Protein PAB, an Albumin-binding Bacterial Surface Protein Promoting Growth and Virulence*  

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Maarten de Château‡§, Elisabet Holst¶, and Lars Björck‡  

From the Departments of §Cell and Molecular Biology and ¶Medical Microbiology, Lund University, P. O. Box 94, S-221 00 Lund, Sweden  

The anaerobic bacterium Peptostreptococcus magnus is a human commensal and pathogen. Previous work has shown that strains of *P. magnus* isolated from patients with gynecological disease (vaginosis) frequently express an immunoglobulin (Ig) light chain-binding protein called protein L. Here we report that strains isolated from localized suppurrative infections bind human serum albumin (HSA), whereas commensal isolates bind neither Ig nor HSA. The HSA-binding protein PAB was extracted from the bacterial surface or isolated from the culture supernatant of the *P. magnus* strain ALB8. Protein PAB was shown to have two homologous HSA-binding domains, GA and uGA. GA is absent in the sequence of a related protein from another *P. magnus* strain and shows a high degree of homology to the HSA-binding domains of streptococcal protein G. Therefore GA is believed to have recently been shuffled as a module from genes of other bacterial species into the protein PAB gene. This GA module was shown to exhibit a much higher affinity for HSA than uGA and was also found to be present in all of the isolates tested from localized suppurrative infections, indicating a role in virulence. Moreover, when peptostreptococci or streptococci expressing the GA module were grown in the presence of HSA, the growth rate was substantially increased. Thus, the HSA binding activity of the GA module adds selective advantages to the bacteria, which increases their virulence in the case of *P. magnus* strains.  

As a rule, anaerobic infections are caused by bacteria that are part of the indigenous flora of mucosal surfaces and the skin. *Peptostreptococcus magnus* is such a commensal, and it belongs to the major group of anaerobic bacterial species causing clinically significant infections (1). Still little is known about the virulence factors of this bacterium. Increased oxygen tolerance, as seen among clinical *P. magnus* isolates, could contribute to the pathogenic potential (2). Other possible virulence factors, apart from the herein discussed bacterial surface proteins, include encapsulation (3) and collagenase production (4).  

Numerous Gram-positive bacterial species and human pathogens express structurally related surface proteins that interact mainly with soluble host proteins (5). Protein A of *Staphylococcus aureus* and protein G of human group C and G streptococci both interact with the Fc region of IgG (6–8). Protein G also has affinity for human serum albumin (HSA) (9), as do members of the M protein family expressed by *Streptococcus pyogenes* (10–12). Albumin binding has also been described for protein PAB (peptostreptococcal albumin binding) of the anaerobic commensal and pathogen *P. magnus* (13). Protein L is another surface protein of certain strains of *P. magnus* that binds to immunoglobulin light chains (14). Protein L has been shown to be a virulence determinant in bacterial vaginosis (15), perhaps due to its histamine-releasing activity (16). M proteins contribute to the virulence of *S. pyogenes* by their antiphagocytic property (17), and also IgGFc-binding proteins of these bacteria have been reported to be virulence factors (18). Experiments with deletion mutants have likewise shown that protein A of *S. aureus* plays a role in virulence, possibly by inhibiting oposonphagocytosis (19).  

Host protein binding cell wall proteins of Gram-positive bacteria share common primary structure motifs, including (from the distal NH$_2$ terminus) a signal sequence, a variable NH$_2$-terminal region, a varying number of repeated domains that independently bind different plasma proteins, and a prolinerich region supposedly intercalating the protein in the Gram-positive cell wall, followed by a COOH-terminal cell wall sorting signal required to anchor the protein to the cell wall (20). The gene structure of the albumin-binding protein PAB has been shown to contain a centrally located functional domain of 45 amino acid residues responsible for the binding of HSA (Fig. 1). This domain has been subject to module shuffling between bacterial species, and was subsequently named the GA module, protein G-related albumin binding module (13). Such shuffling of modules seems to be a persistent activity among this group of genes, and when a consensus sequence of 15 nucleotides (called *recer* sequence) flanking the different modules in the *P. magnus* family of surface proteins was identified, a model for the shuffling of modules was proposed (21). The albumin-binding protein G of group G streptococcal strain G148 carries three GA modules in the NH$_2$-terminal part of the protein showing up to 60% identity to the shuffled module of protein PAB, indicating that protein G might be the source of the GA module in protein PAB. The secondary structure and global fold of the GA module in protein PAB have been determined by NMR and were shown to adopt a 3-helix bundle (22), and the third GA module of protein G was recently shown to exhibit the same structure (23). The predecessor of protein PAB has also been identified. This protein, called urPAB, is expressed by a strain that binds less albumin to its surface. Protein urPAB lacks the shuffled GA module (21), but in the NH$_2$-terminal region, a domain (uGA) was identified showing 38% identity to the GA  

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§ To whom correspondence should be addressed. Tel.: 46-46-222-4488; Fax: 46-46-157756.  

