Immunoglobulin Binding by the Regular Surface Array of Aeromonas salmonicida*

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The cell surface of Aeromonas salmonicida is covered by a regular surface array composed of a single species of protein, the A-protein (Phipps, B. M., Trust, T. J., Ishiguro, E. E., and Kay, W. W. (1983) Biochemistry 22, 2934-2939). The array, known as the A-layer, is the key virulence factor for this organism. Cells containing the A-layer specifically bound rabbit IgG and human IgM with high affinity ($K_D = 1.0 \times 10^{-10} M$ and $3.3 \times 10^{-10} M$, respectively), but neither isogenic A-protein-deficient strains nor an Aeromonas hydrophila strain also possessing a regular surface array had binding activity. Selective removal of A-protein at pH 2.2 inactivated IgG binding. Structurally intact IgG was requisite for binding since both Fab and Fc fragments were inactive. Aeromonas A-protein did not share the same IgG binding sites as Staphylococcus aureus protein A. Purified A-protein bound IgG only weakly, but reassembled A-layer regained binding activity. Protein modification and perturbation of the A-layer indicated that no single amino acid residue was critical for binding, and that the binding site consisted of a native arrangement of at least four A-protein monomers in the layer.

Several Gram-positive bacterial species produce surface-exposed proteins which exhibit nonimmune binding to the Fc portion of a variety of immunoglobulin classes—predominantly the IgGs (1). Protein A of Staphylococcus aureus, especially of strain Cowan 1, is the best known of these Fc receptor proteins and has become essential to a wide variety of immunochimical studies. Recently, the gene (spa) for protein A has been cloned, and its sequence has been determined (2, 3). It is now known to contain four highly homologous regions, each of which is able to bind the IgG Fc fragment (4). Protein A has been speculated to be a virulence factor, yet no correlation of the absence or presence of protein A with any pathogenic property has been reported (5). Immunoglobulin binding proteins of even broader specificity are produced by a variety of streptococcal species (6, 7). One of these, known as protein G, has been purified (8) and recently the DNA sequence of the gene responsible (spg) was determined (9).

The Gram-negative bacterium Aeromonas salmonicida is a well-known fish pathogen. It produces the fatal septicemic disease furunculosis in salmonid fishes (10), which is a major problem in commercial salmon culture. The so-called “atypical” strains are responsible for several ulcerative diseases in a broad range of salmonid and nonsalmonid fishes (10). A. salmonicida possesses a regularly arranged surface protein array known as the A-layer (11), comprising a single species of protein of $M_r = 49,000$, the A-protein, which forms a paracrystalline two-dimensional cylindrical shell that completely encloses the cell (12). The chemical properties of the A-protein and its interactions with the outer membrane components have been characterized in some detail (13, 14), and mutants lacking the protein or LPS' O-polysaccharide chains have been studied (15). The actual role of the A-layer appears to be manifold. It is the primary virulence factor of this organism as it protects the bacterium from both serum and antibody-mediated killing (16), and in addition facilitates the spread of infection by enabling the organism to associate with host macrophages (17).

A. salmonicida is the first gram-negative bacterium to exhibit Ig binding and the only bacterium in which the protein responsible has been shown to be essential for virulence. Here we describe the characteristics of the immunoglobulin binding process.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—A. salmonicida A449, A450, and A451 are virulent A-layer-producing (A') strains originally supplied by M. Michel, Grignon, France. Strains A449-3, A450-3, and A451-3 are isogenic A' derivatives containing normal smooth LPS, isolated as previously described (18). Strain A451-70 is an isogenic A-protein-excreting derivative deficient in LPS polymeric O-polysaccharide. A. hydrophila TF7 is a protein regular surface layer (RS-layer)—producing strain obtained from R. Lalier, St. Hyacinthe, Quebec.

Luria-Bertani broth in which the NaCl was replaced with 0.5% dextrose and used as a complete medium for all strains. When cells were to be labeled with $[32P]P$-orthophosphate, 40 Ci was included per 200 ml of culture medium. Growth was at 20 °C on a reciprocating water bath-shaker to a density of 7.5 mg/ml (wet weight) (late exponential phase). Cells were harvested by centrifugation at 9000 X g and washed twice with PBS and once with PBS/TBA.

