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Regulation of proteolysis is a critical element of the host immune system and plays an important role in the induction of pro- and anti-inflammatory reactions in response to infection. Some bacterial species take advantage of these processes and recruit host proteinases to their surface in order to counteract the host attack. Here we show that Thrombin-activatable Fibrinolysis Inhibitor (TAFI), a zinc-dependent procarboxypeptidase, binds to the surface of group A streptococci of an M41 serotype. The interaction is mediated by the streptococcal collagen-like surface proteins A and B (SclA and SclB), and the streptococcal-associated TAFI is then processed at the bacterial surface via plasmin and thrombin-thrombomodulin. These findings suggest an important role for TAFI in the modulation of host responses by streptococci.

Streptococcus pyogenes is an important human Gram-positive pathogen that mainly causes throat and skin infections. Although these conditions are normally superficial and self-limiting, they can occasionally turn into invasive and life-threatening diseases such as sepsis and necrotizing fasciitis (1). In order to colonize the human host, S. pyogenes expresses so-called adhesins, which allow the bacterium to attack to the extracellular matrix or to cell surface structures. So far, at least 20 adhesins have been described in S. pyogenes, including for instance M proteins and protein F1 (for a review see Ref. 2). The two related streptococcal collagen-like surface proteins ScIA and ScIB, which were first described in 2000 and 2001, also belong to this family (3–7). Although their extracellular parts differ in size and primary sequence, ScIA and ScIB are organized into a similar “lollipop”-like structure. The stalk is made up of a collagen-like region with varying numbers of GAY repeats, whereas the globular head consists of a non-collagenous amino-terminal variable region. Both proteins have a conserved signal peptide and a carboxyl-terminal region that is attached to the cell wall via an LPATG anchor. The collagen-like regions of ScIA and ScIB have been shown to mediate adhesion to human lung epithelial cells (5) and fibroblasts (4). It has also been reported that ScIA from M type 41 activates the collagen receptor α5β1, integrin on fibroblasts (8) and interacts with the low density lipoprotein in human plasma (9).

Proteolysis plays an important role in host parasite interactions. Although some immune defense mechanisms such as complement, coagulation, and fibrinolysis are dependent on their activation by limited proteolysis, bacteria have evolved strategies to benefit from these host systems by assembling host proteinases at their surface. Probably the best studied interaction in this respect is the binding of plasminogen to the bacterial surface, which is thought to be a mechanism for the bacteria to trigger their dissemination in the human host (10). Thrombin-activable fibrinolysis inhibitor (TAFI),2 also known as procarboxypeptidase B, procarboxypeptidase R, and procarboxypeptidase U, is an arginine- and lysine-specific procarboxypeptidase. The protein is synthesized in the liver and secreted into plasma, where it circulates as a zymogen at concentrations between 60 and 275 nmol/l (11). The most potent activator of TAFI is the thrombin-thrombomodulin complex (12), but plasmin, trypsin, and neutrophil elastase have also been reported to function as activators (13–15). Active TAFI (TAFIA) has anti-fibrinolytic properties as the enzyme removes carboxyl-terminal lysine residues from fibrin, thereby attenuating accelerated plasmin formation. Apart from its role in regulating fibrinolysis, TAFI can also modulate inflammatory responses. For instance, TAFIA has been shown to inactivate the anaphylatoxins C3a and C5a by removing their carboxyl-terminal arginine residues (16), and

2 The abbreviations used are: TAFI, thrombin-activatable fibrinolysis inhibitor; CFU, colony-forming unit; PAB, peptostreptococcal albumin binding; PBS, phosphate-buffered saline; ScI, streptococcal collagen-like surface protein.
Binding of TAFI to SclA and SclB from S. pyogenes

it has been suggested that TAFI is involved in the conversion of the fibrinopeptide B (FPB), a potent neutrophil chemoattractant, to its inactive metabolite des-Arg FPB (17). In another study, it was found that the removal of the carboxy-terminal arginine from thrombin-cleaved osteopontin by TAFI impairs the adhesive function of the protein (18). Moreover, TAFI also has pro-inflammatory properties, because it can convert bradykinin into des-Arg⁹bradykinin, a selective agonist of the kinin B₁ receptor, which has an important role in chronic inflammatory processes (16, 19). However, the pathophysiological effects of these modifications are not completely understood.

The present study was undertaken to investigate the interaction between TAFI and S. pyogenes. Here we find that streptococci of serotype 41 are able to bind TAFI and that activation of TAFI can occur on the bacterial surface, which might be an important mechanism for bacteria to modulate inflammatory reactions of the host.

EXPERIMENTAL PROCEDURES

Reagents—TAFI and PAB were produced and purified as described elsewhere (20, 21). Plasminogen and plasmin were purchased from Sigma, and thrombin was from Chemicon, respectively. Rabbit lung thrombomodulin was purchased from American Diagnostica (Greenwich, CT), hirudin-arginine and H-o-Phe-Pro-Arg-chloromethylketone from Bachem (Bubendorf, Switzerland), and potato carboxypeptidase inhibitor from Calbiochem (La Jolla, CA). For the TAFI activity assays, thrombin was used that was a generous gift from Dr. W. Kiesiel (University of New Mexico, Albuquerque, NM), while plasmin and t-PA (actilyse) were purchased from Roche Applied Science.

