Structure, Specificity, and Mode of Interaction for Bacterial Albumin-binding Modules*

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We have determined the solution structure of an albumin-binding domain of protein G, a surface protein of group C and G streptococci. We find that it folds into a left-handed three-helix bundle similar to the albumin binding domain of protein PAB from Peptostreptococcus magnus. The two domains share 59% sequence identity, are thermally very stable, and bind to the same site on human serum albumin. The albumin-binding site, the first determined for this structural motif known as the GA module, comprises residues spanning the first loop to the beginning of the third helix and includes the most conserved region of GA modules. The two GA modules have different affinities for albumin from different species, and their albumin binding patterns correspond directly to the host specificity of CG streptococci and P. magnus, respectively. These studies of the evolution, structure, and binding properties of the GA module emphasize the power of bacterial adaptation and underline ecological and medical problems connected with the use of antibiotics.

In the complex molecular interplay between a pathogen and its human host, protein-protein interactions play important roles. For instance, bacteria express surface proteins that interact with abundant human extracellular proteins with high affinity and specificity. In human plasma, albumin (HSA)1 and immunoglobulins (Ig) are the quantitatively dominating proteins, and significant human pathogens have developed surface proteins that bind these and other plasma proteins (for references, see Ref 1). Two of the most well known such proteins are protein A of Staphylococcus aureus (2) and protein G of group C and G streptococci (3, 4), which both bind to the Fc region of IgG. In 1980, Myhre and Kronvall (5) reported that HSA could bind to the surface of various streptococcal species, including group C and G streptococci. It was later found that protein G was responsible also for the interaction with HSA (6). Protein G has separate binding domains for IgG and HSA (7, 8), and as a result C and G streptococci are in vivo covered with an inner layer of IgG and an outer layer of HSA. Peptostreptococcus magnus are strictly anaerobic bacteria that are part of the indigenous human flora of the skin, oral cavity, and gastrointestinal and urogenital tracts. Some isolates of this species bind HSA (9), and notably these isolates are mostly from patients with deep wound infections (10), suggesting that HSA binding turns the commensal P. magnus into a potential pathogen. The surface protein of P. magnus binding HSA is called PAB, and it contains a domain of 45 residues showing a high degree of sequence homology with the HSA binding domains of protein G (11). Analysis of the gene encoding PAB suggested that this domain originates from protein G and has been transferred to and introduced into the pab gene through the action of a conjugational plasmid (related to pCP10 of Enterococcus faecalis) followed by a recombinational event (11). This interspecies exchange of a structurally well defined motif represents the first example of so-called module shuffling (12) in prokaryotic cells, and the motif was designated the GA (protein G-related albumin binding) module. Functionally, GA-expressing bacterial strains show increased growth rates in the presence of HSA, whereas strains that do not bind HSA are unaffected (10). This suggests that the HSA binding property adds selective advantages to the bacteria, which may also explain why HSA-binding P. magnus strains are more virulent than nonbinding strains (10).

The solution structure of the GA module of protein PAB originating from protein G, designated ALB8-GA, has been reported previously (13). Protein G from strain G148 (3) contains three GA modules (14), and the one nearest the C terminus of these (G148-GA3) exhibits 59% sequence identity at the protein level with ALB8-GA (see Fig. 1A). To enable a structural comparison of the two GA modules, the solution structure of G148-GA3 has now been determined using NMR spectroscopy; H-D; hydrogen-deuterium; HPLC, high-performance liquid chromatography; IC50, concentration necessary to inhibit 50% of binding; Ig, immunoglobulin; NMR, nuclear magnetic resonance.
copy. NMR methods were also used to identify the albumin binding site of the two GA modules. This structural information, together with data obtained by competitive binding experiments, offers a perspective for understanding the specific interactions between the albumin and the GA modules. Our results suggest that the structure and albumin binding properties of the two GA modules studied here reflect bacterial host

EXPERIMENTAL PROCEDURES

Expression and Purification—Expression and purification of ALB8-GA will be described elsewhere.2 A peptide corresponding to a truncated ALB8-GA lacking the six N-terminal residues (26) will be described elsewhere.2 A peptide corresponding to a truncated ALB8-GA lacking the six N-terminal residues (26) was purchased from Clinical Chemistry, Malmo General Hospital, where it was synthesized and subsequently purified using high performance liquid chromatography. The expression and purification of G148-GA3 were carried out as described previously (15).