Chemical and Immunological Reagents—Rabbit IgG used in initial experiments and rabbit anti-mouse IgG Fab were the kind gift of T. W. Pearson (this department). Rabbit IgG, rabbit IgG Fc fragment, and human serum IgA were from Jackson Immunoresearch. Human IgG and human IgM were supplied by CooperBiomedical. All the above immunoglobulins and fragments were purified by a combination of affinity and conventional chromatography techniques from

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†Recipient of a scholarship from the Natural Sciences and Engineering Research Council of Canada.
§Supported by the Natural Sciences and Engineering Research Council of Canada.

1 The abbreviations used are: LPS, lipopolysaccharide; PBS, phosphate-buffered saline; pH 7.4; PBS/TBA, PBS containing 0.05% Tween 20, 0.2% bovine serum albumin, and 0.02% sodium azide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TEPA, 20 mM Tris-HCl, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 0.02% sodium azide, pH 8.0.

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normal rabbit or human serum. Horseradish peroxidase-conjugated goat anti-rabbit IgG for Western blotting was supplied by Tago Inc. Fixed protein A-bearing SAC cells were from Behring Diagnostics. Protein A was from Sigma, and cyanogen bromide-activated Sepharose was from Pharmacia LKB Biotechnology Inc. NaOH was from Amer sham and [125I]orthophosphate from Du Pont-New England Nuclear. N,N,N-trimethylenediamine-N,N-dithiocarbamic acid was from Pierce; trinitrobenzenesulfonic acid, tetrarnitromethane, iodosacetic acid, diethylpyrocarbonate, 2-hydroxy-5-nitrobenzyl bromide, and detergents were from Sigma; guanidine hydrochloride and urea were ultrapure grade from Canadian Scientific Products. All other reagents were commercially available. Sulfanilic acid was diazotized just before use.

Removal of A-layer by Glycine Extraction—PBS-washed A450 cells were washed twice with 20 mM sodium phosphate, pH 7.0. The cell pellet was gently suspended in ice-cold 0.2 M glycine-HCl, pH 2.15, at a ratio of 1.0 ml of glycine buffer per 125 mg (wt weight) of cells. The suspension was stirred at 0 °C for 2-30 min. The cells were centrifuged and washed once in PBS and once in PBS/TBA before use in 125I-IgG binding assays. Whole cell lysates were subjected to SDS-PAGE and Western-blotting with affinity-purified polyclonal anti-A-protein Ig to determine the extent of removal of A-protein from the cells.

Electrophoretic Techniques—Whole cell lysates were subjected to SDS-PAGE in 15% gels according to the system of Laemmli as modified by Aanes (21). Gels were stained with Coomassie Brilliant Blue R-250. Electrophoretic transfer of proteins from SDS-PAGE to nitrocellulose and development of the blot with affinity-purified polyclonal anti-A-protein Ig was carried out according to Towbin et al. (22), except that 0.05 M Tris-HCl, 0.005 M EDTA, 0.15 M sodium chloride, pH 7.4, containing 0.5% Tween 20 was used at all steps after the initial blocking step.

Iodination—Immunoglobulins. A-protein, and bovine serum albumin were iodinated by reacting 50 μg of protein with 1 mCi of Na125I in 0.3 M sodium phosphate, pH 7.3, in a test tube containing 4 μg of immobilized IODO-GEN iodination reagent. Residual 125I was reacted with excess L-tyrosine. Labeled protein was separated from unreacted iodine by chromatography on a disposable 9-ml Sephadex G-25 column (Pharmacia) and eluted with PBS containing 1% bovine serum albumin and 0.1% sodium azide.

