Streptococcal Protein G

GENE STRUCTURE AND PROTEIN BINDING PROPERTIES

(Received for publication, July 23, 1990)

Ulf Sjöbring§§, Lars Björck††, and William Kastern**

From the Department of Medical Microbiology, University of Lund, S-223 62 Lund, Sweden, and the Hagedorn Research Laboratory, Gentofte, Denmark

Protein G was solubilized from 31 human group C and G streptococcal strains with the muraytic enzyme mutanolysin. As judged by the mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the binding patterns of the solubilized protein G molecules in Western blot experiments, the strains could be divided into three groups, represented by the group C streptococcal strains G148 and G43 and the group G streptococcal strain C40. The 65-kDa G148 protein G and the 58-kDa C40 protein G showed affinity for both immunoglobulin G (IgG) and human serum albumin (HSA), whereas the 40-kDa G43 protein G bound only IgG. Despite the different molecular patterns, the three protein G species had identical NH2-terminal amino acid sequences. Apart from the 65-kDa peptide, digestion of G148 streptococci with mutanolysin also produced a 52-kDa IgG- and HSA-binding peptide and a 14-kDa HSA-binding peptide. It was demonstrated that these peptides resulted from cleavage of 65-kDa protein G by proteolytic components in the mutanolysin preparation. The protein G genes of the C40 and G43 strains were cloned and sequenced, and their structure was compared to the previously published sequence of the G148 protein G gene. As compared to G148, both the C40 and G43 genes lacked a 210-base pair fragment in the IgG-binding region, accounting for the 10-fold lower affinity of these proteins for IgG. The G43 gene also lacked a 450-base pair fragment in the 5'-end of the gene, explaining why the G43 protein G did not bind HSA. The differences in protein G structure did not correlate with the clinical origin of the strains used in this study. The IgG-binding region of protein G was further mapped. Thus, a peptide corresponding to a single IgG-binding unit was obtained by the cloning and expression of a 303-base pair polymerase chain reaction-generated DNA fragment. The affinity of this 11.5-kDa peptide for human IgG was 8.0 x 10^5 M^-1, as determined by Scatchard plots. Finally, a 55-amino acid-long synthetic peptide, corresponding to one of the three repeated domains in the COOH-terminal half of strain G148 protein G, effectively blocked binding of protein G to IgG.

* This work was supported by grants from the Swedish Medical Research Council (Project 7480), King Gustav V:s 80 years Foundation, and the A. Osterlund Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§§To whom correspondence and reprint requests should be addressed.
††Present address: Dept. of Medical and Physiological Chemistry, University of Lund, Box 94, S-221 00, Lund, Sweden.
**Present address: University of Florida, College of Medicine, Dept. of Pathology, Box J-275 JHMHC, Gainesville, FL 32610.

Protein G is the immunoglobulin G (IgG)-binding protein of group G streptococci (1, 2) and consists of repetitively arranged domains (3, 4). The COOH-terminal domains are responsible for IgG binding, whereas domains in the NH2-terminal half of the protein have been found to bind human serum albumin (HSA) (5-7). In the present study, we have solubilized and analyzed protein G from 31 group C and G streptococcal strains. We find that solubilized protein G molecules can be divided into three groups with respect to molecular weight and binding properties. To investigate the molecular basis for the observed differences, we have cloned protein G genes from isolates representative of each group and compared the binding properties of the three different protein G molecules expressed in Escherichia coli. One of the genes has previously been sequenced (8), whereas the two other genes were sequenced in this study. The structures of the genes were related to the protein binding characteristics of the three gene products. In the present study, we further define and analyze the IgG-binding region of protein G by characterization of a single IgG-binding unit.

MATERIALS AND METHODS

Bacteria and Growth Conditions—The group G streptococci G43, G148, and the group C streptococci C40 are type isolates of human tonsillar origin used in our laboratory (1, 9). Six group C and twelve group G streptococcal isolates from the blood of patients with sepsis were collected at the Laboratory of Clinical Bacteriology, Lund, 1983-87, as were five tonsillar specimens of each group. Serogrouping of streptococci was performed by the co-agglutination method (10). The streptococci were cultured in Todd-Hewitt broth (Difco) at 37 °C. The streptococci were heat killed, washed, and resuspended to 10% (v/v) in 10 mM Tris-HCl, at the pH to be used in the experiments. The bacterial concentration was measured by determining the optical density at 620 nm. One ml of 10% bacteria was equal to 2 x 10^9 bacteria.