CD Measurements of G148-GA3—The thermal stability of G148-GA3 at a concentration of 25 μM in 20 mM phosphate buffer (pH 7.2) was monitored by monitoring the ellipticity at 222 nm between 4 and 95 °C. The ellipticity corresponds to a subtraction of six from every residue number in our previous papers regarding ALB8-GA (15, 26). The ALB8-GA construct contains residues 212–285 of intact protein PAB (11). The first 19 residues (MKAIFVLANQHDEAVDANS, denoted by a star) in the G148-GA3 sequence are a remnant of the cloning procedure, and the sequence is followed by residues 141–186 of intact protein G (7). References to other GA modules can be found elsewhere (13). The helices, defined as described under "Results and Discussion," are identified by boxes at the top.

Panel B, melting curve of G148-GA3 (filled circles) as obtained from the temperature dependence of the ellipticity at 222 nm. Panel C, competitive binding experiments between different GA modules and HSA. The binding of 15N-labeled ALB8-GA to HSA-beads was blocked with different amounts of unlabeled ALB8-GA (filled squares), G148-GA3 (filled circles), and a truncated ALB8-GA lacking the six N-terminal residues (open squares).

RESULTS AND DISCUSSION

G148-GA3 Exhibits High Thermal Stability—CD measurements were performed to investigate the thermal stability of G148-GA3 and to enable comparison with the remarkable thermal stability of ALB8-GA (26). Both G148-GA3 (Fig. 1D) and ALB8-GA (26) start to unfold only at high temperatures. However, because of the gradual unfolding over a large temperature range it is not possible to determine the transition midpoint of the thermal denaturation for either of the two GA modules.

Structure Determination of G148-GA3—The assignment of ALB8-GA has been reported previously (15). The calculation of the G148-GA3 solution structure described here and evaluation of the structures obtained, based on agreement with experimental data, precision, and geometric quality is summarized in Table I. Of the final 50 structures computed, 30 structures having lowest energy and which simultaneously exhibited no distance violations greater than 0.5 Å or dihedral angle violations greater than 5°, were chosen for further analysis.

The lack of distance violations greater than 0.5 Å or dihedral

2 M. U. Johansson, H. Nilsson, J. Evenäs, S. Fursén, T. Drakenberg, L. Björek, and M. Wikström (2002) J. Mol. Biol., in press.
TABLE I

Restraints and structural statistics of G148-GA3

| All variances are | ± 1 S.D. |
|------------------|----------|
| Restraints used in the structure calculation |  |
| No. of distance restraints |  |
| Intraresidue | 294 |
| Sequential | 207 |
| Medium range | 230 |
| Long range | 108 |
| Total | 839 |
| No. of dihedral angle restraints |  |
| Distance restraint violations (>0.5 Å) | 0 |
| Dihedral angle violations (>5°) | 0 |
| r.m.s. deviations from experimental restraints |  |
| Distance restraints (Å) | 0.028 ± 0.003 |
| Dihedral restraints (°) | 0.09 ± 0.14 |
| r.m.s. deviations from idealized geometry |  |
| Bonds (Å) | 0.0028 ± 0.0001 |
| Angles (°) | 0.37 ± 0.02 |
| Improper s (°) | 0.31 ± 0.04 |
| Precision |  |
| r.m.s. deviations from average coordinates (residues 1–45) |  |
| Backbone atoms (Å) | 0.51 |
| All heavy atoms (Å) | 1.04 |
| Geometric quality |  |
| Lennard-Jones van der Waals energy (kcal mol⁻¹) | 202 ± 9 |
| Ramachandran analysis |  |
| Residues in most favored regions (%) | 90.0 |
| Residues in additionally allowed regions (%) | 8.0 |
| Residues in generously allowed regions (%) | 1.7 |
| Residues in disallowed regions (%) | 0.3 |