Purification of A-protein—A-protein from A450 was purified by homogeneity by previously published methods (13), except that the guanidine hydrochloride concentration used to extract and disperse the A-protein was changed from 6 M to 2 M to minimize possible denaturation. A-protein from the excreting mutant A451-70 was obtained from the culture supernatant after growing the cells to late exponential phase in a medium comprising 2% casamino acids, modified Davis minimal medium, and 0.5% dextrose. The culture supernatant was desalted on a Sephadex G-25 column (Pharmacia) and eluted with PBS containing 1% bovine serum albumin and 0.1% sodium azide.

Modification of Cell Surface Protein—A450 cells were grown in the presence of [125I]orthophosphate and washed with PBS. They were washed once with modification reaction buffer, which was chosen to maximize the modification reaction rate and specificity for target amino acids while minimizing damage to the cells. The pH was kept in the range of 5.0 to 8.5. Cells (100 mg wet weight) were suspended to 2.0 ml in the same buffer and the protein modification reagent added as a small volume of concentrate. For most reagents, two or more concentrations in the range 0.1 to 10 mM were used. Incubation was at 0 °C or room temperature with gentle shaking. In all cases, a control was run with no modification reagent to determine the effect of buffer alone or the cells. Treated cells were centrifuged, washed twice, resuspended in PBS/TBA to 75 mg/ml (wt weight) (based on the assumption that no cell lysis had occurred), and 125I-IgG binding was assayed. Cell mass was followed as sedimentable 32P; the amount of IgG bound (32P) was corrected to account for any loss in cell mass. The data were determined by Cerenkov counting in a Beckman LS8100 scintillation counter.

Perturbation of Cell Surface—A450 cells labeled with [125I]orthophosphate were washed, and 100 mg (wt weight) of cells were suspended in 2.0 ml of PBS/A. 2.0 ml of perturbant (chaotrope, detergent, or EDTA) in PBS/A was added while mixing. The mixture was incubated at room temperature for 30 min with shaking.

RESULTS

Whole Cell Immunoglobulin Binding—S. aureus SAC cells bind IgG in a cooperative manner (23), as shown in Fig. 1. A. salmonicida A450 cells also bound IgG, but the binding was noncooperative (Fig. 1). The isogenic A-layer-negative strain A450-3 was virtually devoid of IgG binding activity (Fig. 1). This implicated the A-layer as being responsible for the binding phenomenon.

Specificity of Immunoglobulin Binding—The ability of A450 cells to bind a series of affinity-purified polyclonal immunoglobulins (rabbit IgG, rabbit IgG Fc, human IgG, human serum IgA, human IgM) and rabbit anti-mouse IgG Fab was investigated. Only rabbit IgG and human IgM were found to bind effectively. A low level of binding was observed with the other Ig classes and Ig fragments (approximately 10-fold lower), but saturation was not found at high Ig concentrations. The cells did not appear to bind proteins nonspecifically since 125I-labeled bovine serum albumin did not bind. Isogenic A-protein-negative cells (A450-3) were unable to bind any of the immunoglobulin classes. Unlike SAC cells which have specific IgG Fc receptors (4), A450 cells were unable to bind either the Fc portion or the Fab portion of IgG indicating that the intact molecule was required for binding.

Parameter of Immunoglobulin Binding—The binding of rabbit IgG and human IgM to A450 and A450-3 cells was analyzed. Binding of both immunoglobulins to A450 cells exhibited saturation (Figs. 2 and 3). A450-3 cells were unable to bind the immunoglobulins effectively at any concentration of IgG studied. The apparent affinity for the immunoglobulins was high with a Kd of 1.0 × 10⁻⁶ M for rabbit IgG and 3.3 × 10⁻⁶ M for human IgM. A Scatchard analysis (24) of these data (Fig. 2, inset and Fig. 3, inset) determined that the [P],...
IgG concentrations ranging from 1.0 \times 10^{-7} \text{ M} to 2.0 \times 10^{-6} \text{ M}, in 0.4 \text{ ml of PBS/TBA}. The A-protein on the surface of A450 cells represented a concentration of approximately 1.5 \times 10^{-6} \text{ M}. After incubation for 40 min at room temperature, cells were centrifuged and washed. From the bound label, the concentration of bound IgG was calculated, and free IgG was obtained by subtraction from the known total concentration of IgG. All data points represent two determinations.

