INFECTION AND IMMUNITY, Sept. 1992, p. 3601–3608
0019-9567/92/093601-08$02.00/0

Vol. 60, No. 9

Isolation and Molecular Characterization of a Novel Albumin-Binding Protein from Group G Streptococci

ULF SJÖBRING

Department of Medical Microbiology, University of Lund, S-22362 Lund, Sweden

Received 19 February 1992/Accepted 16 June 1992

Many streptococcal strains are known to bind the two most abundant plasma proteins, namely, immunoglobulin G and albumin. Protein G isolated from group C and G streptococci has been demonstrated to have separate binding regions for each of these proteins. However, some group G streptococcal strains bind only serum albumin. This report describes the isolation of a 48-kDa albumin-binding protein from such a strain (DG12). The affinity constant of this protein for human serum albumin was determined to be 5×10^9 M^{-1}, and the protein interacted strongly also with serum albumin from several other mammalian species. The gene encoding the albumin-binding protein was cloned and expressed in *Escherichia coli*. DNA sequence analysis of this gene revealed a unique NH_2-terminal sequence and three types of repeats in the encoded protein. One of these repeated sequences has significant homology with the albumin-binding domains of protein G, and it was demonstrated that the albumin binding of the DG12 protein was localized within these domains. Another type of repeat is localized in the putative wall-spanning region of the molecule. This repeat sequence, which has the length of only 4 amino acids (LysProGluVal), is repeated 14 times. The relationship of the albumin-binding protein to other cell-wall-associated proteins of pathogenic streptococci is discussed.

Albumin is the most abundant plasma protein. It is, therefore, of interest to study various interactions between albumin and bacteria and to characterize the structures involved. Such studies have demonstrated that pathogenic group A, C, and G streptococci express surface proteins that bind serum albumin (18, 36). Thus, protein G, the immunoglobulin G (IgG)-binding protein of group C and G streptococci (3, 24), has been shown to bind serum albumin in addition to IgG (2). Different parts of the protein G molecule were shown to be responsible for the IgG binding and the albumin binding (1, 2). The albumin binding was subsequently located in a region with three repeated sequences in the amino-terminal half of the molecule (1, 20, 32). However, it has also been demonstrated that some group G streptococcal strains of bovine origin lack the IgG-binding phenotype (37). Furthermore, these strains were reported to bind albumin with a specificity which is different from that seen in streptococci expressing protein G (23, 38). These observations made it of interest to isolate and characterize the albumin-binding protein expressed by these streptococcal strains and to compare it with protein G.

MATERIALS AND METHODS

Bacterial strains and media. The group G streptococcal strains DG8 and DG12 of bovine origin have been previously described (37) as has the human group G streptococcal isolate G148 (3). These bacteria were cultured in Todd-Hewitt broth (Difco). *Escherichia coli* JM105 was grown in LB medium.

Binding assay. Heat-killed streptococci were resuspended in PBSA (0.12 M NaCl, 0.03 M phosphate, 0.02% NaN_3 [pH 7.2]) to a concentration of 10^8 cells ml^{-1}. Dilutions of the bacteria (200 μl) were incubated with ^{125}I-labeled protein (200 ng) at 20°C for 30 min in a total volume of 225 μl in plastic tubes. Dilutions of streptococci were made with the nonbinding strain *Staphylococcus epidermidis* L603 at 10^8 cells ml^{-1}. After incubation, the cells were washed twice in PBSA with 0.05% Tween 20 (PBSAT). The radioactivity in the pellet was counted, and the binding was expressed as the percentage of the added radioactivity. The nonspecific uptake to plastic tubes containing 200 μl of 10^8 cells of strain L603 ml^{-1} was less than 4%.

Adsorption experiments. Bacteria (10^{10} cells) were incubated in 1 ml of human plasma diluted 1:20 in PBSA, or they were incubated in PBSA alone, for 15 min at 37°C. The cells were pelleted and washed four times with 25 ml of PBSAT. To elute adsorbed proteins, the cells were resuspended in 0.5 ml of 0.1 M glycine (pH 2.0), left at 20°C for 10 min, and centrifuged. The proteins in the supernatant were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Human serum albumin (HSA) and α_2 macroglobulin eluted from the bacteria were identified by probing electroblotted replicas of the separated glycine eluates with rabbit antisera prepared against these plasma proteins.

Purification of HSA-binding proteins. Digests of streptococci, supernatants from streptococcal cultures, or bacterial shock lysates were subjected to affinity chromatography on columns (20 ml) of HSA coupled to Sepharose CL-4B (Pharmacia). Bound material was eluted with 0.1 M glycine-HCl (pH 2.0). The pH of the eluted fractions was adjusted to 7. Proteins used for the determination of affinity constants were further purified on Sephadex G-200 (Pharmacia). The albumin-binding proteins expressed by the DG12 and DG8 strains were purified from culture supernatants of these streptococcal strains. The yields were, in both cases, 50 μg/10 liters of streptococcal culture. Recombinant DG12 protein was expressed in *E. coli* JM105 carrying the streptococcal gene in the plasmid pKK233-2; the cultures were induced at an A_{620} of 0.6 by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) at 1 mM. After incubation at 37°C for 3 h, the cells were harvested by centrifugation. The bacteria were then lysed (4), and the albumin-binding protein was purified from the bacterial lysate. The yield was 0.5 mg of HSA-binding protein per liter of culture. The A1B HSA-binding peptide fragment was purified from lysates of *E. coli* transformed with the plasmid pHD389 (4) containing a

relevant insert (see Results); the bacteria were grown in LB broth at 28°C, expression was induced by raising the temperature to 42°C, and the culture was continued for 3 h at this temperature. The yield of the HSA-binding peptide in the lysate was 20 mg/liter of culture.

Electrophoresis and electroblotting. SDS-PAGE was performed as described by Neville (19). Before loading, the samples were boiled for 3 min in a sample buffer containing 2% SDS and 5% mercaptoethanol. Molecular weight markers were from Sigma. Gels were stained with Coomassie blue. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon, Millipore) as described previously (34) or manually applied to membranes by using a dot blot apparatus (Bio-Rad). The membranes were blocked, incubated with radiolabeled proteins, and washed as previously described (32).

