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INTERACTIONS BETWEEN STAPHYLOCOCCAL PROTEIN A AND IMMUNOGLOBULIN DOMAINS

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SUMMARY

The affinity and stoichiometry of interaction between staphylococcal protein A and different domains of immunoglobulins have been studied. Light scattering and tryptophan fluorescence quenching titrations along with direct binding assays were performed. The lack of binding to protein A of pFc' fragment (corresponding to C_H3 domain of IgG) or of Facb derivative of rabbit IgG (which is devoid of the C_H3) suggests that the locus of protein A binding is at the interface between the C_H2 and C_H3 domains. This assignment is also supported by results of the tryptophan fluorescence quenching and $C\bar{1}$ binding experiments.

INTRODUCTION

Protein A (SPA), a major cell wall component of most strains of Staphylococcus aureus, binds specifically to the Fc region of immunoglobulins (1-3). It is a single polypeptide chain of 42,000 Daltons having a rather extended shape as judged from its hydrodynamic properties (4). SPA has been shown to consist of five regions. Four highly homologous domains are Fc-binding, approximately 60 residues long, and consecutively arranged from the N-terminus of the protein (5). The fifth, C-terminal domain is approximately 150 residues long, cell wall bound and Fc nonbinding (6). The specific binding of SPA to immunoglobulins is finding an expanding use in immunology for both preparative and analytical purposes (3). The present study attempts to clarify the stoichiometry and affinity of this interaction as well as the location of the SPA binding site on the Fc. The fact that protein A is devoid of tryptophan residues (4) allowed convenient monitoring of the rabbit Fc intrinsic emission which is specifically quenched upon binding (7).

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MATERIALS AND METHODS

Human myeloma IgG1 was isolated on a QAE-Sephadex A-50 column (8). Its Fab and Fc fragments were generated by trypsin digestion (9) and purified by DEAE-cellulose chromatography (10). Pepsin digestion was carried out according to (11) and was terminated by raising the pH to 9.0. Upon gel filtration on a Sephadex G-100 superfine column (3.0 x 90 cm, 0.01 M Tris-HCl, 0.15 M NaCl, 2 mM EDTA pH 8.2), the pFc' fragment eluted as a well resolved peak. By pooling only the descending portion of the peak eluting at half the column volume, $F(ab')_2$ free of residual IgG was also obtained. Rabbit IgG 168 specific for Micrococcus Lysodeicticus cell wall saccharides was a kind gift of Dr. A.D. Strosberg and A. Schreiber. The Facb fragment was prepared according to (12) from specific antibodies raised in rabbits against poly-D-alanyl diphteria toxoid (13). Staphylococcal Protein A and its fragment B (FB) were prepared as described (5,6). All measurements were performed in 0.01 M sodium phosphate buffer pH 7.4, 0.15 M NaCl (PBS).

Radiolabeling of proteins (^{125}I) was accomplished using the lactoperoxidase procedure (14). Binding to Staph. Aureus was performed according to Brunda et al (15). 20µl of Aureus suspension (10% w/w, heat killed and formalin fixed binding 1 mg IgG per 1 ml hence roughly 10^{-5} M in SPA sites) were added to 180µl of 10^{-6} M solution of ^{125}I labeled (14) IgG derivative containing 1 mg/ml BSA. The final dilution was thus equimolar in SPA and IgG sites.

Light scattering was measurred on a Perkin Elmer MP44A spectrofluorimeter, in a 3 x 3 mm quartz cuvette (25° C). Illumination was at 296 ± 1.5 nm and the scattered light was measured at 90° at the same wavelength. Concentrations were 5 - 8 x 10^{-6} M sites of Ig or fragments thereof, and a roughly equivalent concentration of SPA (assuming 2 and 4 sites per molecule respectively). At these concentrations, the absorbance of the Ig never exceeded 0.3 and that of SPA was negligible. Fluorescence titrations of immunoglobulins and their fragments with the monovalent fragment FB of SPA were performed in a similar fashion, the emission being monitored at 330 nm. SPA is devoid of Trp residues and therefore has no contribution to the fluorescence. $C\bar{l}$ binding by nonaggregated human IgG was measured by a modification (10) of the method of Augener et al (16) IgG used in $C\bar{l}$ binding studies were gel filtered on Sephadex G-200 in 1.0 M guanidine HCl-0.2 M EDTA pH 4.5, in order to remove the trace amounts of $C\bar{l}$. The guanidine HCl was later removed by dialysis (10).