Proteins and Radiolabeling of Proteins—HSA, human polyclonal IgG, and serum albumin (several lots) were purchased from Sigma. Proteins were labeled with 125I-iodine (Amersham) using a lactoperoxidase/glucose oxidase Enzymobead kit from Bio-Rad, following the instructions supplied by the manufacturer. Gelatin was used as a carrier. Free iodide was separated from protein-bound iodine by chromatography on 9-ml columns of Sephadex G-25 (PD-10, Pharmacia LKB-Biotechnology Inc.). The proteins were radiolabeled to a specific radioactivity of 1-5 mCi/mg.

IgG-Binding Assay—The level of cell-bound protein G activity before and after treatment with various amounts of mutanolysin was determined by the binding of 125I-IgG to whole streptococci as described (1).

Determination of Affinity Constants—Constant amounts of HSA and human polyclonal IgG, coupled to Immunobeads (Bio-Rad) and 125I-protein G were incubated with varying amounts of nonteleblotted protein G for 2 h at 25 °C. The radioactivity bound to the beads was determined in a γ-counter. Details of the procedure and calculations, the abbreviations used are: IgG, immunoglobulin G; HSA, human serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.
using the formula of Scatchard (11), have been reported earlier (6).

Preparative Chromatography—Affinity chromatography was performed on columns of IgG- or HSA-Sepharose (1, 7). Bound material was eluted with 0.1 M glycine-HCl, pH 2.0. Gel filtration was done on Sephadex G-200 (Pharmacia) in 0.02 M Tris-HCl, pH 8.0, containing 0.15 M NaCl and 0.02 M NaN3. Eluted fractions were analysed for total protein by measuring the absorbance at 280 nm and screened for IgG- and HSA-binding peptides by SDS-PAGE and Western blot analysis.

SDS-PAGE—SDS-PAGE was run as described by Neville (12). Samples or minigel slabs were run, using a total acrylamide concentration of 10% with 3.3% cross-linking, unless stated otherwise. Samples were pretreated by 3-min boiling in a sample buffer containing 2% SDS and 5% 2-mercaptoethanol. Molecular weight marker kits were purchased from Sigma. Gels were stained with Coomassie Blue.

Western Blots and Analysis of Proteins Applied to Nitrocellulose or Polyvinylidene Difluoride Membranes—Proteins were applied to nitrocellulose (Schleicher & Schuell) or polyvinylidene difluoride membranes (Immobilon), as described by Towbin et al. (13), or manually in dots using a manifold (Schleicher & Schuell). The nitrocellulose membranes were blocked, incubated with radiolabeled proteins, and washed as previously described (5). Autoradiography was done at -70°C using Kodak X-Omat S films and Cronex Xtra Plus intensifying screens. Proteins transferred to polyvinylidene difluoride membranes were visualized with Coomassie Blue, cut out, and subsequently subjected to NH2-terminal amino acid sequence analysis, as described by Studara (14).

Amino Acid Sequences—Sequence analysis was performed on an Applied Biosystems gas-phase Sequencer model 470A equipped with an on-line PTH analyzer model 120A and a Data Module model 900A, using chemicals and software supplied by the manufacturer.

Peptide Synthesis—A 55-amino acid peptide (-H-TYKLILNGKTLKGETTTEAVNAATAEKVFQYANDNGVDGEW-)

was eluted with 0.1 M NaCl and 0.02 M NaN3. Eluted fractions were analysed for total protein by measuring the absorbance at 280 nm and screened for IgG- and HSA-binding peptides by SDS-PAGE and Western blot analysis.