Determination of affinity constants. One-tenth milliliter of ^{125}I-DG12 protein, 0.1 ml of HSA-coupled polyacrylamide beads (Immunobeads, Bio-Rad), and 0.2 ml of nonlabeled DG12 protein in different concentrations (0.5 to 2,000 ng/ml) were mixed, and the mixture was incubated for 2 h at 20°C. After washing twice to remove unbound protein, the radioactivity bound to the beads was determined in a gamma counter. Incubations and washes were done in phosphate-buffered saline (PBS; pH 7.2) with 0.25% gelatin and 0.25% Tween 20. Details of the procedure and calculations, using the formula of Scatchard (28), have been reported earlier (32).

DNA isolation and hybridization. Isolation and purification of DNA from streptococci was performed as described previously (30). Hybridization of restriction fragments was performed as described previously (33). Oligonucleotides were labeled with [^{32}P]ATP (Amersham) by using T4 polynucleotide kinase. All hybridizations were carried out at 25°C in the presence of 50% formamide. The filters were washed in 0.1% SDS–1× SSC at 25°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

PCR. Polymerase chain reactions (PCR) were performed in a thermocycler (Hybaid) by using thermostable DNA polymerase (AmpliTaq, Perkin-Elmer Cetus) as described previously (25). The oligonucleotides PG1 (5′-CCCCATG GGGTTAGCATCCGTATCAGCT-3′) and PG2 (5′-CCCCA TGGGTTAAGCTGCTGTGAAGAATGGGTT-3′), D1 (5′-A/CGNGCA/TCAA/GGCA/TAAAGCA/TGC-3′) and D2 (5′-AGC/TTGC/TTTCACCTGTTGATGGTAA-3′), or A1 (5′-GCGCCGGCTTTAGATCAAGCTAAGCAAGCT-3′) and B_{rev} (GCGGGATCCTTATTTAATTGATTTAAGGGT GTCTTC-3′) were used as primers with genomic DNA from the DG12 strain as template. Different cycling conditions were tried. For cloning purposes, the following parameters were used: 20 cycles with 1 min of denaturation at 92°C, 1 min of annealing at 58°C, and 1 min of extension at 72°C. The PCR products were analyzed on agarose gels (NuSieve, FMC).

Cloning procedure. The PCR-generated PG1-PG2 fragment was eluted from a 1% agarose gel, digested with *Nco*I, and ligated to the *Nco*I-digested expression vector pKK233-2 (Pharmacia). The ligation mixtures were used to transform *E. coli* JM105. *E. coli*-containing plasmids with relevant inserts were selected by colony blot experiments. The transformants were cultured on culture plates with LB medium containing ampicillin (100 μg ml^{-1}) and 2 mM IPTG. The colonies were transferred from the culture plates to nitrocellulose filters. The bacteria were lysed on the filters with 10% SDS and subsequently probed with ^{125}I-HSA. PCR with the oligonucleotides A1 and B_{rev} resulted in a fragment of approximately 220 bp. The fragment had primer-directed terminal recognition sites for *Hpa*II and *Bam*HI as well as two stop codons in the 3′-end of the coding strand. The PCR fragment was eluted from a 3% agarose gel and digested with *Hpa*II and *Bam*HI. The fragment was ligated to pHD389 (4) digested with *Nar*I and *Bam*HI. Fragments inserted in the *Nar*I site of this vector will be translated fused to the signal sequence of *E. coli* outer membrane protein A (OmpA). Inserted sequences will be under transcriptional control of the p_R promoter. The gene encoding the heat-labile cI857 repressor, which regulates p_R, has also been introduced into pHD389. Standard procedures for ligation and transformation of DNA were used (26). Cloning of the G148 protein G gene was described previously (2). Restriction enzymes were from Promega.

DNA sequence analysis. Double-stranded DNA sequencing was performed by using the dideoxy nucleotide chain termination method (27) with Sequenase version 2.0 (U.S. Biochemicals). Both strands were sequenced.

Other methods. Immunoglobulins and albumins were purchased from Sigma. IgG Fc fragments were from Calbiochem. Purification of protein G from *E. coli* containing the streptococcal gene has been described (2). Proteins were radiolabeled to a specific activity of 1 to 5 mCi/mg with ^{125}I (Amersham) by using an Enzymobead kit from Bio-Rad. For determination of NH_2-terminal amino acid sequences, proteins were transferred to Immobilon membranes, visualized by Coomassie blue, cut out, and analyzed by the method of Matsudaira (15). Sequence analysis was performed on an Applied Biosystems gas-phase sequencer (model 470A) equipped with an on-line amino acid analyzer (model 120A) and a data module (model 900A) and by using chemicals and software supplied by the manufacturer.

Nucleotide sequence accession number. The sequence is available in GenBank under accession number M95520.

RESULTS

Binding of plasma proteins to group G streptococci. Human and bovine isolates of group G streptococci were examined for the binding of radiolabeled HSA and IgG. The human isolates consistently showed high levels of IgG binding (^{125}I-IgG uptake ranging from 60 to 80%) but variable HSA binding (10 to 60%). In contrast, all bovine strains bound a large fraction of the added HSA (60 to 80%) but either low amounts of IgG (20 to 30%), as in the case of strain DG8, or no IgG at all, as in the case of strain DG12. Selected strains were then tested for their ability to adsorb proteins from human plasma (Fig. 1). As expected, the protein G-expressing strain G148 adsorbed both IgG and albumin (Fig. 1, lane A), whereas strain DG12 only adsorbed albumin (Fig. 1, lane B). These results confirm the results of the tube binding assays.