* The abbreviations used are: HSA, human serum albumin; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; PAGE, polyacrylamide gel electrophoresis; FFA, free fatty acids; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)methyl]glycine.
mids strain L603 (10^9 bacteria/ml). 200 µl of these mixed bacterial suspensions were then incubated with 10 µM of 125I-HSA or IgG for 30 min. Cells were spun down, and the radioactivity of the pellet was measured in a γ counter and expressed as percentage of added radioactivity.

Solubilization of Peptostreptococcal Proteins—Peptostreptococci were grown as described until the stationary phase was reached and then harvested by centrifugation. Subsequently, 10% solutions of the bacteria were boiled in HCl, pH 2.0, vortexed, chilled, and treated with mutanolysin or trypsin to extract surface proteins. Acid and alkali extractions were performed by boiling the bacteria for 3 min in 0.1 M HCl or NaOH, whereafter the sample was neutralized by the addition of 0.1 volume of 0.1 M Tris-HCl, pH 8.0. Extraction was done by vortexing the bacterial suspension in PBS. Mutanolysin digestions were performed in 0.01 M phosphate buffer, pH 6.8, followed by addition of NaHCO₃ to pH 7.5 and by cooling on ice. Trypsin digestions were done at pH 6.1 in 0.05 M phosphate buffer containing 5 mM EDTA. Reactions were inhibited by adding benzamidine to a final concentration of 5 mM. Enzyme incubations were at 37 °C for 1–2 h.

Affinity Chromatography of Peptostreptococcal Protein PAB and an E. coli-expressed Protein PAB Fragment—Culture medium from peptostreptococci (strain ALB8) expressing protein PAB or periplasmic E. coli lysates of clones expressing the 5'-end (nucleotides 1–857) of the protein PAB gene (pab) were used as starting materials and subjected to affinity chromatography on HSA-Sepharose CL-4B (Pharmacia Biotech Inc., Uppsala, Sweden). Columns of Sepharose, coupled with 3–5 mg of HSA/ml of packed gel, were equilibrated in PBS. The sample, in 0.01 M phosphate buffer, pH 7.5, was applied, and the column was rinsed with PBS and then eluted with 0.1 M glycine buffer, pH 2.0. Eluates were immediately neutralized by the addition of 0.1 volume of unbuffered 1 M Tris.

Electrophoresis and Blotting of Proteins—Proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (29) and transferred to PVDF membranes (30). Agarose gel electrophoresis was performed as described (31). Transfer of proteins from agarose gels was done by applying PVDF membranes on top of the gels under pressure (1–2 kg).

Absorption Experiments with Peptostreptococci and Human Plasma—P. magnus bacteria of strain 312 (10^9 cells) were incubated in 1 ml of human plasma for 2 h at 37 °C. The cells were spun down and washed five times in PBS. The bacterial pellet was then resuspended in 50 µl of PBS and boiled in an equal volume of SDS sample buffer. Cell debris was spun down, and the supernatant was subjected to SDS-PAGE and Western blot analysis (9).

Equilibrium Constants—Equilibrium constants were determined by incubating constant amounts of Immuno beads (Bio-Rad), coupled with HSA, together with 125I-labeled protein PAB or GA and varying amounts of non-labeled proteins for 16 h at room temperature after washing, the amount of bound radioactivity was measured. Using the formula of Scatchard, the calculations were done as previously reported (32).

Mutanolysin Extraction of an Albumin Binding Surface Component and Slot Binding Experiments—10% (v/v) solutions of strain ALB8 peptostreptococci were incubated for 2 h at 37 °C with mutanolysin in 0.01 M phosphate buffer, pH 6.8. The reaction was blocked with NaHCO₃ to pH 7.5 followed by cooling on ice. Cells were spun down, and material from the supernatant was applied to PVDF membranes using a dot blot apparatus from Schleicher & Schuell (Dassel, Germany). Incubation with radiolabeled HSA was followed by autoradiography to visualize binding.