**RESULTS**

**Solubilization of Streptococcal Protein G with Mutanolysin**—The muramyl enyme mutanolysin was used to solubilize protein G from group G streptococci of the G148 strain. Conditions for the treatment were studied by measuring the 125I-IgG binding capacity of digested bacteria and by Western blots of supernatants from digested streptococci, using 125I-human IgG as a probe. The most effective mutanolysin concentration was 160 units (32 μg of protein) per 2·109 streptococci at pH 9.0 for 12 h (Fig. 1). Further studies were carried out at the conditions indicated by these experiments. The Western blot experiments (Fig. 1) suggested that mutanolysin solubilized two major IgG-binding molecules from G148 streptococci. These peptides, with molecular masses 65 and 52 kDa, respectively, were purified from the supernatants of mutanolysin-digested G148 streptococci by affinity chromatography on IgG-Sepharose, followed by separation on a Sephadex G-200 column (300·15 mm). When radiolabeled, both these peptides bound to IgG and HSA on nitrocellulose filters. In addition, a 14-kDa peptide which only bound HSA was isolated as previously described (7) when the supernatant was passed over HSA-Sepharose. The NH2-terminal amino acid sequence of the mutanolysin-solubilized 65-kDa protein (Fig. 2) was identical with the sequence of a 65-kDa protein G molecule purified from G148 culture medium and to that of the protein G expressed by E. coli containing the cloned G148 protein G gene (6). Comparison of the NH2-terminal amino acid sequences of the 65-, 52-, and 14-kDa peptides (Fig. 2) with the deduced amino acid sequence of protein G of G148 (8) indicated that the 52- and 14-kDa peptides resulted from proteolytic activity in the mutanolysin preparation on the 65-kDa protein. The effect of mutanolysin on purified 65-kDa protein was studied in a series of experiments. The curve shown in Fig. 1 shows the extent of solubilization of protein G with mutanolysin. Group G streptococci (strain G148, 2·1010 bacteria/ml) were digested with mutanolysin at final concentrations of 5·1000 units/ml in 0.05 M phosphate buffer, pH 9.0, for 12 h at 37°C. The curve shows the extent of binding of 125I-IgG to bacteria subjected to mutanolysin at the indicated concentrations. The autoradiographs (inset) shows the size and the relative amount of protein G in 50 μl of the supernatants of mutanolysin-digested bacteria as judged by Western blots of SDS-PAGE gels probed with 125I-IgG.
Protein G Genes

FIG. 2. NH2-terminal amino acid sequences and map of the protein G peptides produced by mutanolysin digestion of G148 streptococci. The NH2-terminal amino acid sequence of the protein G molecules resulting from treatment of G148 streptococci with mutanolysin was determined. The size of the fragments was estimated by mobility in SDS-PAGE. The nomenclature of Olsson et al. (8) is used for designating the domains of protein G.

Protein G Gene (G148)

| Domain | Amino Acid Sequence |
|--------|---------------------|
| Ss     | VDSPIEDTPIRNGG      |
| E      | ALRELKDGYVGYDHLYK   |
| A1     | ALRNEESATDLTAA      |
| B1     |                        |
| A2     |                        |
| A3     |                        |
| A4     |                        |
| B2     |                        |
| A3     |                        |
| B3     |                        |
| B4     |                        |
| C1     |                        |
| p1     |                        |
| p2     |                        |
| C2     |                        |
| p3     |                        |
| C3     |                        |
| M      |                        |
| W      |                        |

Mr 66 50 25 kDa

FIG. 3. Cleavage of protein G (65 kDa) with mutanolysin. 1 µg of purified protein G (65 kDa) was incubated with different amounts of mutanolysin in phosphate buffer, pH 9.0, for 12 h at 37 °C. The reactions were terminated by boiling the samples for 3 min with an equal volume of a sample buffer containing 5% 2-mercaptoethanol and 2% SDS. The samples were then separated on SDS-PAGE (13.5% T, 3.3% C). Lane A, protein G incubated with buffer; lane B, protein G incubated with 1 unit of mutanolysin; lane C, protein G incubated with 2 units of mutanolysin; lane D, 6 units of mutanolysin. One unit of mutanolysin corresponds to 0.2 µg of protein.

kDa protein G supported this notion (Fig. 3). Moreover, the first three NH2-terminal amino acids of the 14-kDa fragment (Ala-Leu-Arg-) resemble corresponding residues in the NH2 terminus of the 52-kDa fragment (Ala-Asn-Arg-), and the NH2 termini are in both cases preceded by a Leu residue, suggesting a possible site of recognition for a peptidase.