Isolation of the albumin-binding protein of strain DG12. Albumin-binding polypeptides have been isolated from bovine group G streptococci by using various heat extraction methods (23, 37). However, these molecules showed considerable size heterogeneity. Attempts to solubilize the albumin-binding protein from strain DG12 with *N*-acetylmuraminidase (mutanolysin) yielded a number of albumin-binding peptides, the dominating one of which had a molecular mass of 48 kDa, as judged by SDS-PAGE. To obtain a homogeneous material suitable for further analysis, we therefore used an alternative approach based on the assumption that the protein would be present in the culture supernatant. By using affinity chromatography on HSA-

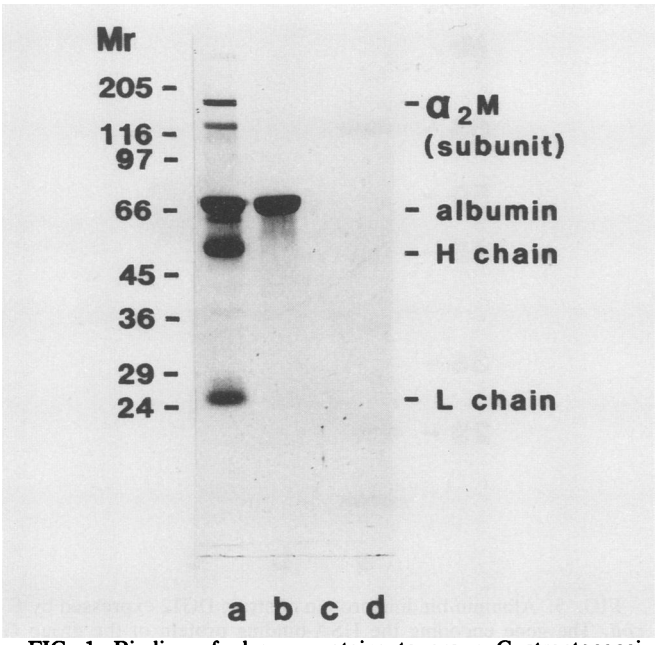

FIG. 1. Binding of plasma proteins to group G streptococci. Group G streptococci were incubated with human plasma diluted 1:20 in PBSA or with PBSA (pH 7.2) for 15 min at 37°C, washed, and finally pelleted. To elute adsorbed proteins, the cells were incubated in 0.1 M glycine (pH 2.0) for 10 min and subsequently centrifuged. The supernatants were analyzed by SDS-PAGE. Lanes: a, G148 cells plus plasma; b, DG12 cells plus plasma; c, G148 cells plus PBS; d, DG12 cells plus PBS. The positions of the 185-kDa subunit of α_2 macroglobulin (α_2M), albumin, and heavy- (H) and light- (L) chain IgG are indicated.

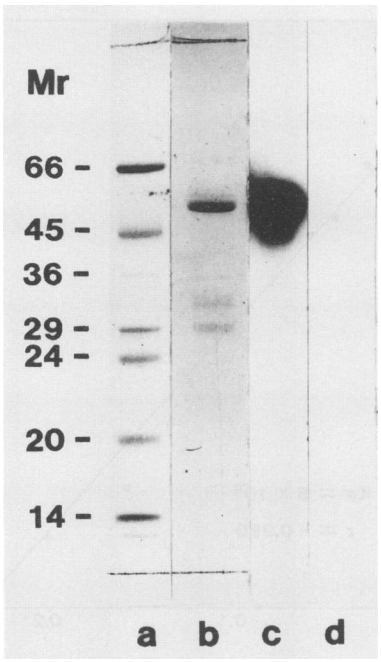

FIG. 2. Western blot analysis of the purified albumin-binding DG12 protein. The supernatant from a culture of the group G streptococcus DG12 was subjected to chromatography on HSA-Sepharose. Eluted material was analyzed by SDS-PAGE (12% total acrylamide) (lane b), and blotted to nitrocellulose filters, which were subsequently probed with ^{125}I-HSA (lane c) and ^{125}I-IgG (lane d). Molecular weight markers were separated in lane a.

Sepharose, we could indeed isolate an albumin-binding protein with a molecular mass of 48 kDa from supernatants of strain DG12 (Fig. 2). This protein will be referred to as the DG12 protein. In Western blots, the protein showed affinity for radiolabeled HSA but not for IgG (Fig. 2). The NH_2-terminal amino acid sequence of the DG12 protein (GlnValThrThrArgAlaGlnAlaLysAlaAlaArg) demonstrated no apparent homology to published sequences of bacterial cell-wall-associated proteins. A 48-kDa albumin-binding peptide was also purified from the culture supernatant of another bovine group G streptococcal isolate (DG8). The NH_2-terminal amino acid sequence of that protein (AlaThrThrArgSerMetThrAlaGlu) has a tripeptide sequence (ThrThrArg) in common with the DG12 sequence but shows no homology to other streptococcal proteins.

Binding properties of the DG12 albumin-binding protein. The DG12 protein was radiolabeled and used to probe proteins immobilized on nitrocellulose. It bound HSA as well as serum albumin from different mammalian species (Fig. 3). The species reactivity pattern was identical to that of protein G (data not shown; see also reference 21). We did not detect binding of the purified and labeled DG12 protein to bovine serum albumin. In contrast, other authors have reported binding of radiolabeled BSA to group G streptococci (23, 38). No binding was seen to IgG from any of the tested species (humans, rabbits, mice, rats, cats, dogs, guinea pigs, goats, cows, horses, or sheep). The affinity constant for the binding of the DG12 protein to HSA (5×10^9 M^{-1}; Fig. 4) is somewhat lower than the affinity constant reported for the interaction between protein G and HSA (3×10^{10} M^{-1} [1]).