Values of $K_d$ were calculated from double-reciprocal plots ($1/bound \text{ IgG} versus 1/free \text{ IgG};$ not shown); binding stoichiometries $N$ were determined from Scatchard plots (inset). A450, --; A450-3, - -; A451-3, O-O. Inset, Scatchard analysis of IgG binding to A450.

values were $0.22 \times 10^{-6} \text{ M}$ and $0.41 \times 10^{-6} \text{ M}$ for rabbit IgG and human IgM, respectively. In the case of IgG, this indicated the binding stoichiometry to be 1 IgG molecule to 4 or 5 A-protein monomers. For IgM, the ratio is approximately 1 IgM to 2–3 A-protein molecules. Thus, a simple 1:1 association of immunoglobulin with A-protein does not exist.

**Bacterial Strain Specificity**—Various *A. salmonicida* strains were examined for their ability to bind IgG. The A-layer-containing strains A449 and A451 bound approximately twice as much as A450. Isogenic mutants devoid of A-layer (A449-3, A450-3, A451-3) were binding-negative. Strain A451-70, a mutant which excretes A-protein rather than assembling it on the cell surface (15), was also unable to bind. A related pathogen, *Aeromonas hydrophila* TF7, containing its own brand of RS-layer (25), was also unable to bind immunoglobulin. Thus, there is some strain variation in binding but in each case the binding specifically requires the presence of an A-layer on the surface of the cell.

**Selective Removal of A-protein**—During the course of this investigation, we discovered a selective chemical method for removal of A-protein from the surface of the cell. Briefly, when cells of A-layer-containing strains were treated in 0.2 \text{ M} glycine buffer at pH 2.2 and 0 °C, the A-protein layer disassembled into A-protein monomers which could be removed by centrifugation. The resulting cells (Fig. 4) can be seen with diminished A-protein content. Approximately 10–20% of the A-protein is not removed by this method. That the residual band corresponded to A-protein and not another protein of the same $M_t$ was shown by the positive reaction of the band in a Western blot against anti-A-protein antibody. In a series of experiments, the ability to bind rabbit IgG diminished corresponding to the loss of the A-protein from the layer (Table I). These results strongly suggest that A-layer is responsible for IgG binding.

* A. salmonicida A-protein versus *S. aureus* Protein A Binding—Reciprocally competitive inhibition experiments using either *S. aureus* protein A or *A. salmonicida* A-protein showed that protein A completely inhibited the binding of rabbit IgG to SAC cells but had little or no effect on binding to A450 cells (Fig. 5, left panel). A-protein had no effect on IgG binding to SAC cells and could only inhibit binding to A450 cells at very high A-protein concentrations and only to a level of 50% (Fig. 5, right panel). These results indicate that SAC cells and A450 cells recognized different binding sites on IgG.

**Requirement of a Supramolecular Structure for IgG Binding**—As noted above, A-protein only prevented the binding of immunoglobulins to cells when present at unusually high concentrations. Direct demonstration of IgG binding to soluble A-protein proved to be very difficult. When A-protein was covalently cross-linked to cyanogen bromide-activated Sepharose and the beads used as a sedimentable form of A-protein in an $^{125}$I-IgG binding assay, the amount of immunoglobulin bound was negligible. Very weak binding (Fig. 6) was observed

$^2$ W. W. Kay, unpublished results.