Preparation of Oligonucleotides, PCR Procedures, and Cloning and Expression in E. coli—Five oligonucleotides were synthesized, all of which include restriction site linkers (NruI and SalI) for cloning in the expression vector pHD 389 (33): RXN1, dGCT CAG GCG CGC CGG ACG AAC CCG GGG CAC CCA A; RX3, dCAG CAG GTC GTA TTA TTA GAG GTC TGC TGG TTA AAT TAC TTT GTT, 1118, dCAG GTC GAC TTA TTA TTC GTC TTC TAC TCG TGA TAA TAC; RC3, dGCT GTT CTA GAT TAT TAT (TG/TT TCT/C) GCT (TG/TT TCT TCT TCT TT; RXN°, dGCG AAT TCG CCG CAT GAA AAT TAA TAA GAA ATT ATT. These oligonucleotides were used as primers with genomic DNA from P. magnus strains ALB8 and ALB1B as templates to generate inserts for cloning. PCR products were purified by chloroform/phenol extraction and ethanol precipitation and were subjected to restriction enzyme cleavage before a second purification and ligation to the vector. Subsequently, the ligation mixes were transformed into competent E. coli strain JM109 cells. Clones were selectively grown on ampicillin-containing plates and were screened by PCR and for expression of HSA binding peptides. HSA binding peptides were then purified by affinity chromatography on HSA-Sepharose and on a gel chromatography col-

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**Protein PAB, a Growth Stimulator and Virulence Determinant**

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**Fig. 1. Schematic representation of proteins PAB, urPAB, and G and recombinant fragments.** Schematic gene structures and structures of recombinant fragments are given in box form. Common features for proteins PAB, urPAB, and G are the signal peptide (ss), cell wall (W), and membrane-spanning regions (M) as well as the different HSA-binding GA domains (GA, uGA, GA1–3). The C domains of proteins PAB and urPAB have no known function. The C domains (C1–3) of protein G, interspersed by linking domains (D1–2), bind IgGFc (39, 40). Numbers refer to amino acid positions. Accession numbers of the sequences in the GenBank®EBI and PIR data banks are: protein PAB, GB X77864 and PIR A53586; protein urPAB, GB Z48975; and protein G, GB X04015.

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**Table:**

| Protein PAB gene | Fragment AC | Fragment GA | Protein urPAB gene | Fragment GA-K |
|------------------|-------------|-------------|-------------------|---------------|
| (P. magnus strain ALB8) | ![Fragment AC](image1) | ![Fragment GA](image2) | (P. magnus strain ALB1B) | ![Fragment GA-K](image3) |

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**MATERIALS AND METHODS**

**Bacteria—**P. magnus strains were clinical isolates from the Department of Clinical Microbiology, Lund University Hospital. The peptostreptococci were grown under strictly anaerobic conditions at 37 °C in Todd-Hewitt broth (Difco).

**Proteins, Sera, and Labeling of Proteins—**HSA and human IgG were purified from human plasma (28). Other serum albumins and sera were from Sigma. Protein L was prepared from peptostreptococcal growth medium (15), and recombinant protein G was from Escherichia coli lysates (9). Proteins were radiolabeled with 125I using the Bolton-Hunter reagent (Amersham Corp., Buckinghamshire, Great Britain), chloramine T, or lactoperoxidase.

**Binding Assay—**Bacteria were suspended, heat killed (80 °C, 5 min), and washed in phosphate-buffered saline (PBS) containing 0.02% NaN₃ and 0.5% Tween 20. Bacterial suspensions of different concentrations in a volume of 100 µl were mixed with 900 µl of Staphylococcus epidermidis.
compared with the commensal strains, there was a significant and none bound HSA. Finally, neither HSA nor IgG binding one isolate showed significant IgG binding. In the vaginosis infection isolates, 16 (53%) were HSA binding, whereas only human IgG was tested (Fig. 2). Among the 30 suppurative healthy carriers. The ability of these strains to bind HSA and

Forty-eight suppurative infections—

Strains of P. magnus Isolated from Patients with Localized suppurative infections

P. magnus

Isolation and characterization of the HSA-binding proteins showing intermediate binding (27%) were chosen for further

bacterial cells (Fig. 3). Among the HSA-binding strains, strain ALB8 showing maximal binding (70%) and strain ALB1B showing intermediate binding (27%) were chosen for further isolation and characterization of the HSA-binding proteins PAB and urPAB, respectively.