Cloning and expression of the DG12 albumin-binding protein. A comparison of a number of cell-wall-associated proteins from group A (9, 10, 12, 13), C (31), and G streptococci (5, 11) reveals a common stretch of amino acids (AsnProPhePheThrAlaAla) in the C-terminal region of these proteins. Close to this region, another conserved sequence (LeuProSerThrGlyGlu) is found in cell surface proteins of gram-positive bacteria (8). The corresponding DNA sequences are also highly conserved in both cases. Two oligonucleotides were constructed, one of which (D2) was based on the LeuProSerThrGlyGlu sequence and another of which (D1) was based on the obtained NH_2-terminal amino acid sequence of the DG12 protein. The use of these two

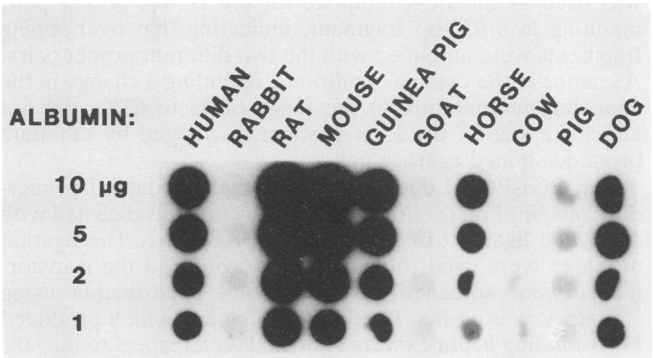

FIG. 3. Species specificity of the albumin-binding DG12 protein. Serial dilutions of serum albumin from different mammalian species were applied to a nitrocellulose filter. The filter was probed with the radiolabeled DG12 protein.

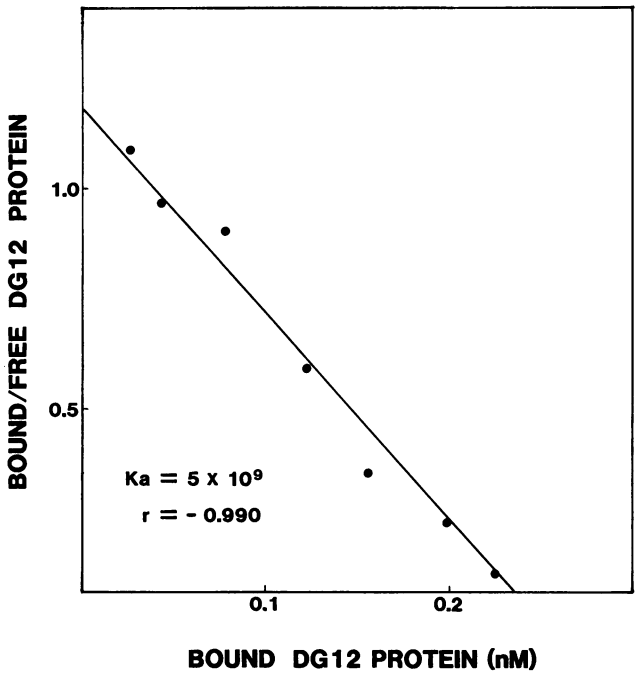

FIG. 4. Scatchard plot for the reaction between the DG12 protein and HSA. The affinity constant for the reaction between the 50-kDa DG12 protein and HSA was determined. HSA coupled to polyacrylamide beads was mixed with a constant amount of ^{125}I-labeled DG12 protein and different amounts of nonlabeled DG12 protein and incubated for 2 h at 20°C. The beads were subsequently washed to remove unbound DG12 protein. The beads were then pelleted, and the radioactivity in the pellet was counted. Bound and free proteins were plotted as a function of bound protein. The equilibrium constant was then equal to the absolute value of the slope of the curve.

primers (D1 and D2) in PCR, with chromosomal DNA from the DG12 strain as the template, resulted in a 950-bp amplification product. In a second set of reactions, a primer (PG2) based on the AsnProPhePheThrAlaAla sequence was combined with a primer (PG1) which is homologous to part of the DNA sequence that encodes the signal sequence of protein G, assuming that the DG12 protein and protein G would have similar signal peptides. PCR with the primers PG1 and PG2, with DG12 DNA as target, indeed resulted in a 1.0-kb PCR product. Finally, the PG1-PG2 1.0-kb product was used as the PCR template for the D1-D2 primer pair, resulting in a 950-bp fragment, indicating that overlapping fragments were amplified with the two different primer pairs. A change in the cycling conditions, including a change in the annealing temperature in the range of 45 to 62°C, did not affect the size of the PCR product, as judged by ethidium bromide-stained agarose gels.

The PG1-PG2 1.0-kb fragment which contained primer-directed *Nco*I restriction sites in both ends was digested with *Nco*I and ligated to *Nco*I-digested pKK233-2. The ligation mixtures were used to transform *E. coli*, and the transformants were screened in colony blot experiments using ^{125}I-HSA as a probe. Twelve of the clones which produced HSA-binding peptides were analyzed with respect to the size of the insert: all carried 1.0-kb inserts. Four of the clones which produced HSA-binding protein were chosen for further analysis. The HSA-binding proteins produced by these clones were purified by affinity chromatography on HSA-

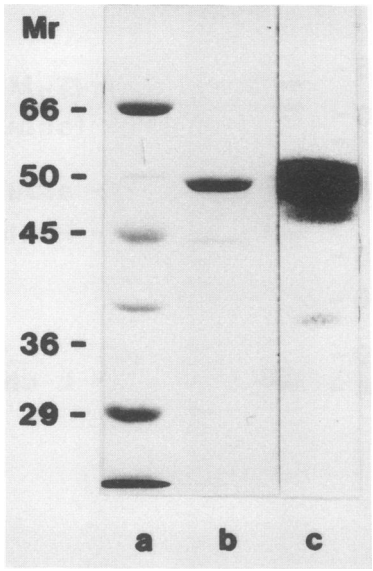

FIG. 5. Albumin-binding protein of strain DG12 expressed by *E. coli*. The gene encoding the HSA-binding protein of the group G streptococcal strain DG12 was introduced in the expression vector pKK233-2. Albumin-binding proteins were purified from *E. coli* transformed with this construct by affinity chromatography on HSA-Sepharose. The proteins eluted from the HSA-Sepharose were analyzed by SDS-PAGE (lane b) and Western blots using ^{125}I-HSA as a probe (lane c). Molecular weight markers were separated in lane A.