Comparison of the Protein G Molecules of Group C and G Streptococci—Protein G from 30 additional group C and G streptococcal strains of different clinical origin was solubilized using mutanolysin. The resulting supernatants were separated on SDS-PAGE in duplicate, electroblotted to nitrocellulose membranes, and probed with either radiolabeled IgG or HSA. Three different patterns were seen, each represented by one of the strains in Fig. 4. As with G148, digestion of the C40 type isolates with mutanolysin produced two IgG- and HSA-binding peptides. The larger of the C40 peptides had a molecular mass of about 58 kDa. The G43-type protein G was considerably smaller (40 kDa) and appeared as a single band which bound only 125I-IgG. The molecular pattern did not correlate with either the serological group of the tested C or G streptococci or the clinical source of the isolates. The mutanolysin-solubilized 58-kDa C40 and 40-kDa G43 protein G molecules were purified. The proteins were radiolabeled and used as probes to IgG and HSA on nitrocellulose. Labeled C40 protein G showed affinity for both IgG and HSA, whereas the G43 protein bound only IgG. The NH2-terminal amino acid sequences of the purified C40 58-kDa and the G43 40-kDa protein G molecules were identical with that of the G148 65-kDa protein (VDSPIEDTPIRNGG).

Cloning of the Protein G Genes of a Group C (C40) and a Group G (G43) Streptococcus—The protein G genes of the C40 and G43 strains were cloned. An oligonucleotide which was homologous to a sequence in the IgG-binding region of the G148 protein G (C1 domain) was shown to hybridize to a 2.2-kilobase C40 HindIII chromosomal DNA fragment, as well as to a 1.8-kilobase G43 HindIII DNA fragment (19). These fragments were cloned into the HindIII site of pUC18, and the E. coli strain JM105 was transformed with the resulting plasmids. To identify clones with protein G inserts, the oligonucleotide 5'-GCTGCTACTGCAGAAAAAGTTCAAA-3', a sequence found in the C1 domain of the G148 protein G gene (3), was used as a probe in colony blot experiments. Clones which reacted with the probe were grown, and the lysates were applied to nitrocellulose and screened for IgG binding using 125I-IgG as a probe.
Characterization of C40 and G43 Protein G Genes Expressed in E. coli—Lysates of the E. coli clones with the genes for C40 and G43 protein G were purified on IgG-Sepharose, followed by chromatography on Sephadex G-200. Fig. 5 shows the protein G molecules expressed by these clones and analyzed by SDS-PAGE and Western blot experiments, using $^{125}$I-IgG and $^{125}$I-HSA as probes. The patterns obtained were similar to those found when separating the mutanolysin-released proteins (Fig. 4). Thus, the cloned C40 molecule appeared smaller than G148 protein G by approximately 7 kDa, but still reacted with both probes. The cloned G43 protein was considerably smaller (40 kDa) and bound only $^{125}$I-IgG. The three different protein G molecules were radiolabeled and used as probes to IgG and HSA on nitrocellulose. Again, the G148 and C40 protein G molecules showed affinity for both IgG and HSA, whereas the G43 protein G bound only IgG (Fig. 6). Scatchard plots for the reactions between the three protein G species and IgG revealed that both the C40 and G43 protein G molecules bound to IgG with an affinity 10 times lower than the G148 protein G (Fig. 7). The G148 and C40 molecules both had the same affinity for HSA, whereas the G43 protein did not bind to HSA (Fig. 7).

Nucleotide Sequences of the C40 and G43 Protein G Genes—To study the structural basis for the different binding properties exhibited by the three protein G species in more detail, the nucleotide sequences of the C40 and G43 protein G genes were determined and compared to the previously reported sequence of the G148 gene (8).

The C40 and G43 protein G genes (Fig. 8) were both highly similar to the G148 gene in the regions which do not encode IgG or HSA binding. Thus, only conserved base pair substitutions were recognized in the signal peptide (Ss), the alanine-rich region (E), the sequence which separates the albumin- and IgG-binding regions (S), the wall-spanning region (W), and the membrane anchor (M). Since the NH$_2$-terminal amino acid sequences of the mutanolysin-solubilized protein G molecules of the G148, C40, and G43 strains were identical, it is unlikely that translational or post-translational events involving the NH$_2$-terminal part of the proteins account for the different molecular weights. The cell walls of group C and G streptococci have a very similar structure (20) and a high degree of similarity in the wall-spanning regions of the protein G molecules expressed by strains of the two different streptococcal groups was not unexpected.