Isolation of the HSA-binding Protein PAB from P. magnus

After anaerobic growth of P. magnus strain ALB8 bacteria for 3 days in Todd-Hewitt broth, cells were collected by centrifugation and washed in PBS. Different procedures to solubilize HSA binding materials were tested. Boiling of the bacteria at low or high pH in HCl and NaOH, respectively, released substantial amounts of fragmented HSA-reactive material in the range of 14—45 kDa (Fig. 4). Similarly, degraded material could be obtained by treatment of intact bacteria with the muranolytic enzyme mutanolysin, whereas trypsin digestion resulted in extensive degradation. A higher proportion of larger fragments could be obtained by simply vortexing the bacteria in PBS. Still, a full-length molecule of 47 kDa was only seen when the HSA-binding protein was isolated from the culture supernatant by affinity chromatography on HSA-Sepharose. The protein yield from the culture supernatant was approximately 1 mg/liter. The NH₂-terminal sequence of this 47-kDa material has been shown to correspond to that of the NH₂-terminal sequence of protein PAB as deduced from the gene sequence (13). Mild treatment of the material coming off the HSA-Sepharose column with trypsin resulted in a single HSA binding fragment of 23 kDa.

Protein PAB-expressing Bacteria and Purified Protein PAB

Bind HSA with High Specificity—Protein PAB-expressing bacteria were incubated with human plasma. After incubation and washing, the bacteria were boiled in SDS-PAGE sample buffer. Cells were spun down, and the supernatant was run on a gel that was also blotted onto a PVDF membrane and probed with radiolabeled protein PAB. As seen in Fig. 5, only a single band at 66 kDa representing HSA could be adsorbed to the bacterial surface, and this band also reacted with protein PAB in the Western blot experiment. The conclusion is that neither the bacteria nor protein PAB will interact with any other plasma protein than HSA. Subsequent tests of the binding of ¹²⁵I-
labeled protein PAB to a number of purified proteins (i.e. IgG, IgA, fibrinogen, and fibroactin) in slot binding experiments were also negative (not shown). In order to further analyze the specificity of the interaction between protein PAB and HSA, human plasma was run on agarose gels. After transfer to PVDF membranes, the plasma proteins were probed with either protein L or protein PAB. As seen in Fig. 6, protein L bound to the cathodal immunoglobulin region of the plasma sample, whereas protein PAB bound to the anodal albumin region. Proteins PAB and L were also applied to the gel and were found to migrate in opposite directions to their respective ligands. These results reflect that protein PAB is a basic protein (net charge of +7, pI of 9.78), and protein L is an acidic protein (net charge of −54).

Binding of Protein PAB to Mammalian Sera and Purified Albumins—Sera from 10 different mammalian species were subjected to SDS-PAGE. The separated proteins were blotted onto a PVDF membrane and probed with radiolabeled protein PAB (Fig. 7). Binding to the albumin band in serum from man, baboon, rhesus monkey, rat, and cat was seen. Fig. 8 demonstrates that, also among purified albumins from 12 mammalian species, protein PAB has affinity for albumin from man, baboon, rhesus monkey, and rat (purified cat serum albumin was not available).

Characterization of Protein PAB Fragments Identifies Two Distinct HSA-binding Sites—If P. magnus strain ALB8 bacteria are grown in Todd-Hewitt broth until stationary phase, HSA binding activity can be detected not only on the surface of the bacteria but also in the culture supernatant. This material can be adsorbed to HSA-Sepharose and then eluted at pH 2.0. The eluted proteins were separated by SDS-PAGE and blotted onto two PVDF membranes. One was probed with radiolabeled HSA, and the results demonstrated that the majority of the bands still bind HSA (not shown). The other membrane was stained with Coomassie Blue, and as seen in Fig. 9, the HSA binding material coming off of the column is often size heterogeneous. This could be due to partial hydrolysis at low pH or due to proteolytic activity at the bacterial surface or in the culture medium. The albumin binding bands at 47 (I), 24 (II), and 16 kDa (III) were cut out and subjected to NH2-terminal sequencing. All three bands had the same amino acid sequence, identical to the NH2 terminus of protein PAB. This shows that the fragments are derived from protein PAB and that there must be an HSA-binding site in the most NH2-terminal 130–140 amino acids, giving an approximate molecular mass of 16 kDa. The same material was then subjected to mild trypsin treatment, yielding a single major albumin-bind-
strains were tested for the presence of the GA module. Chromosomal DNA from the different strains was used as templates in polymerase chain reactions. An oligonucleotide (RX2N) representing the 5'-end of the recently introduced GA module of protein PAB and a second oligonucleotide spanning the very well conserved cell wall anchor motif (769) were used as primers in these reactions. DNA from the protein PAB strain, which was used as a positive control, yielded a product of approximately 450 base pairs. All 15 HSA-binding strains yielded products of equal or near equal (± 100 base pairs) sizes, whereas none of the 10 non-binding strains yielded any PCR products with these primers.