Sepharose. The protein had a molecular mass of 48 kDa as judged by SDS-PAGE (Fig. 5, lane b). When separated on the same gel, the HSA-binding protein expressed by *E. coli* and that purified from the streptococcal culture medium showed the same mobility (not shown). The protein expressed in *E. coli* bound ^{125}I-labeled HSA (Fig. 5, lane c) but not ^{125}I-IgG in Western blots. When radiolabeled, the DG12 protein bound HSA, but not IgG or IgG Fc fragments, on nitrocellulose (Fig. 6). The protein had the same reactivity

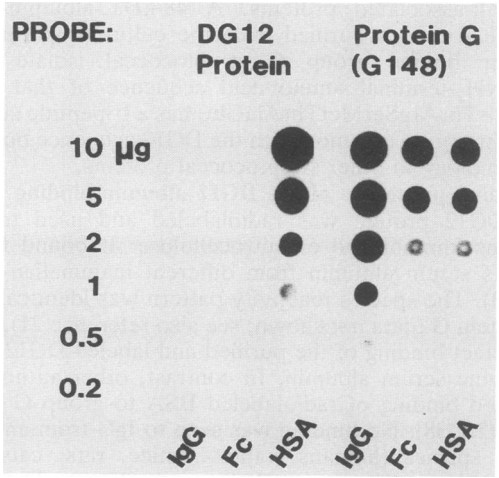

FIG. 6. Binding properties of the albumin-binding DG12 protein expressed in *E. coli*. Serial dilutions of human polyclonal IgG, purified IgG Fc fragments, or HSA were applied to nitrocellulose filters. The filters were probed with the DG12 protein or with protein G, both expressed in *E. coli*.

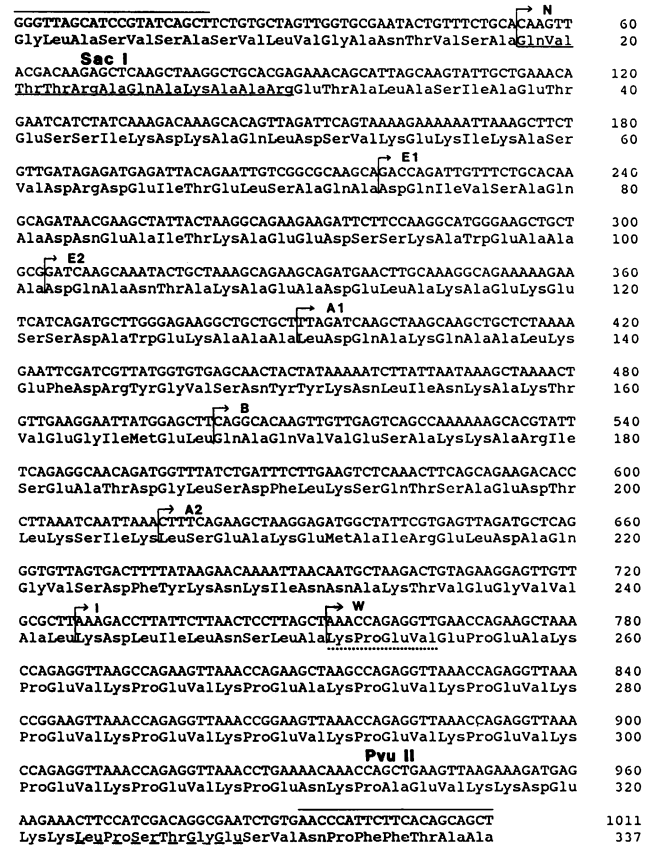

FIG. 7. Nucleotide sequence and deduced amino acid sequence of the DG12 albumin-binding streptococcal protein. Oligonucleotides used to amplify the gene for the DG12 protein are indicated with a line over the sequence. The NH_2-terminal amino acid sequence obtained from the protein isolated from the DG12 streptococci is underlined. The first repeat in the four-residue periodic structure is indicated with a dotted line. The LeuProSerThrGlyGlu motif (8) is marked by a dashed line. Two unique restriction sites are indicated.

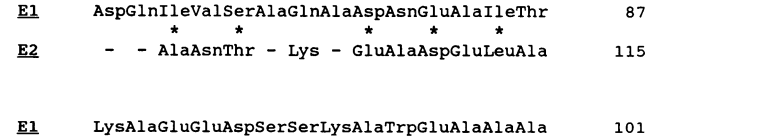

FIG. 8. Comparison of the two E repeats of the DG12 protein. Identical residues are denoted with a bar. Replacements which do not affect the charge or polarity are marked with an asterisk.

with mammalian albumins as the protein isolated from streptococci did (Fig. 3). The protein produced by one of the clones (pKK/DG7) did not inhibit the binding of protein G to Fc fragments coupled to polyacrylamide beads (not shown). The affinity of this peptide for HSA on polyacrylamide beads was $6 \times 10^9\ M^{-1}$, very close to the value obtained for the reaction between the protein isolated from streptococci and HSA (Fig. 4).