The $M$, of the protein G molecules encoded by these genes were 50,424 and 31,683 for C40 and G43, respectively, as compared to 59,260 for G148. For all three molecular species, the molecular mass was overestimated on SDS-PAGE by 7 to 9 kDa. The reason for this is unclear, but it has been suggested that the relatively large number of proline residues in the wall-spanning regions of peptidoglycan-associated proteins could explain this discrepancy (3, 21). We do not favor this explanation since the $M$, values of protein G peptides devoid of the wall-spanning region were also overestimated by SDS-PAGE (22 and present investigation). It is possible that the special amino acid composition (low content of hydrophobic residues) of these molecules possibly results in poor binding of SDS (22). Another feature which may influence the migration in SDS-PAGE is the extremely elongated shape of protein G (23).

The parts of the gene which encode the albumin-binding (A and B repeats) and IgG-binding (C and D repeats) properties however were subject to variation. Thus, the C40 gene in comparison to the G148 protein G gene contained all three A repeats, as well as the two B repeats. A single amino acid substitution was found in the A3 repeat. This finding was in agreement with the fact that the C40 protein G bound to HSA with an affinity comparable to that of the G148 protein G (Fig. 7). The gene had, however, lost one D and one C repeat (corresponding to C2 of G148), together 210 base pairs. The remaining two C repeats were identical with the corresponding repeats of the G148 gene. The loss of a domain in the IgG-binding region of the C40 protein G would account for the lower affinity for IgG, as well as for its lower molecular weight. The part of the G43 protein G gene which encodes IgG binding was identical with that of the C40 gene, i.e. it had two C repeats, homologous to the C1 and C3 repeats of G148, flanking a single D repeat. However, the G43 gene only retained one A repeat, which was identical with the A3 region of C40, and it had no B repeat. This indicates that a single A domain is not sufficient for albumin binding to protein G.

Expression and Characterization of a Single IgG-binding Protein G Unit—A 55-residue peptide, corresponding to the C1 domain of protein G, was synthesized. This 7.2-kDa peptide effectively inhibited the binding of protein G to IgG coupled to polyacrylamide beads, but exhibited very weak binding when radiolabeled. The peptide showed no affinity for HSA. To further analyze the binding properties of a single IgG-binding protein G domain, we therefore amplified a DNA fragment comprising the D1, C2, and D2 domains by polymerase chain reaction, using G148 DNA as template.
Protein G Genes

To facilitate cloning of the polymerase chain reaction-generated DNA in different expression vectors, several restriction sites (HindIII, EcoRI, and NcoI) were introduced in both ends of the polymerase chain reaction-generated DNA fragments through the primers. An ATG-codon (as part of an NcoI site), immediately preceding the protein G sequence, would also make it possible to cleave off extra amino acids in the NH₂-terminal end of the expressed polymerase chain reaction-generated fragment with cyanogen bromide (no methionine residues are present in the C and D repeats of protein G). One of the expression systems used was the secretion vector pIN-III-ompA-Hind (18). In this vector, the signal sequence of the E. coli outer membrane protein A (ompA) is placed immediately in front of the sequence to be expressed. Cleavage of the signal peptide occurs between an alanine and a serine residue, and the codon TCC (serine) was therefore included in the 5'-end of the protein-encoding strand to ensure correct processing of the ompA signal-sequence-protein G fusion. Two stop codons were introduced in the 3'-end of the protein-encoding strand.

The resulting polymerase chain reaction-generated 303-base pair DNA fragment was cloned into the HindIII site of pIN-III-ompA-Hind, followed by transformation of E. coli. Clones expressing IgG-binding peptides were picked by colony blots using the ³²P-labeled oligonucleotide 5'-GCTGCTACT-GCAGAAAAAGTCTTCAAACAA-3' and ³²P-IgG as primary and secondary probes, respectively. Three clones producing IgG-binding peptides were selected and sequenced and the DNA sequences of these were all completely correct. The clones were grown, cells were lysed by osmotic shock, and the resulting supernatants were subjected to affinity chromatography on IgG-Sepharose. The peptides eluted appeared larger (14 kDa) than would be expected from the sequence data (the M, calculated from the sequence was 11,495), when separated on SDS-PAGE (Fig. 9). The NH₂-terminal amino acid sequence (SEFSMEKPEVIDASELTP) of the IgG-binding peptide produced by one of the clones (pinDCD2) showed that the signal peptide had been cleaved at the expected position. The peptide was radiolabeled and used as a probe to proteins on nitrocellulose. Binding occurred both to IgG and purified Fc fragments of IgG, whereas no binding was seen to purified Fab fragments. The affinity of the radiolabeled protein G peptide for human polyclonal IgG coupled to polyacrylamide beads was calculated as $9.0 \times 10^7 \text{ M}^{-1}$ (Fig. 10).