**TABLE I**

<table>
<thead>
<tr>
<th>$A. \text{ salmonicida A}^+$ cells</th>
<th>IgG binding</th>
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<tr>
<td>5-min extraction</td>
<td>23</td>
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<tr>
<td>30-min extraction</td>
<td>10</td>
</tr>
<tr>
<td>Double 30-min extraction</td>
<td>8</td>
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**FIG. 2**. Analysis of binding of $^{125}$I-rabbit IgG to *A. salmonicida* cells. 15 mg (wet weight) of cells were incubated with $4 \times 10^6$ cpm of $^{125}$I-IgG, at IgG concentrations ranging from $1.0 \times 10^{-7}$ M to $2.0 \times 10^{-6}$ M, in 0.4 ml of PBS/TBA. The A-protein on the surface of A450 cells represented a concentration of approximately $1.5 \times 10^{-6}$ M. After incubation for 40 min at room temperature, cells were centrifuged and washed. From the bound label, the concentration of bound IgG was calculated, and free IgG was obtained by subtraction from the known total concentration of IgG. All data points represent two determinations. Values of $K_d$ were calculated from double-reciprocal plots ($1/bound \text{ IgG} versus 1/free \text{ IgG}$; not shown); binding stoichiometries $N$ were determined from Scatchard plots (inset). A450, --; A450-3, - -; A451-3, O-O. Inset, Scatchard analysis of IgG binding to A450.

**FIG. 3**. Analysis of binding of $^{125}$I-human IgM and $^{125}$I-human IgG to *A. salmonicida* cells. Experiments were performed, and binding parameters were determined as described in the legend to Fig. 2. IgM binding to A450, --; IgM binding to A450-3, - -; IgG binding to A450, A-A. Inset, Scatchard analysis of IgM binding to A450.

**FIG. 4**. Removal of A-protein from the surface of *A. salmonicida* A450 cells by low pH extraction. Washed cells were extracted with 0.2 M glycine, pH 2.15, at 0 °C for 2 min or 30 min. Cells were centrifuged, washed, and lysed in SDS-PAGE sample buffer. Supernatants containing extracted A-protein were precipitated with trichloroacetic acid. The extracted A-protein shown was from the 2-min extraction. Samples were subjected to SDS-PAGE and stained with Coomassie Blue. The $M_t$ of A-protein is 49,000.

**FIG. 5**. Inhibition of *A. salmonicida* A-protein binding to SAC cells by A450. A-protein had no effect on IgG binding to SAC cells and could only inhibit binding to A450 cells at very high A-protein concentrations and only to a level of 50% (Fig. 5, right panel). These results indicate that SAC cells and A450 cells recognized different binding sites on IgG.

**TABLE I**

<table>
<thead>
<tr>
<th>Effect of 0.2 M pH 2.2 glycine extraction on rabbit IgG binding by $A^+$ Aeromonas cells</th>
<th>IgG binding</th>
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<td>Untreated</td>
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<td>5-min extraction</td>
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<td>Double 30-min extraction</td>
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and incubated 40 min at room temperature. The final concentration of $^{125}$I-IgG (5 x 10$^4$ cpm) was mixed with 0.05 ml of either protein A (1–20 μg) or A-protein (4-100 μg) and incubated 20 min at room temperature. 0.4 ml (10 mg) of SAC cells or A450 cells were added and incubated 40 min at room temperature. The final concentration of IgG was $3.5 \times 10^{-7}$ M, of protein A was $5 \times 10^{-8}$ M to $1 \times 10^{-7}$ M, and of A-protein was $1.0 \times 10^{-7}$ M to $8.0 \times 10^{-9}$ M. Left panel, effect of S. aureus protein A on $^{125}$I-IgG binding to SAC cells (A – A) and A450 cells (A – O). Enhanced binding to A450 cells at low protein A concentrations is probably due to the formation of complexes of S. aureus protein A on 9-IgG binding to SAC cells (A – A).

Fig. 6. Analysis of binding of rabbit IgG to $^{125}$I-labeled A. salmonicida A-protein. $4 \times 10^4$ cpm of A-protein at a final concentration of $5.0 \times 10^{-7}$ M to $1.0 \times 10^{-5}$ M and unlabeled rabbit IgG at a final concentration of $3.0 \times 10^{-5}$ M were combined in 0.2 ml of PBS/TBA. After incubation at room temperature for 40 min, 0.2 ml of SAC cells was added to precipitate free IgG and IgG bound to $^{125}$I-A-protein. The cells were sedimented and washed, and total cell-bound label was determined. Concentrations of bound and free A-protein were calculated from the known total concentration of A-protein. Inset, double reciprocal plot (1/bound A-protein versus 1/free A-protein) from which $K_D$ was calculated. All assays were run in duplicate.