Deduced amino acid sequence of the DG12 albumin-binding protein. The DNA sequence of the PCR-generated insert was obtained for four individual clones, with identical results (Fig. 7). Thus, no PCR-generated mutation was detected. To further exclude PCR-generated deletions, an oligonucleotide, the sequence of which is found as the first 23 nucleotides of the B domain of the DG12 gene (Fig. 7), was radiolabeled and used as a probe in Southern blot experiments. The probe hybridized with a single 870-bp band resulting from digestion of chromosomal DG12 DNA with *Sac*I and *Pvu*II (Fig. 7), indicating that no major deletion had occurred during the amplification and cloning procedures. The molecular weight of the HSA-binding DG12 protein calculated from the deduced amino acid sequence was 36,366 Da, which is considerably lower than that estimated from the relative mobility of the protein by SDS-PAGE. Overestimation of the molecular weight by SDS-PAGE is common to several cell-wall-associated proteins of gram-positive bacteria (5, 10, 13, 14a). It has been suggested (13) that the anomalous migration may be explained by a high content of prolines in the wall-spanning regions of these proteins, a requirement which is well fulfilled by the albumin-binding protein (see below). The deduced amino acid sequence reveals several interesting features. After the PG1 primer sequence, one finds a sequence (11 amino acids) which is compatible with the last part of a leader peptide, i.e., it contains a row of hydrophobic residues with a small uncharged amino acid at the end (35). This sequence is followed by a unique 55-amino-acid-long sequence designated N, the first 12 amino acids of which are identical to the NH_2-terminal sequence obtained for the secreted DG12 HSA-binding protein. It is followed by two repeats designated E1 and E2, which are 28 and 29 residues long, respectively. Fourteen of the amino acids in these sequences are identical; another 6 residues are conserved with respect to charge and polarity (Fig. 8). There is a homology of both E domains of the DG12 protein to the C-terminal part of the single E domain of protein G (see Fig. 9), which is 70 residues long. Thus, the sequence AlaTrpGluAlaAlaAla in the E domain of protein G is found in the E1 of the DG12 protein, and a similar sequence (AlaTrpGluLysAlaAlaAla) is present also in E2. The second E domain is immediately followed by two 37-residue-long repeated domains (A1 and A2), which are separated by a 38-amino-acid-long unrepeated sequence (B). The A1-B-A2 region is highly homologous to the albumin-binding region of protein G (Fig. 9). After the second A repeat is a 9-residue sequence (I), which contains a tetrapeptide (LeuIleLeuAsn) also found in the first of the IgG-binding domains of protein G. In the next sequence, designated W, one finds a unique periodic structure, where a tetrapeptide sequence (LysProGluVal) is repeated 12 times. Moreover, four other short peptide sequences (GluProGluAla, LysProGluAla, LysProGluAsn, and LysProAlaGluVal), which are reminiscent of the LysProGluVal repeat, are also found in the C-terminal part of the DG12 protein. Regions with evenly spaced prolines are found in several surface proteins from gram-positive bacteria and may be involved in associating these proteins with the cell wall (13). Thus, the sequence LysProGluAla is found twice in the putative wall-spanning region of protein G. Interestingly, the sequence LysProGluVal, is also found as part of 15-residue structures (D repeats; Fig. 9), which separate the IgG-binding domains of protein G (5, 11).

Definition of the albumin-binding domains. A DNA sequence encoding the A1 and B domains of the DG12 protein (Fig. 9) was amplified by PCR. The amplified fragment was cloned into the secretion vector pHD389 (4). The construct was used to transform *E. coli* JM105. The transformants

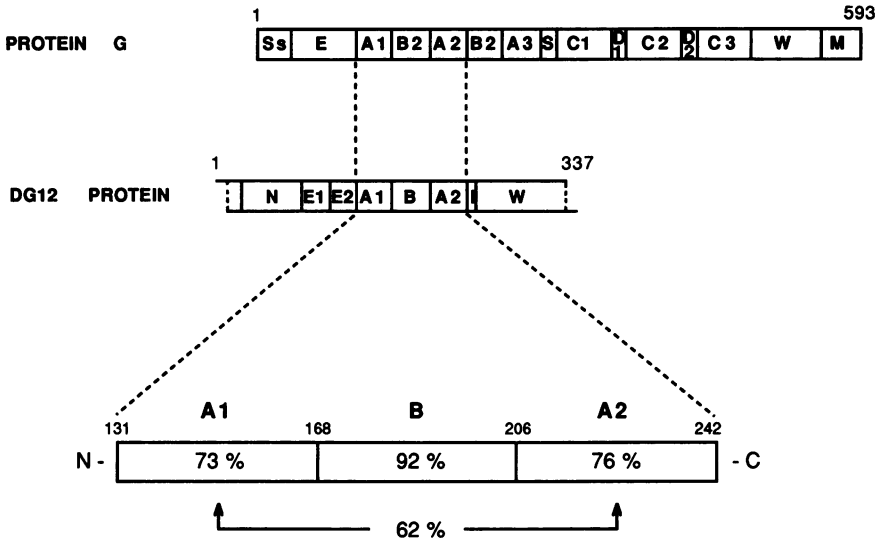

FIG. 9. Structure of albumin-binding proteins of group G streptococci. Schematic drawing of the different regions of the DG12 protein and of protein G. The A and B domains are responsible for the albumin binding of protein G, whereas the IgG binding has been localized to the C domains. The single lines in the ends of the DG12 protein indicate residues encoded by the PCR primers which were used to amplify the gene for the DG12 protein. The percentages in the enlarged A1-B-A2 domains indicate the degrees of homology of the respective domains to corresponding domains of protein G. The percentage in the arrow connecting the A repeats indicates the homology between the two A domains of the DG12 protein. The positions which delineate the domains of protein G agree with those proposed by Fahnestock et al. (5), whereas the nomenclature is that suggested by Olsson et al. (22).

were induced and screened in colony blot experiments using ^{125}I-HSA as a probe. Several reactive clones were found. The albumin-binding peptides produced by one of the reactive clones was purified on HSA-Sepharose. The peptide had an apparent molecular size of 13 kDa as judged by SDS-PAGE (Fig. 10), whereas the molecular weight of the A1B peptide as deduced from the amino acid sequence is 8,549. Studies of other peptides expressed in the pHD389 vector indicate that this disagreement in size is due to an incomplete processing of the OmpA signal peptide (21 amino acids) (14a). By using the same expression system, a functional A1B1 (Fig. 9) peptide fragment of protein G was also produced (data not shown).

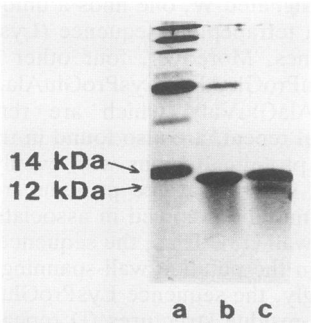

FIG. 10. Localization of the albumin-binding domains in the DG12 protein. A peptide fragment consisting of the A1 and B domains of the DG12 protein (Fig. 9) was expressed in *E. coli*. The peptide was purified on HSA-Sepharose and analyzed by SDS-PAGE (18% total acrylamide concentration) (lane c). Molecular size markers were separated in lane a, and β_2 microglobulin was separated in lane b.