**DISCUSSION**

β-Hemolytic streptococci are differentiated into groups in which the group-specific antigens are carbohydrates. Protein
Protein G Genes

**Fig. 8.** Schematic drawing of the protein G genes of one group C (C40) and two group G streptococcal (G148 and G43) strains. The position in the G148 protein G gene of the oligonucleotide 5'-GCTGCTACTGCAAAGACCTTCAAACAA-3' used in the cloning of the C40 and G43 protein G genes is indicated. The nomenclature is according to Olsson et al. (8).

**Fig. 9.** Polymerase chain reaction-generated expression of a single IgG-binding protein G unit. The oligonucleotides 5'-CCAAGCTTTCAATGCCTTGCATGGAACCCACAGTGATGATGCG-3' and 5'-CGAAGCTTCCATGGTTATTATGTCACGGCTGGTGTTAATTCAGA-3' were used as primers in polymerase chain reaction, using a plasmid containing the G148 protein G gene as template. The polymerase chain reaction-product was cloned into the HindIII site of the secretion vector pLN-III-ompA-Hind. After transformation of E. coli, clones which expressed IgG-binding peptides were identified by colony blots, using 125I-IgG as a probe. IgG-binding peptides were purified from lysates of positive clones by affinity chromatography on IgG-Sepharose. Lane A, molecular weight markers; lane B, human β2-microglobulin; lane C, IgG-binding peptide purified from a positive clone (pINDCD).

G was originally defined as the IgG Fc-binding protein of both group C and G streptococci (1, 24), and subsequent work supported the notion that C and G streptococci produce protein G with similar structure and binding properties (9, 19, 25). In the present study, it was found that although protein G molecules of different sizes and binding properties were produced by different strains of group C and G streptococci, the same pattern of heterogeneity was seen in both groups. The work also contains the first sequence of a protein G gene from a group C streptococcal strain, and this sequence establishes that protein G molecules with the same principal structure are produced by group C and G streptococci.

Group C and G streptococci cause indistinguishable clinical symptoms (26), the most common being pharyngitis. Sometimes, however, these bacteria cause more severe infections such as septicemia (27). The 31 group C and G strains used in this study were isolated either from the pharynx (12 strains) or from the blood of patients with streptococcal septicemia (19 strains). No difference in protein G structure was seen when tonsillar strains were compared to blood isolates, suggesting that protein G is not a virulence determinant. The results rather indicate that protein G could participate in more basic biological events.

The difference in size between the protein G molecules solubilized from the G148, G43, and C40 strains by mutanolysin, correlated with differences in binding properties. Nucleotide sequence data verified this correlation. For both the C40 and G43 protein G genes, the nucleotide differences involved at least one of the repeats in the IgG- and/or HSA-binding encoding parts of the gene, and the duplications/deletions were confined to the boundaries of the repeats. Our findings support the suggestion that the repeats of these genes have arisen by gene duplications of the protein-binding re-
Whether protein G and other Ig-binding bacterial proteins are involved in essential functions, such as multiplication, is currently being investigated. This work has been stimulated by the observation that, although proteins A and G show no similarity in their IgG-binding repeats (3, 33), they both bind to the C2-C3 domain interface region of IgG Fc (34). This is also true for protein H, a recently isolated IgG-binding protein from group A streptococci (35). Thus, protein H binds to the same region of human IgG Fc but shows no similarity within its IgG-binding region to the IgG-binding domains of proteins A and G (36). This represents an unusually clear example of convergent evolution, where three nonhomologous genes of Gram-positive bacteria have evolved to encode cell wall proteins with very similar binding properties. This information together with other data, including those of the present study, supports the notion that the primary function of Ig-binding bacterial proteins is to be found in connection with essential bacterial functions.

Acknowledgment—We express our gratitude to Dr. F. Rientier-Delrue for kindly providing the secretion vector plN-III-ompA-Hind.