* Idealized covalent geometry is based on the parallhdg.pro force-field in X-PLOR (20).
* Residue 18 is missing in the G148-GA3 sequence.
* The program PROCHECK_NMR (18) was utilized in the Ramachandran analysis.

angle violations greater than 5° in combination with data for average root mean square (r.m.s.) deviations from experimental restraints and average r.m.s. deviations from idealized geometry suggest that the computed structures do indeed agree well with experimental data (Table I).

Average r.m.s. deviation from the average coordinates is 0.51 Å for the backbone (N, C', C) atoms (Table I) when overlaying all residues of the defined GA module sequence (Fig. 1A). All three helices are well defined with r.m.s. deviations of 0.39, 0.21, and 0.33 Å (the extents of helices are defined below). As for ALB8-GA, the relative orientations between the helices of G148-GA3 are also well defined, although not as well defined as the individual helices, as seen from the slightly higher r.m.s. deviation of 0.50 Å when superimposing all helices simultaneously. A stereo view of the superposition of the ensemble of 30 structures onto their unminimized average coordinates is shown in Fig. 2A, wherein the unordered character of the long N terminus is readily apparent, as is the distinct three-helix bundle structure formed by the remaining residues. Observed ¹³C chemical shifts (15) correlate well with the computed structures, having typical random coil values for the first 16–17 residues and showing typical helix values for three regions of consecutive residues.

The geometric quality of the structures is manifested by a large negative Lennard-Jones van der Waals energy and by a large portion of the non-glycine residues having their ϕ and ψ angles in the most favored regions of a Ramachandran plot (Table I).

As seen from Fig. 2A, G148-GA3 is a three-helix bundle with a left twist. Segments 1–15, 20–27, and 32–45 in G148-GA3 are determined to be helical, and the relative orientations of the three helices are nearly anti-parallel; Ω₁₂ = 171 ± 3, Ω₁₃ = 13 ± 2, and Ω₂₃ = 159 ± 3°. The secondary structure assignment was calculated using the method of Kabsch and Sander (27) within the PROCHECK_NMR (28) program.

Solution Structure of G148-GA3 versus ALB8-GA—Ribbons images of the GA modules shown in Fig. 2, B and C, and tube representations shown in Fig. 2D illustrate the overall structural similarity of the two modules. However, there are differences between the structures. The lengths of the helices in the two GA modules are not the same according to Kabsch and Sander secondary structure assignment calculations of each ensemble. The first helix in G148-GA3 starts already at residue –1, which is in the segment not belonging to the actual protein G sequence (see legend to Fig. 1A), and continues until residue 15. By contrast, the first helix in ALB8-GA runs between residues 4 and 13, i.e. this helix is seven residues shorter than the corresponding helix in G148-GA3. Helix 2 starts at residue 20 in both GA modules and ends at residue 27 in G148-GA3 but at residue 28 in ALB8-GA. The third helix starts at residue 32 and ends at residue 45 in both GA modules. Thus, the lengths and locations of helices 2 and 3 are nearly identical. Interestingly, this is the region hosting the albumin binding site (see below) and also the most conserved region of GA modules.

The relative helix orientations in both GA modules are similar, with Ω₁₂ = 171 ± 3, Ω₁₃ = 13 ± 2, and Ω₂₃ = 159 ± 3° for G148-GA3, and Ω₁₂ = 167 ± 4, Ω₁₃ = 26 ± 4, and Ω₂₃ = 158 ± 4° for ALB8-GA. The largest difference is observed for Ω₁₃ of the two sets of solution structures. The r.m.s. deviation of backbone atoms for residues belonging to the defined GA module sequence was 1.94 Å, whereas the r.m.s. deviation of backbone atoms for the 32 residues determined to be helical in both GA modules, segments 4–13, 20–27, and 32–45, was 1.69 Å. For both G148-GA3 and ALB8-GA, the structure closest to the mean was utilized for the r.m.s. deviation calculations.