DISCUSSION

The albumin-binding DG12 protein has several distinctive features. Thus, the protein has a unique NH_2-terminal domain (N), which shows sequence variation between different isolates. Sequence variation in the amino terminus is a typical trait of the M proteins of group A streptococci (7) but is not seen in protein G (5, 6, 22, 31). M-related proteins have been isolated from group G streptococci (14), but there is no evidence that the albumin-binding protein described here is such a protein. However, the sequence variation suggests that the albumin-binding proteins of group G streptococci, like the M proteins, may be targets for the immune response. One also finds the tandemly repeated E domains, which are present neither in protein G nor in other known proteins, after the unique N domain. Finally, the pronounced periodicity in the putative wall-spanning region is another unique feature of this streptococcal protein.

However, the DG12 protein also has a definite structural relationship to streptococcal protein G, which also binds albumin. The fact that the gene encoding the DG12 albumin-binding protein could be amplified by using a nucleotide sequence derived from part of the protein G leader sequence as one of the primers indicates that similarities exist in this region. The highly repetitive LysProGluVal sequence of the DG12 protein is present as the first four amino acids of the sequence (15 residues) which separates its IgG-binding domains. However, the most striking similarity is the extensive homology of the A1-B-A2 sequence to corresponding HSA-binding structures of protein G. The A1B fragment of the DG12 protein and the A1B1 peptide fragment of protein G were both expressed in *E. coli* and were both found to bind HSA, demonstrating that the existing sequence differences do not drastically influence the function of these domains.

The DG12 protein has little sequence homology with the IgG-binding domains of protein G. However, hybridization data demonstrate that there are other nucleotide sequences present in the DG12 genome, which have homology with sequences encoding the IgG-binding region of protein G (not shown). Preliminary data indicate that these sequences are localized immediately downstream from the gene for the albumin-binding protein (29a). Although the DG12 strain does not bind IgG, other bovine strains do, e.g., strain DG8. Thus, the HSA-binding 50-kDa protein purified from the IgG-binding DG8 strain did not bind IgG, but low-molecular-weight IgG-binding peptides could be isolated from that strain (29a). Taken together, these data indicate that some bovine group G streptococci have two protein G-related genes, which separately encode albumin binding and IgG binding. The dual binding function of protein G may thus be the result of a recombination event between two adjacent genes, each encoding a protein with only one of the two binding properties.

The homology found between the HSA-binding domains of human and bovine group G streptococci is in agreement with published immunological data, which point to some cross-reactivity between these structures (23). The structural homology also corresponds to a functional similarity. Thus, both the DG12 protein and protein G bound strongly to albumin from humans, rats, mice, guinea pigs, horses, and dogs. However, previous experiments with whole bacteria have demonstrated that bovine group G streptococci, contrary to human isolates, also bind albumin from cows, goats, and rabbits (23, 38). This suggests that the specificity of the protein expressed by bovine strains is different from that of protein G. We did not detect binding of these albumins to the purified DG12 protein. However, it is possible that the binding of cow, goat, and rabbit albumin may require cooperation between two or more albumin-binding molecules exposed on the streptococcal cell surface. Alternatively, other structures on the bacterial surface, such as M-like proteins (36) or lipoteichoic acid (32), may contribute to the binding of albumin isolated from these species.

Group C and G streptococci bind a much wider range of mammalian serum albumins than group A streptococci do (36). Functionally, the broader species reactivity, shown by albumin-binding proteins of group C and G streptococci, probably reflects the wider range of mammalian species which can be infected by group C and G streptococci, as compared with group A strains. Similar differences in the IgG-binding patterns are also shown by these strains (17), further supporting the notion that the albumin- and the IgG-binding properties have arisen as a result of a common evolutionary pressure.

One may speculate on the function of the interactions between streptococcal surface proteins and major plasma proteins. The most simplistic explanation proposes that such bacterial structures could serve to disguise the parasite and thus help it evade the defense mechanisms of the host. The high affinity interaction with albumin, the most abundant extracellular protein, makes the albumin-receptor-expressing bacteria very potent in this respect. However, other functional consequences of these interactions can be conceived. Thus, albumin is a major transport protein and could, after binding to the streptococcal surface, provide substances, e.g., fatty acids, to be used by the bacterium. Moreover, it has been demonstrated that the physicochemical properties of streptococci are changed as host proteins, including HSA, are bound to the cells (16). Such changes are likely to affect the adherence between bacteria, and between bacteria and host cells, and could influence important factors governing the establishment of an infection, such as colonization and spreading of the parasites. A detailed molecular knowledge, to which the present study contributes, of the structures involved may help to further elucidate the role of these protein-protein interactions in the host-parasite relationship.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (Project B92-16X-09926-01A), the Alfred Österlund Foundation, the Kock Foundation, the Royal Physiographic Society, and the Medical Faculty, Lund University.

I am indebted to Henrik Dalböge for providing the secretion vector pHD389, Lars Björck for valuable advice, and Gunnar Lindahl for careful reading of the manuscript.

