobulins were purified from sera, obtained from the various species, by protein-A-Sepharose and gel filtration. Monoclonal IgG from all species used was obtained from Hybridoma banks or purchased from suppliers. Recombinant human IgG was obtained from Amersham Sweden. horse IgG was obtained from Sigma Chemical Company.

DNA preparations. Plasmid DNA from E. coli was prepared by the alkaline extraction method (Birnboim and Doly 1979). Plasmid transformation and other DNA techniques were as described by Maniatis et al. (1982). Restriction endonucleases, 74 DNA ligases, DNA polymerase I, Klenow fragment of DNA polymerase I and other enzymes were used as specified by suppliers.

DNA constructions. The coding sequence of the three IgG binding regions from protein G is contained within the E. coli-BAC fragment of the gene between nucleotides 1,322 and 1,652 (Nilsson et al. 1981). The BAC site was converted to a KpnI site by a ligation of a synthetic oligonucleotide corresponding to the KpnI site and a 5' linker. For phosphorothioate linkage, the synthetic oligonucleotides were phosphorylated with polynucleotide kinase and oligonucleotides were linked with T4 DNA ligase. The mutant protein G was sequenced to verify the desired substitutions. The recombinant proteins were produced by growing E. coli harboring the recombinant plasmid in an isopropyl thiogalactoside-induced culture and isolating the recombinant plasmid DNA.

The IgG-binding assay. Precipitation of IgG by the Fc-receptor complexes was studied by radial immunodiffusion as described by Ouchterlony (1958). The binding between the subclasses of immunoglobulin and immobilized protein A was assayed by the competitive ELISA technique as described by Guss et al. (1984).

RESULTS

Construction of gene fusions encoding chimeric proteins. E. coli strain 115 has been manipulated to express a plasmid-encoded fusion protein containing a gene coding for the IgG binding region of a human or a rat protein. Details of the constructions are described in Materials and Methods. In all cases, the base pairs encoding the IgG-binding region of protein G were inserted into pGEM-3 (-3894-5000) using the SmaI-SnaBI restriction sites.

Expression and purification. Since all three constructs contain the protein A-coding region and signal sequence, it is possible to express the products in E. coli. The IgG-binding region was expressed in E. coli strain 115 harboring the recombinant plasmid. The fusion proteins were purified by isoelectric focusing and gel filtration chromatography. The proteins were eluted from the gel filtration column using 0.5 M NaCl. The eluted material was studied using a Staphylococcus aureus (strain 8325) cell extract as a source of protein A.

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Antigenic properties of the chimeric receptors. The immunological reactivity of the purified proteins (Fig. 1) were tested using a non-competitive ELISA technique. The receptors were allowed to bind to the immobilized Fab' fragments of anti-protein G rabbit IgG. Thereby only the receptors containing protein G determinants would bind. In the next step, anti-protein G Fab IgG antibodies were added, followed by the addition of goat anti-rabbit IgG antibodies. Thus, only proteins containing both protein A and protein G epitopes were detected. Positive reactions were only obtained for protein A and 125I (data not shown). This shows that the chimeric receptors have antigenic properties of both the parental proteins. It is noteworthy that the synthetic domain 2, which has been designed by protein engineering to differ from the native protein A by its resistance to chemicals such as cyanogen bromide and hydroxylamine, still reacts with the antibodies directed towards native protein A.

Competitive binding using rabbit IgG. A competitive ELISA was used in which the receptors were immobilized to microtiter wells and the immunoglobulins were allowed to compete with enzyme conjugated rabbit IgG for binding. The affinity was measured as the relative amount of IgG required to give 50% inhibition of the binding of rabbit polyclonal IgG to the immobilized receptors. Rabbit polyclonal IgG has been shown to bind with similar strength with both proteins A and G. Line D was used as a negative control. The reaction mixture was used as a tracer in the assay. To eliminate the effect of variations in the binding of the receptors to the solid support, all values are normalized to the amount of rabbit IgG needed to inhibit the rabbit IgG conjugate. This assay thus gives the relative binding strength of a recombinant receptor towards the various immunoglobulins.

Fig. 1. SDS-PAGE of the purified IgG-binding receptors. The recombinant proteins (lane B, C and D) were purified from the culture medium of E. coli by IgG affinity chromatography as described in Material and Methods. Lane A: protein A (clone 2, Sweden), lane B: protein G, lane C: protein A and lane D: protein ZZ. Lane M: marker proteins with the size shown as molecular weight x 10^6.