The ensembles of structures have also been compared through a distance difference matrix (29) (DDM). The DDM of average intramolecular C'–C' distances (Fig. 2E) reveals that a majority of the significant differences are located in the proximity of residue 18 in ALB8-GA (Thr-18), at which position there is a deletion in the G148-GA3 sequence (Fig. 1A).

ALB8-GA and G148-GA3 Exhibit Different HSA Binding Affinity—To analyze the interaction between each GA module and HSA, a competitive binding assay was used. In this assay, the binding of radiolabeled ALB8-GA to HSA-beads was blocked with unlabeled ALB8-GA and G148-GA3, respectively (Fig. 1C, filled squares and circles, respectively). The inhibition curves obtained had similar shapes, suggesting the same binding site on HSA for ALB8-GA and G148-GA3. However, compared with ALB8-GA, a 100-fold higher concentration of G148-GA3 was required for efficient inhibition. Thus, the results from this competitive binding assay indicate that ALB8-GA has higher affinity for HSA than G148-GA3 has.

G148-GA3 Exhibits Broad Albumin Binding Specificity—To determine relative affinities, a competitive binding assay was again utilized. Purified albumin and serum from different mammalian species were used to block the binding of radiolabeled ALB8-GA and radiolabeled G148-GA3, respectively, to HSA-beads. Table II demonstrates that for each GA module the affinities for purified albumin from human, baboon, and rhesus monkey are of the same magnitude. The interactions between...
mouse, rabbit, and bovine albumin and the GA modules are noticeably weaker. The discrepancy in affinity for HSA in Table II versus Fig. 1C could be caused by the different assays that were used. Fig. 1C shows the GA modules competing with ALB8-GA for binding to HSA. Table II describes albumin from different mammalian species competing with HSA in binding to ALB8-GA and G148-GA3, respectively. Thus, the numbers in Table II reflect interactions between each GA module and different albumins.

Among the different mammalian sera that were tested, human, baboon, rhesus monkey, rat, and cat could inhibit the interaction between ALB8-GA and HSA-beads quite efficiently, whereas a weaker inhibition was obtained with mouse, rabbit, bovine, guinea pig, dog, and horse sera (Table III). Goat, swine, and sheep sera have too low affinity for ALB8-GA to be detected in these experiments. As shown in Table III, the interaction between G148-GA3 and HSA-beads, on the other hand, could be inhibited by all mammalian sera tested, although rabbit, bovine, and goat sera were weak inhibitors. Taken together the data indicate that the GA module from C and G streptococci, G148-GA3, has broader albumin binding specificity compared with the ALB8-GA module from P. magnus.

**Table II**

| Species         | ALB8-GA  | G148-GA3 |
|-----------------|----------|----------|
| Human           | 3.1 nM   | 1.6 nM   |
| Baboon          | 2.3 nM   | 2.0 nM   |
| Rhesus monkey   | 4.0 nM   | 3.0 nM   |
| Mouse           | 11.2 μM  | 10.2 μM  |
| Rabbit          | 46.7 μM  | 1.3 μM   |
| Cow             | 8.7 μM   | 0.3 μM   |

**Table III**

| Species         | ALB8-GA  | G148-GA3 |
|-----------------|----------|----------|
| Human           | 1:10,000 | 1:100,000|
| Baboon          | 1:10,000 | 1:100,000|
| Rhesus monkey   | 1:10,000 | 1:100,000|
| Mouse           | 1:10     | 1:10,000 |
| Rat             | 1:10,000 | 1:100,000|
| Rabbit          | 1:10     | 1:100    |
| Cow             | 1:10     | 1:100    |
| Guinea pig      | 1:10     | 1:10,000 |
| Dog             | 1:100    | 1:10,000 |
| Cat             | 1:1,000  | 1:10,000 |
| Goat            | 1:100    | 1:100    |
| Horse           | 1:10     | 1:1,000  |
| Swine           | 1:1,000  | 1:1,000  |
| Sheep           | 1:1,000  | 1:1,000  |

*a* Dilution 1:10 gives 22% inhibition.