REFERENCES

1. **Åkerström, B., E. Nielsen, and L. Björck.** 1987. Definition of the IgG- and the albumin-binding regions of streptococcal protein G. J. Biol. Chem. **262:**13388–13391.
2. **Björck, L., W. Kastern, G. Lindahl, and K. Widebäck.** 1987. Streptococcal protein G, expressed by streptococci or by *Escherichia coli*, has separate binding sites for human albumin and IgG. Mol. Immunol. **24:**1113–1122.
3. **Björck, L., and G. Kronvall.** 1984. Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. J. Immunol. **133:**969–974.
4. **Dalböge, H., E. Bech Jensen, H. Töttrup, A. Grubb, M. Abrahamson, I. Olafsson, and S. Carlsen.** 1989. High-level expression of active human cystatin C in Escherichia coli. Gene **79:**325–332.
5. **Fahnestock, S., P. Alexander, J. Nagle, and D. Filipula.** 1986. Gene for an immunoglobulin-binding protein from a group G streptococcus. J. Bacteriol. **167:**870–880.
6. **Filpula, D., P. Alexander, and S. Fahnestock.** 1987. Nucleotide sequence of the protein G gene from streptococcus GX7805, and comparison to previously reported sequences. Nucleic Acids Res. **15:**7210.
7. **Fischetti, V.** 1989. Streptococcal M protein: molecular design and biological behaviour. Clin. Microbiol. Rev. **2:**285–314.
8. **Fischetti, V., V. Pancholi, and O. Schneewind.** 1990. Conservation of a hexapeptide sequence in the anchor region of surface proteins from Gram-positive cocci. Mol. Microbiol. **4:**1603–1605.
9. **Frithz, E., L.-O. Heden, and G. Lindahl.** 1989. Extensive sequence homology between IgA receptor and M proteins in Streptococcus pyogenes. Mol. Microbiol. **3:**1111–1119.
10. **Gomi, H., T. Hozumi, S. Hattori, C. Tagawa, F. Kishimoto, and L. Björck.** 1990. The gene sequence and some properties of protein H — a novel IgG binding protein. J. Immunol. **144:**4046–4052.
11. **Guss, B., M. Eliasson, A. Olsson, M. Uhlen, A.-K. Frej, H. Jörnvall, I. Flock, and M. Lindberg.** 1986. Structure of the IgG-binding regions of streptococcal protein G. EMBO J. **5:**1567–1575.
12. **Heath, D., and P. Cleary.** 1989. Fc-receptor and M-protein genes of group A streptococci are products of gene duplication. Proc. Natl. Acad. Sci. USA **86:**4741–4745.
13. **Hollingshead, S., V. Fischetti, and J. Scott.** 1986. Complete nucleotide sequence of type 6 M protein of the group A streptococcus. J. Biol. Chem. **261:**1677–1686.
14. **Jones, K., and V. Fischetti.** 1987. Biological and immunochemical identity of M protein on group G streptococci with M protein on group A streptococci. Infect. Immun. **55:**502–506.

14a. **Kastern, W., U. Sjöbring, and L. Björck.** Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain. J. Biol. Chem., in press.

15. **Matsudaira, P.** 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. **262:**10035–10038.

16. **Miörner, H., E. Myhre, L. Björck, and G. Kronvall.** 1980. Effect of specific binding of human albumin, fibrinogen, and immunoglobulin G on surface characteristics of bacterial strains as revealed by partition experiments in polymer phase systems. Infect. Immun. **29:**879–885.
17. **Myhre, E., and G. Kronvall.** 1977. Heterogeneity of nonimmune immunoglobulin Fc reactivity among gram-positive cocci: description of three major types of receptors for human immunoglobulin G. Infect. Immun. **17:**475–482.
18. **Myhre, E., and G. Kronvall.** 1980. Demonstration of specific binding sites for human serum albumin in group C and G streptococci. Infect. Immun. **27:**6–14.
19. **Neville, D. M., Jr.** 1971. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinous buffer system. J. Biol. Chem. **246:**6328–6334.
20. **Nygren, P.-Å., M. Eliasson, L. Abrahamsen, and M. Uhlén.** 1988. Analysis and use of serum albumin binding domains of streptococcal protein G. J. Mol. Recog. **1:**69–74.
21. **Nygren, P.-Å., C. Ljungquist, H. Trömborg, K. Nustad, and M. Uhlén.** 1990. Species-dependent binding of serum albumins to the streptococcal receptor protein G. Eur. J. Biochem. **5:**143–148.
22. **Olsson, A., M. Eliasson, B. Guss, B. Nilsson, U. Hellman, M. Lindberg, and M. Uhlén.** 1987. Structure and evolution of the repetitive gene encoding streptococcal protein G. Eur. J. Biochem. **168:**319–324.
23. **Raeder, R., R. Otten, M. Boyle.** 1991. Comparison of albumin receptors on bovine and human group G streptococci. Infect. Immun. **59:**609–616.
24. **Reis, K., E. Ayoub, and M. Boyle.** 1984. Streptococcal Fc receptors. I. Isolation and partial characterization of the receptor from a group C streptococcus. J. Immunol. **132:**3091–3097.
25. **Saiki, R., D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. Horn, K. Mullis, and H. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
26. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
27. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
28. **Scatchard, G.** 1949. The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. **51:**660–672.
29. **Simpson, W., I. Ofek, and E. Beachey.** 1980. Binding of lipoteichoic acid to the fatty acid binding sites on serum albumin. J. Biol. Chem. **55:**6092–6097.
29a. **Sjöbring, U.** Unpublished data.
30. **Sjöbring, U., L. Björck, and W. Kastern.** 1989. Protein G genes: structure and distribution of IgG-binding and albumin-binding domains. Mol. Microbiol. **3:**319–327.
31. **Sjöbring, U., L. Björck, and W. Kastern.** 1991. Streptococcal protein G: gene structure and protein binding properties. J. Biol. Chem. **266:**399–405.
32. **Sjöbring, U., C. Falkenberg, E. Nielsen, B. Åkerström, and L. Björck.** 1988. Isolation and characterization of a 14 kDa albumin-binding fragment of protein G. J. Immunol. **140:**1595–1599.
33. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
34. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
35. **von Hejne, G.** 1984. How signal sequences maintain cleavage specificity. J. Mol. Biol. **173:**243–251.
36. **Widebäck, K., J. Havlicek, and G. Kronvall.** 1983. Demonstration of a receptor for mouse and human serum albumin in Streptococcus pyogenes. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **91:**373–382.
37. **Widebäck, K., and G. Kronvall.** 1987. Isolation of a specific albumin receptor from a group G streptococcal strain. Acta Pathol. Microbiol. Scand. Sect. B **95:**203–210.
38. **Widebäck, K., U. Seal, and G. Kronvall.** 1982. Receptor in group C and G streptococci detects albumin structures present in mammalian species. Infect. Immun. **36:**469–475.