using an assay in which $^{125}$I-A-protein was allowed to bind to rabbit IgG and sedimented with SAC cells. Thus, A-protein appears to have a binding affinity for IgG which is weak unless the protein is assembled in an ordered arrangement as it is in the A-layer. Additional evidence comes from reconstitution experiments in which soluble A-protein was mixed with isogenic A-layer-negative cells and allowed to assemble on their surfaces. The association appeared to be specific, i.e. A-protein did not associate with cells of other bacterial strains, and a tetragonally arranged layer was formed. However, the reconstituted array had a distinctly altered appearance compared to native arrays, and no immunoglobulin binding occurred. Taken together, these data suggest that a special molecular arrangement of A-protein monomers in a native A-protein layer is required for effective immunoglobulin binding.

**TABLE II**

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<th>Modification or perturbation with</th>
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<td>2-Hydroxy-5-nitrobenzyl bromide</td>
<td>Trp</td>
<td>15</td>
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<tr>
<td>Formaldehyde</td>
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<tr>
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<tr>
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*The abbreviations used are: TNM, tetranitromethane, DEPC, diethyl pyrocarbonate; EDAC, i-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NBS-F, p-nitrobenzenesulfonfluoride; DOC, deoxycholate.

**Effects of Protein Modification Reagents and A-layer Perturbants on IgG Binding**—In an attempt to determine whether specific amino acid residues of A-protein were responsible for IgG binding, we first subjected A450 cells to a series of protein modification reactions. In some cases, protein modifications on the whole cells caused cell lysis and thus fewer cells were available to bind IgG. For an accurate measure of cell mass, we labeled the cells by growth in media containing $^{32}$P orthophosphate and determined sedimentable $^{32}$P as Cerenkov radiation. $^{125}$I-IgG binding was then expressed as a function of cell mass by calculating the ratio of $^{125}$I (IgG-bound) to $^{32}$P (cell mass). Table II lists the results of these binding experiments. Traditional single residue modification reagents were largely ineffective in preventing IgG binding. This is in contrast to S. aureus protein A-containing cells, in which modification of a critical tyrosine residue drastically reduces binding (26). Only those agents which modify a wider spectrum of residues such as diazosulfanilic acid or formaldehyde caused significant inhibition of IgG binding.

In a similar series of experiments, we treated A450 cells with reagents known or suspected to perturb the A-layer without fully dissociating it. A sample of treated cells was subjected to SDS-PAGE to verify that the layer had not been removed from the cells. Guanidine, a known A-layer perturber (12, 13), and heating at 80 °C were effective in disrupting IgG binding. Thus, it appeared that no single residue was responsible for binding. Instead, either structural perturbation of the intact A-layer or more widespread amino acid modification led to inhibition of IgG binding. The data suggest that there is no single easily definable binding site on the A-protein monomer responsible for the observed high degree of IgG binding.

**DISCUSSION**

To the best of our knowledge, A. salmonicida is the first Gram-negative bacterium shown to be able to bind immunoglobulins. Binding does not appear to be as extensive with...
respect to immunoglobulin types as that exhibited by staphylococcal protein A or streptococcal protein G from Gram-positive cells. So far, effective binding to A. salmonicida IgG has only been demonstrated for rabbit IgG and human IgM, although low levels of binding were detected with other immunoglobulin classes and subclasses. The affinity for binding of IgG to A. salmonicida cells (Kd = 1.0 x 10^-9 M) was approximately 2 orders of magnitude lower than that exhibited for S. aureus protein A (Kd = 2.5 x 10^-6 M - 5 x 10^-6 M) (23), and approximately 3 orders of magnitude less than that of protein G (7). Nevertheless, binding constants in the micromolar range indicate relatively high affinity binding.