RESULTS AND DISCUSSION

Fluorescence titrations of IgG attempted with intact SPA resulted in rather small and irreproducible changes. This is attributed to the interference by light scattering of IgG-SPA aggregates (see below) which has a contribution (Raman scattering) at wavelengths up to 40 nm longer than that of excitation. We therefore performed measurements with the monovalent FB fragment. Titrations in which FB was added to intact IgG (rabbit 168) displayed a curve that levelled off at 7% maximal fluorescence quenching. Due to the small change no detailed analysis was attempted. Since the Fc domains were found to contribute only 10-20% to the total fluorescence intensity of rabbit IgG (17) a larger effect is expected on binding if quenching upon SPA

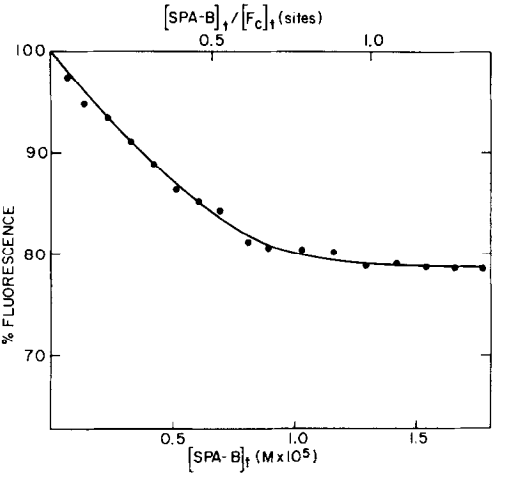

Fig. 1. Titration of an Fc fragment of rabbit IgG with FB. Nominal Fc concentration is 9.3 10^{-6} M. Points are the observed, normalized fluorescence values corrected for light scattering and volume changes. The curve is computed using best fit parameters given in the text.

binding is restricted to Fc tryptophans. This indeed was found to be the case with rabbit (papain) Fc (Fig. 1) where 20% quenching of its fluorescence is attained at saturating concentrations of FB. Analysis of the quenching curve by a non-linear least squares routine (18) yielded a best fit value of K = 3 ± 0.5 x 10^6 M^{-1}, with 1.7 ± 0.3 binding sites per Fc. This constant is more than tenfold smaller than that reported (19) for the intact SPA on the bacterium (4.5 x 10^7 M^{-1}). The difference may be accounted for by the fact that interactions between polyvalent reactants lead to an apparent affinity higher than the intrinsic value measured here (cf. ref. 20). Such interactions may also lead to a non-linear Scatchard plot (20) as is indeed the case in ref. 19. However, it is also possible that the SPA sites are somewhat impaired upon fragmentation.

Experiments were also performed with human Fc (trypsin). These revealed practically no fluorescence changes upon FB addition. Sjöholm (21) also found a rather minimal change at the tryptophan 292nm band in his CD study of human Fc and SPA. Rabbit C_H3 has one more tryptophan (residue 404) than the human

domain which is located close to the C_H2 - C_H3 interface (R. Huber, personal communication). Thus the fluorescence quenching observed for rabbit Fc may tentatively be assigned to changes at Trp 404, reflecting proximity between SPA binding site and this residue.

Light scattering measurements were performed in the 0.1 - 1 minute time range. The results are expressed as the ratio R between the intensity scattered by the SPA-protein complex and that due to the same protein without SPA. For IgG of different specificities from human, rabbit and mouse a value of R = 3.2 ± 1.0 was obtained. Mildly reduced and alkylated rabbit IgG gave R = 3.0. An Fc of rabbit IgG R = 1.2. As these measurements were done under conditions of Rayleigh scattering (low concentration and aggregates small relative to the employed light wavelength), the scattering intensity I is proportional to M·C (M= molecular weight, C = weight concentration). Since at site equivalence M·C for SPA is only 0.5% of that for IgG and 4% of Fc, the SPA contributes a negligible scatter signal. At constant C, any change in I binding of SPA may be attributed to a change in the aggretation state. Hence relatively well defined aggregates are initially formed containing 2-4 IgG or Fc bridged by SPA (Fig. 2). Disruption of the H-H disulfide bond of IgG has little effect.