*b* Dilution 1:10 gives 53% inhibition.
belonging to the defined GA module sequence are shown. The contact surfaces in panels D overlapped, or were not detected at all are shown in magenta. Panel E

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and the GA modules is weak (the IC50 of rabbit serum albumin

min was chosen because the interaction between this albumin

stepwise to a solution of each GA module. Rabbit serum albu-

tion experiments in which rabbit serum albumin was added

left residues with broadened cross-peaks at 37°C, and Val-35) of the residues with broadened cross-peaks (Tyr-22, Phe-23, Asn-24, Ala-25, Ile-26, Lys-28, Ala-29, Glu-34, Val-35, Asn-36, Ala-37, Leu-43, and Lys-44) at 47°C (which were also the residues with most shifted or broadened cross-peaks at 37°C) are also indicated in Fig. 3C. In summary, the 18 significantly perturbed residues of ALB8-GA are Trp(-1), Ser-19, Tyr-22, Phe-23, Asn-24, Ala-25, Ile-26, Lys-28, Ala-29, Glu-34, Val-35, Asn-36, Ala-37, Leu-43, and Lys-44) at 47°C (which were also the residues with most shifted or broadened cross-peaks at 37°C) are also indicated in Fig. 3C. In summary, the 18 significantly perturbed residues of ALB8-GA are Trp(-1), Ser-19, Tyr-22, Phe-23, Asn-24, Ala-25, Ile-26, Lys-28, Ala-29, Glu-34, Val-35, Asn-36, Ala-37, Leu-43, and Lys-44. Using criteria similar to those for ALB8-GA, 20 residues (Glu-11, Asp-13, Val-17, Ser-19, Tyr-22, Asn-24, Leu-25, Asn-28, Ala-29, Thr-31, Val-32, Glu-33, Gly-34, Val-35, Lys-36, Ala-37, Leu-43, Ala-44, Ala-45, and Leu-46) of G148-GA3 were identified as significantly perturbed at 37°C. The obtained chemical shift perturbations are mapped by color-coding onto the surfaces of the GA modules in Figs. 3, D and E.

Perturbations, i.e. chemical shift changes or broadening beyond detection of ligand cross-peaks upon binding, result from a number of direct and indirect structural and dynamic effects, and therefore interaction surfaces described by chemical shift perturbation methods are usually larger than the region in direct contact (30, 31). Not all of the significantly perturbed residues in ALB8-GA and G148-GA3 are surface exposed and thus cannot readily participate in the binding. For example, Ile-26 and Val-32 in ALB8-GA only have a total solvent acces-