The Affinity for HSA Differs among GA Modules—To find an explanation to why a second GA module has been introduced into protein PAB, the affinity of this GA module was determined and compared with the relative affinities of the uGA regions of proteins PAB and urPAB. Recombinant fragments of proteins PAB and urPAB covering the three resident GA modules were expressed in E. coli and purified by affinity chromatography on HSA-Sepharose, followed by gel filtration. Fragment AC corresponds to amino acid residues 27–195 of protein PAB, containing the uGA and C domains. A second fragment (GA) covers residues 213–265 of protein PAB containing the GA module, whereas uGA-K is a fragment corresponding to the uGA region (positions 1–102) of protein urPAB (Fig. 1). These fragments together with proteins urPAB and G were run on a Tricine-SDS-PAGE gel to show sizes and purity (Fig. 10A).

They were also radiolabeled and allowed to bind to HSA in slot binding experiments (Fig. 10B). In these experiments, the AC fragment bound weakly, the GA module bound almost as strong as intact protein PAB (compare with Fig. 8), and the uGA-K bound to an intermediate degree.

A competitive binding assay was utilized to determine the equilibrium constant of the interactions between HSA and fragment GA of protein PAB, as well as the relative affinities of the three peptostreptococcal GA modules. The affinity of frag-
ment GA (the shuffled module) for HSA was determined to be $6.7 \times 10^9$ M$^{-1}$ s$^{-1}$. Furthermore, HSA was coupled to polycrylamide beads and incubated with the radiolabeled GA fragment in the presence of different amounts of non-labeled competing proteins (Fig. 10D). The AC fragment could only interfere with the binding of radiolabeled GA when added at a molar excess of $>1000:1$. Protein urPAB was a much more efficient inhibitor but still not as efficient as GA itself. Based on the amount of inhibitor needed to elicit the same inhibitory effect on the binding of 125I-labeled GA to HSA, the relative affinities were 1, <0.05, and <0.001 for GA, uGA of protein urPAB, and uGA of protein PAB, respectively.

**Growth of HSA-binding Bacteria Is Stimulated by HSA**—The growth rates of albumin-binding strains of *P. magnus* and group G streptococci were markedly increased upon the addition of HSA to the growth medium (Fig. 11). In these experiments, 100 μl of overnight cultures were transferred to 12-ml tubes containing plain Todd-Hewitt broth or broth supplemented with 0.5 mg/ml of HSA (11). Samples were taken at regular time intervals, and culture density was measured by colony counting (colony forming units) or by measuring optical density at 620 nm.