REFERENCES

1. Björck, L., and Kronwall, G. (1984) J. Immunol. 133, 968-974
2. Reis, K. J., Ayoub, E. M., and Boyle, M. D. P. (1984) J. Immunol. 132, 3091-3097
3. Guss, B., Eliasson, M., Olsson, A., Uhlen, M., Frej, A.-K., Jornvall, H., Flock, J. I., and Lindberg, M. (1986) EMBO J. 5, 1067-1075
4. Fahlenestock, S. R., Alexander, P., Nagle, J., and Filipula, D. (1986) J. Bacteriol. 167, 870-880
5. Björck, L., Kastern, W., Lindahl, G., and Widehack, K. (1987) Mol. Immunol. 24, 1115-1122
6. Åkerström, B., Nielsen, E., and Björck, L. (1987) J. Biol. Chem. 262, 13388-13391
7. Sjöbring, U., Falkenberg, C., Nielsen, E., Åkerström, B., and Björck, L. (1981) J. Immunol. 127, 1565-1569
8. Olsson, A., Eliasson, M., Guss, B., Nilsson, B., Hellman, U., Lindberg, M., and Uhlen, M. (1987) Eur. J. Biochem. 168, 719-724
9. Reis, K., Hansen, F., and Björck, L. (1986) Mol. Immunol. 23, 425-431
10. Christensen, P., Kahinmeh, G., Jonasson, S., and Kronwall, G. (1973) Infect. Immun. 7, 881-885
11. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672
12. Neville, D. M., Jr. (1971) J. Immunol. 106, 1677-1686
13. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354
14. Matsudaira, P. (1987) J. Biol. Chem. 262, 10005-10008
15. Stewart, J., and Young, J. (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL
16. Sambrook, J., Fritsch, E., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
17. Saito, R. K., Gefland, D. H., Stoffel, S., Scharf, S., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1986) Science 239, 487-491
18. Rientier-Delrue, F., Swennen, D., and Martial, J. (1988) Nucleic Acids Res. 16, 7829
19. Sjöbring, U., Björck, L., and Kastern, W. (1989) Mol. Microbiol. 3, 319-327
20. Schneider, K., and Kandell, O. (1989) EMBO J. 8, 467-473
21. Hollingsead, S. K., Fischetti, V. A., and Scott, J. R. (1986) J. Biol. Chem. 261, 1677-1682
22. Gowland, C., Murphy, J., Atkinson, T., and Barloutat, D. (1990) Biochem. J. 267, 171-177
23. Åkerström, B., and Björck, L. (1986) J. Biol. Chem. 261, 10240-10247
24. Miyhe, E., and Kronwall, G. (1977) Infect. Immun. 17, 476-482
25. von Mering, G., and Boyle, M. (1986) Mol. Immunol. 23, 811-821
26. Efratiou, A. (1988) J. Med. Microbiol. 29, 207-219
27. Ackert, R., Hermans, P., and Washington, J. A. (1983) J. Rev. Infect. Dis. 5, 196-204
28. Scatchard, F. (1987) Trends Biotechnol. 5, 70-83
29. Wiebevik, L., and Kronwall, G. (1982) Infect. Immun. 38, 1154-1163
30. Sjöbring, U., Tejnor, J., Gotebruck, B., and Björck, L. (1989) J. Immunol. 143, 2948-2954
31. Björck, L. (1990) Mol. Microbiol. 4, 7493-7442
32. Björck, L., Åkerstrand, P., Bobus, M., Tejnor, J., Abrahamsson, M., Olafsson, I., and Grubb, A. (1989) Nature 337, 385-386
33. Uhlen, M., Guss, B., Nilsson, B., Gatenbeck, S., Philipson, L., and Lindberg, M. (1984) J. Biol. Chem. 259, 1663-1702
34. Slone, G. C., Sjöbring, U., Björck, L., Spiegler, J., Barber, C. V., and Nerdello, P. A. (1988) J. Immunol. 141, 565-570
35. Åkerstrand, P., Cooney, J., Kishimoto, F., and Björck, L. (1990) Mol. Microbiol. 4, 1225-1237
36. Gomi, H., Hozumi, T., Hattori, S., Tagawa, C., Kishimoto, F., and Björck, L. (1989) J. Immunol. 144, 4046-4052

2 L. Björck and U. Sjobring, manuscript in preparation.