Fig. 3. Albumin binding site of ALB8-GA and G148-GA3. Panel A, chemical shift perturbations upon addition of 0.6 eq of rabbit serum albumin to ALB8-GA at 37°C. Panel B, same as panel A but for G148-GA3. The chemical shift change was calculated from the chemical shift of the backbone 15N and 1H resonances using the following formula: \( \Delta \delta = ((\Delta \delta(1H))^2 + (0.2\Delta \delta(15N))^2)^{1/2} \) and is indicated with a filled bar at the corresponding residue. Each residue for which the cross-peak is broadened beyond detection upon the addition of albumin is indicated by a green bar at the value of 0.14 ppm (corresponding to the maximum chemical shift change of residues with nonbroadened cross-peaks). Residues that were too weak to give any reliable information, overlapped, or were not detected at all are indicated with outlined circles at a value of 0 ppm. The helices are indicated by boxes at the top. Panel C, overlay of a region of 15N-1H HSQC spectra of 2 eq ALB8-GA at 47°C in the absence (blue) and in the presence (red) of 1 eq of rabbit serum albumin with the residue numbers indicated. For details, see “Results and Discussion.” Panels D and E, contact surfaces displaying the effects of albumin binding shown in two different views differing by a 180° rotation along the y axis. The orientation of the views to the left is the same as in the ribbon representations in Fig. 2, panels C and B, respectively. The 18 (ALB8-GA, panel D) and 20 (G148-GA3, panel E) significantly perturbed residues are indicated in red. Residues that were too weak to give any reliable information, overlapped, or were not detected at all are shown in magenta. The remaining residues are colored blue. To clarify the presentation, only residues belonging to the defined GA module sequence are shown. The contact surfaces in panels D and E were prepared using MOLMOL (40).
sible surface area of 1.1 Å². These two conserved residues also have the least solvent accessible surface area (28.1 and 7.0 Å² for Ile-26 and Val-32, respectively) in G148-GA3. The total solvent accessible surface area of the significantly perturbed residues is 1,300–1,400 Å² in both GA modules, but it should be noted that some chemical shift information is missing (e.g. because of overlap) in one or both GA modules, preventing more extensive comparisons of albumin-binding sites.

**Binding Efficiency Is Reduced Significantly in a Truncated ALB8-GA**—To analyze the importance of the N-terminal residues in the interaction, HSA binding of radiolabeled ALB8-GA to HSA-beads was blocked with a synthetic peptide corresponding to a truncated ALB8-GA lacking the six N-terminal residues (Fig. 1A). The inhibition curve obtained (Fig. 1C, open squares) had a shape similar to those for ALB8-GA and G148-GA3, suggesting the same binding site on HSA. However, compared with ALB8-GA, higher concentrations (~1,000 times more) of the truncated ALB8-GA were required for efficient inhibition. Thus, competitive binding experiments indicate that the N terminus of the ALB8-GA construct is not crucial for the interaction with HSA, but the data show that this part is important for an efficient binding to take place, a fact also put forth by the previous hydrogen-deuterium exchange experiments (13).

It has been shown previously that HSA contains one binding site for protein G, formed by loops 6–8 of HSA (32). This region also contains the binding sites for tryptophan, fatty acids, and thyroxine. ALB8-GA has a tryptophan residue at position −1 which is missing in the truncated ALB8-GA lacking the six N-terminal residues, and this may explain the lower affinity for this peptide. The cross-peak corresponding to backbone 15N and 1H resonances of Trp(-1) was one of the 18 significantly perturbed in the titrations. Unfortunately, no data could be obtained for the cross-peak of the indole 15N and 1H resonances because this cross-peak is not visible at 37 or 47 °C, presumably the result of fast proton exchange with solvent.

**Comparison with Previous Efforts to Map the Albumin Binding Site**—We have tried previously to map the albumin binding site of ALB8-GA by performing hydrogen-deuterium exchange experiments on free and HSA-complexed GA module (13). However, in those experiments, nearly every residue exhibited a decreased amide proton exchange rate in the complex, preventing identification of a distinct albumin binding site. As described above, availability of 15N-labeled ALB8-GA (and the related G148-GA3) has now enabled 15N-1H HSQC NMR titrations yielding a more distinct albumin binding site. In the previous hydrogen-deuterium exchange experiments all residues, except Thr-05, Thr-18, Ser-19, Asp-20, Phe-21, Val-32, and Glu-33, were affected in some way. Even the unstructured and flexible (according to 15N NMR spin relaxation experiments) N terminus was found to be protected in the complex and therefore affected by binding. The large number of affected residues may be the result of albumin binding affecting the conformational dynamics of ALB8-GA, and therefore the binding site could not be determined from hydrogen-deuterium exchange experiments alone. In the present study the importance of the N terminus for binding has been confirmed by the competitive binding experiment (Fig. 1C) as well as by NMR titrations (Fig. 3A).