**DISCUSSION**

The Gram-positive obligate anaerobic peptostreptococci are part of the human indigenous flora on the skin, in the oropharynx, and in the gastrointestinal and genitourinary tracts. Among these commensals, *P. magnus* is the species most often associated with clinically significant infections. It is second in frequency only to *Bacteroides fragilis* among obligate anaerobic bacteria recovered from clinical isolates, accounting for about 10% of anaerobic human infections. The isolates are often part of mixed infections with other anaerobes and facultative anaerobes such as *S. aureus*, but *P. magnus* can also be found in pure isolates, a fact stressing its potential as a pathogen (1). However, like most anaerobes, it is a low grade pathogen that will probably not invade tissues unless preceded by trauma or other more invasive pathogens (34). *P. magnus* will generally cause localized suppurative infections or vaginosis. Localized supplicative infections are understood as infection entities such as abscesses, soft tissue, and wound infections. In the present study, 16 out of 30 isolates from localized suppurative infections were found to bind significant amounts of HSA, whereas none of the vaginosis or commensal isolates showed affinity for HSA (Fig. 2). Statistical analysis of these figures, using the χ² test, demonstrated a correlation between the albumin binding phenotype and suppurative infections ($p < 0.0001$), implying a role for this phenotype in virulence. Previously, *P. magnus* strains expressing the Ig light chain-binding protein L have similarly been associated with bacterial vaginosis, a condition characterized by bacterial overgrowth in the vagina (15). In the current study, all strains were tested for both HSA and Ig binding, and only one strain expressing both activities could be identified. Such a strain expressing a protein with sequence homologies both to the Ig light chain-binding domains of protein L (35) and the GA module of protein PAB was recently described (25). By PCR, the genes for proteins PAB, urPAB, and L have been amplified with chromosomal DNA from the corresponding strains as templates and by using the same sets of oligonucleotides, representing both intra- and extragenic sequence homologies (13, 21). These sequence homologies in the framework regions of the genes suggest a common evolutionary origin. Applying the same PCR reactions to the collection of non-binding strains even at low stringency yielded no specific products. This indicates that there are no silent or down-regulated copies of the genes in non-binding strains. Moreover, the occurrence of these proteins appears to be a down-regulated copies of the genes in non-binding strains even at low stringency yielded no specific products. This indicates that there are no silent or down-regulated copies of the genes in non-binding strains. Moreover, the occurrence of these proteins appears to be a frequent event. These results are particularly significant for the *P. magnus* strain of G148-GA3, which was the only one of the two strains tested for the expression of all three proteins in this study (22) that showed significant binding to HSA. The relative affinities of the peptostreptococcal GA modules derived from Fig. 10D are shown to the far left. Three residues (TSR) in ALB8-uGA and one (F) in ALB1B-uGA are shown in bold because they are suggested to be responsible for the lowered affinities for HSA in these modules as compared with ALB8-GA. The equilibrium constant for the interaction between a protein G fragment containing G148-GA3 and HSA has been determined previously to be 2.6 × $10^9$ M$^{-1}$ (36).
emphasized since these two GA modules show equally strong affinity for HSA, 6.7 × 10^5 and 2.6 × 10^5 M^-1 (36), respectively. Assuming that the different GA modules compete for the same binding sites on HSA, the NH2-terminal uGA domains of proteins PAB and urPAB were shown to have much weaker affinities for HSA. This fact has implications for the interpretation of why the GA module was probably shufﬂed into protein PAB more recently. The uGA domains are located in analogous positions in proteins PAB and urPAB and can be assumed to have evolved divergently in situ from a common ancestor. uGA in protein PAB can therefore be said to be “older” than the more recently acquired GA. A reason for the organism to acquire a new GA module could be that the old one had lost most of its function, hence the difference in affinities.

uGA of protein PAB has low afﬁnity for HSA and differs in four amino acid positions that are all conserved in the other three GA modules with higher afﬁnities (Ala-4, Glu-11, Lys-30, and Thr-31). A ﬁfth conserved position (Val-32) is changed to a proline residue in the uGA domain of protein urPAB. The octapeptide region constituting residues Ile-26 to Glu-33 is the only longer conserved region in 16 GA modules from seven proteins compared. The GA modules of protein PAB (GAI) and protein G (GAS) have been investigated by NMR and were found to adopt antiparallel 3-helix bundle structures (22, 23). In these structural determinations, it was shown that residues 30–32 are part of a loop between helix II and III and should therefore be accessible for solvent. Taken together, these ﬁndings indicate that the binding site for HSA is to be found in this octapeptide region of the GA module.

Bacteria are well adapted to exploit their nutritional environment, and their growth rates can vary over more than a 10-fold range. The results of Fig. 11 show that HSA-binding strains of P. magnus and human group G streptococci grow with higher growth rates and to higher maximum cell densities when HSA is present in the culture medium. However, addition of IgG to the growth medium of comparable Ig-binding strains of P. magnus (50% of all lipid transportation in the human body is carried out by HSA and its ligands. It is also likely that FFA associated with HSA bound to the bacterial surface can be utilized as nutrient. Thus, in the case of hepatocytes, the binding of HSA to a surface protein is followed by the rapid diffusion of FFA across the plasma membrane (38).

Previous studies have described the dynamic on-going evolution of HSA- and Ig-binding surface proteins of P. magnus (13, 21). These studies have also identiﬁed interdomain sequences, so-called recer sequences, with intron-like function that have promoted the evolution of this protein family (21). Here we ﬁnd that the binding of HSA stimulates bacterial growth. This growth stimulation could constitute the selective pressure behind the evolution of protein PAB as the shufﬂing of an additional GA module into protein PAB has dramatically increased its afﬁnity for HSA. Moreover, the presence of GA in all of the isolates from patients with localized suppurative infections supports the notion that this module adds to the pathogenicity of these strains. Thus, as a consequence of the shufﬂing of the GA module and the subsequent growth stimulation, protein PAB-expressing strains have become more virulent.
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Maarten de Château, Elisabet Holst and Lars Björck

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