The direct binding assay of radiolabelled IgG and its fragments to whole S. aureus bacteria shows that binding of more than 90% occurs for both human IgG1 and rabbit IgG, and 82% for Fc of human IgG1 whereas only about 4, 6, 8, and 20% of the pFc', $F(ab')_2$, Fab and Facb respectively are bound. Data for the first six agree with previous reports (1,2,22-24). Significantly there is no binding of pFc' while there is a small extent of binding of Facb. The latter however is atributed to an impurity of intact IgG in the Facb preparation detected by SDS acrylamide gel electrophoresis. These results together with the fluorescence quenching observed for rabbit Fc support the localization of the binding site for SPA at the C_H2 - C_H3 domains interface. Earlier reports (22-24) indi-

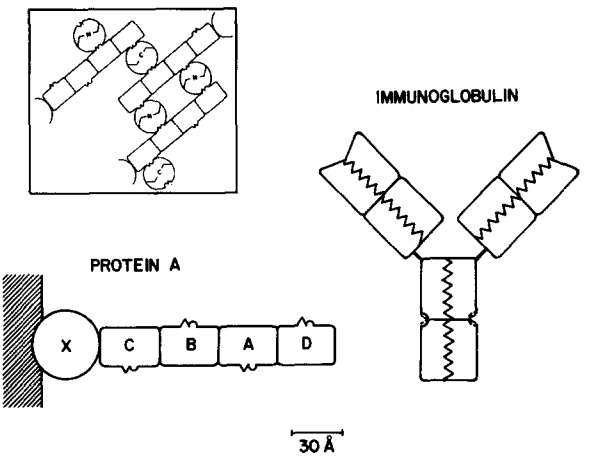

Fig. 2. Schematic representations of SPA and IgG, made to scale, assuming cylinder-shaped domains and using the literature values of the molecular weight (a partial molar volume of 0.72 is assumed). The dimension for IgG domains are roughly as obtained by x-ray crystallography with an axial ratio of 1 for each domain. For SPA an axial ratio of 5 is assumed, which is a minimal value, considering the high frictional ratio of SPA (4). The hypothetic binding sites (2 per Fc and 4 per SPA) are also drawn. In Fc they are related by a two fold rotation axis. We assume that the domains in SPA, are related by a pseudo 2-fold screw axis, so that the binding sites are staggered.

The insert shows a hypothetical shape of an Ig-SPA aggregate. The hypothetic symmetry properties of both molecules result in arrangement where alternative IgG molecules point up and down and alternate SPA molecules point right and left, with minimal steric hindrance.

cated that neither C_H2 nor C_H3 (human IgG) bind to SPA. Our results show the rabbit Facb is essentially incapable of binding SPA implying a role for the missing C_H3. On the other hand the C_H3 does not bind at all. Therefore both domains must be involved in forming the binding site. (A possible alternative that the site is localized on one domain and that mutual domain interactions are essential, can not be excluded.)

The effect of binding FB on the ability of monomeric human IgG to interact with the $C\bar{1}$ component of the complement system was also measured. No alteration in the amount of IgG known capable of binding 50% of the added $C\bar{1}$ was found even at FB concentrations sufficient to saturate 80% of the sites on IgG (assuming similar affinity to the human and rabbit Fc fragments). Previous $C\bar{1}$ studies showed competitive blocking by high SPA/IgG (or Fc) ratios

(26-27). Since the $C\bar{1}$ binding site is entirely localized on the C_H2 domain (12,28,29) this competition was interpreted in terms of steric hindrance reflecting proximity between SPA and $C\bar{1}$ sites. However since the latter experiments were performed with intact multivalent SPA, steric hindrance might have been caused indirectly. Indeed the monovalent fragment FB causes no interference and the locus of SPA binding must therefore be more distant from the $C\bar{1}$ binding site.

In conclusion all the above data are consistent with the localization of the SPA binding site at the C_H3 - C_H2 interface. While this report has been written we have been informed about the direct structure determination of the Fc-FB complex (30) which indeed shows the binding to occur at the locus suggested above.

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