Sequence differences between GA modules with different affinities for HSA (13) indicated that a conserved region (residues 26–33) in the end of helix 2 and the beginning of helix 3 could contribute to the albumin binding. Especially Val-32, although unprotected in the hydrogen-deuterium experiments, was assumed to be part of a region crucial for the interaction with HSA. An explanation for the fact that surface-exposed residues, such as Val-32, at the putative binding interface are less protected than some buried residues was proposed previously (33). Solvent penetration may be facilitated by transient opening of the complex allowing contact surface amide-hydrogens to exchange, whereas motions required for exchange of core amide-hydrogens would still be more restricted. In this study, Val-32 was shown to be affected in the 15N-1H HSQC NMR titration experiments for both GA modules, indicating the importance of this residue.

One of the main conclusions from the present study, and a point of difference from the previous study (13), is the distinctness with which it is seen that helix 1 is essentially unaffected by albumin binding for both GA modules (Fig. 3, A and B).

**Comparison with the Solution Structure and Binding Site of the IgG Binding Domain(s) of Protein A—G148-GA3** (Ref. 15 and present study), like ALB8-GA (13, 26), has a similar three-helix bundle structure as the IgG binding domains (34) of protein A from *S. aureus* (designated E, D, A, B, and C from the N terminus), even though they display different binding properties and show no sequence homology. These three-three-helix bundles all show high temperature stability (Refs. 13 and 35 and present study), most likely because of hydrophobic residues forming the core of the protein domains. The most tangible structural difference is the relative helix orientations. The Fe binding site of protein A is defined by helices 1 and 2 (36, 37), whereas the Fv binding site is defined by helices 2 and 3 (38). Thus, in all the three-helix bundles discussed here (ALB8-GA, G148-GA3, and the IgG binding domain(s) of protein A), a single face of the molecules, composed of residues from two helices, forms the corresponding binding site.

**Evolutionary and Biological Implications**—The GA module has been subject to shuffling between C/G streptococci and *P. magnus* (11). The predecessor of protein PAB, called urPAB, has been identified (10). It lacks the shuffled GA module but contains an analogous domain, uGA, showing 38% sequence identity with the GA module. The uGA domain, which is found also in protein PAB, binds HSA with much lower affinity than the GA module (10). In proteins PAB and urPAB, the uGA domain is located in the same position. Moreover, the presence of a gene sequence adjacent to the GA module with homology to a region of the conjugal plasmid pCF10, and the fact that *P. magnus* strains expressing protein PAB are tetracycline-resistant, suggest that the uGA domain is older than the more recently acquired GA module and that antibodies could represent the selective pressure behind the shuffling. This notion is supported further by the observation that strains of *P. magnus* expressing protein PAB, a more efficient albumin-binding surface protein, grow better and are isolated from patients with deep wound infections (10) who are often treated with broad spectrum antibiotics such as tetracycline. In this dynamic and ongoing evolution, interdomain so-called *recer* sequences with intron-like function appear to play an important role (39). In relation to the present study, it is noteworthy that C/G streptococci infect virtually all mammalian species, whereas *P. magnus* has been isolated only from humans. During the relatively short time period when the protein G-GA of C/G streptococci has been part of protein PAB of *P. magnus*, its only function has therefore probably been to bind HSA. As a result, PAB-GA binds non-primate albums with much lower affinity than it binds primate albums, whereas for G-GA the difference in affinity between non-primate and primate is much less pronounced (Tables II and III). The studies of the evolution, structure, and binding properties of the GA module emphasize the power of bacterial adaptation. They also underline ecological

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3 E. Holst and L. Björck, unpublished data.
and medical problems connected with the use of antibiotics. The results suggest that under the selective pressure of antibiotics, *P. magnus*, a member of the normal bacterial flora, can turn into a potential pathogen through module shuffling.

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