The binding to A. salmonicida cells is specifically determined by the presence of A-layer on the cell surface. Isogenic mutants devoid of this layer were totally unable to bind immunoglobulins and when A-protein was selectively extracted from the cell surface at pH 2.2, IgG binding was strongly diminished. Mutants of either Staphylococcus or Streptococcus devoid of binding activity have not been reported. In contrast to proteins A and G, which are covalently bound to the cell wall and require digestion for liberation (8, 27), A-protein is noncovalently associated with the surface of A. salmonicida cells and easily removed (12). Among various strains of A-layer-containing A. salmonicida, the binding activity varied somewhat. However, all isogenic mutants devoid of the layer were unable to bind immunoglobulin.

The binding of IgG or IgM does not appear to be nonspecific since 125I-bovine serum albumin does not bind to A. salmonicida cells. IgG Fc or Fab fragments are also unable to bind, which indicates that the intact immunoglobulin molecule is required for binding to the cell surface. In addition, other immunoglobulins such as human IgA and IgG do not bind to A. salmonicida.

The nature of IgG binding by the A-layer is distinct from that of S. aureus protein A. The requisite protein from either cell does not compete for immunoglobulin binding to the other strain. Both S. aureus cells and streptococcal protein G-containing cells bind to the Fc portion of immunoglobulins (8).

For effective immunoglobulin binding, it appears that A-protein must be organized into its native regular surface array. Purified A. salmonicida monomeric A-protein does not bind well to immunoglobulins either immobilized on Sepharose or as SAC-precipitated nonimmune complexes. The binding data that were obtained, albeit with some difficulty, indicated a 5- to 10-fold reduction in affinity for immunoglobulins compared to whole cells. A-protein competed only weakly for binding of immunoglobulins to whole cells. Attempts at cellular reconstitution demonstrated that A-protein monomers will specifically absorb to A. salmonicida cells as long as cells contain LPS O-polysaccharide. Interestingly, this surface-reconstituted A-protein does not bind IgG. In keeping with our hypothesis that binding requires a native array-like arrangement of A-protein, these reconstituted cells exhibit an altered tetragonal array structure when examined by electron microscopy.

Fc receptor sites on S. aureus protein A can be specifically chemically modified and thus inactivated (26, 28). Since poor binding was demonstrated with purified A-protein, we tried to identify amino acid residues in the putative binding site by modifying the protein in situ on the cell surface and looking for loss of binding. In general, specific amino acid modification reagents were ineffective in preventing binding and only those reagents with lesser specificity caused some inactivation. The two reagents having the greatest effect were formaldehyde, which has been shown to modify as many as 6 amino acid residues, as well as to form covalent cross-links between residues (29), and diazosulfanilic acid, which modifies Lys, His, and Tyr and perhaps other residues (20). Perturbation of A-layer structure with guanidine or by heating at 80 °C were also effective in inhibiting immunoglobulin binding. Taken together, the data suggest that an intact and organized A-layer is required for immunoglobulin binding and that a specific three-dimensional arrangement of adjacent A-protein monomers, as found in the native layer, is required to form the intact immunoglobulin binding site. This interpretation agrees with the binding data (Fig. 2) which indicate that as many as 4-5 A-protein molecules are required to bind a single immunoglobulin molecule. From recent studies on the three-dimensional architecture of the A-layer on the surface of A. salmonicida, it has been concluded that the subunits are arranged in a 4-fold symmetry forming a three-dimensional pit. It is tempting to speculate that this pit may act as a specific trap for immunoglobulin molecules. Alternatively, a fraction of A-specific monomers on the cell surface may be inaccessible due to shielding by LPS O-polysaccharide chains. Obstruction of specific immunoglobulin binding to outer membrane proteins of other Gram-negative bacteria by LPS O-chains has been documented (30, 31). Whatever the mechanism, the binding of immunoglobulin molecules by A. salmonicida may well have functional significance. It may act as a mechanism to shield the bacterium from host immune defenses (16), and/or help the organism enter macrophages by a nonspecific opsonization, to facilitate the spread of an infection (17).

Acknowledgements—We are indebted to the Natural Sciences and Engineering Research Council of Canada for a strategic grant covering part of this research and to T. J. Trust for helpful suggestions.

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Immunoglobulin Binding by Regular Surface Array of A. salmonicida