Bacteria and Culturing Conditions—S. pyogenes AP strains, obtained from the Institute of Hygiene and Epidemiology (Prague, Czech Republic), were grown in Todd-Hewitt broth (BD Biosciences) in 5% CO₂ at 37 °C. To express SclA and SclB recombinantly, the Escherichia coli strain BL21 Star (DE3) was used. Bacteria were grown in Luria Bertani broth (1% (w/v) tryptone (BD Biosciences), 0.5% (w/v) yeast extract (Oxoid Ltd., Basingstoke, Hampshire, UK), and 1% (w/v) NaCl (Merck), supplemented with 1% (w/v) glucose (Prolabo, Fontenay sous bois Cedex, France) and kanamycin 50 µg/ml (Sigma).

Radiolabeling—Proteins were labeled with ¹²⁵I with Iodo-Beads (Pierce) according to the manufacturer’s instructions.

Binding Assays—Overnight cultures of bacteria were washed three times in PBS with 0.02% sodium-azide and 0.05% Tween, adjusted to 0.02–2 × 10⁷ CFU/ml, and incubated with 40,000 cpm of ¹²⁵I-labeled TAFI, plasmin, or thrombin for 1 h at room temperature. Bacteria were washed once in PBS, 0.02% sodium-azide and 0.05% Tween, and the radioactivity of the cell pellets was detected with a γ counter (PerkinElmer). For competition experiments, AP41 bacteria (1 × 10⁷ CFU/ml) were incubated with 50,000 cpm of ¹²⁵I-TAFI in the presence or absence of 40 µg/ml rSclA/AM/P41, rSclB/AP/M41, or protein PAB. Alternatively, bacteria were incubated with 50,000 cpm of ¹²⁵I-plasmin or ¹²⁵I-thrombin in the absence or presence of 12.5–100 µg/ml of unlabeled plasmin or 100–800 µg/ml of unlabeled thrombin, respectively. Bacteria were washed, and bound ¹²⁵I-labeled ligand was determined as above.

Protein Sequence Analysis—TAFI was coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech, Sollentuna, Sweden) according to the protocol provided by the manufacturer. Surface proteins of S. pyogenes AP41 were removed by cleavage with CNBr as described (22). Bacteria were washed and resuspended in PBS (2 ml of PBS/g bacteria, wet weight) and mixed with an equal volume of CNBr 30 mg/ml in 0.2 M HCl. After incubation for 15 h on rotation at room temperature, cells were pelleted by centrifugation. The supernatant was filtered, dialyzed against 0.1 M HCl, neutralized with 1.5 M Tris-HCl, pH 8.8, and finally incubated with TAFI-coupled Sepharose at 4 °C overnight. After extensive washing, bound proteins were eluted with 0.1 M glycine-HCl, pH 2.0. Eluted fractions were examined by SDS-PAGE, and protein bands were excised and sent to Eurosequence (Groningen, The Netherlands) for identification by internal sequencing. The peptide sequences generated by internal sequencing were matched against streptococcal proteins in a BLAST search.

PCR and Sequencing—Genomic DNA was obtained by boiling bacteria in H₂O for 5 min. PCR was performed in a Mastercycler (Eppendorf) using Taq polymerase and buffers from Saveen & Werner AB (Malmö, Sweden). dNTPs were from Fermentas (Burlington, Ontario, Canada). Sequencing was performed using BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol.

Primers with the following sequences were used: SclA forward, CAA CAT ATG TTG ACA TCA AAC AGC, SclA reverse, GCT CTC AAG TTG CTG GTA AAC GTC; SclB forward, CAC AAA AAA GAA CCT ATG AAC CAT TC, SclB forward, CTT ATT GAG TTA AAT ATG TTT G, and SclB reverse, GAT TGG ATC CTT AGC CTG TTG CTC GC.

Recombinant Protein Expression—SclA and SclB from the AP41 strain were produced and purified as described with the following primers: SclA forward, CAC CGA TAT CTG GGA CCA GGA GC, SclA reverse, GCT CTC ATG TTG CTG GAA GAT GTG; SclB forward, CAC CGA TGG CGA AGG TAT CAA AAA G, and SclB reverse, GTT TCT CAT GTT GCT GGC AAT TG. The PCR products were cloned into the pET200 TOPO vector (Invitrogen), resulting in His-tagged fusion proteins. The vector was amplified in E. coli TOP 10 cells and then transformed into E. coli of the BL21 Star (DE3) strain for protein expression.

Recombinant proteins were purified on nickel-nitrilotriacetic acid His-Bind-Sepharose (Novagen, Madison, WI). Recombinant SclA from the M41 strain MGAS6183 and rSclB from the M28 strain MGAS6274 were produced and purified as described previously (23).

Slot Blot Analysis—Different amounts of recombinant SclA and B from different strains were applied to the wells of a MikliBlot (Millipore Corp., Billerica, MA) and transferred to a methanol-activated Immobulone Transfer Membrane (Millipore) by gentle suction. After blocking in VBS buffer (140 mM NaCl, 5 mM sodium-5.5-diethylbarbiturate, pH 7.35) supplemented with 0.25% (w/v) gelatin and 0.25% (v/v) Tween 20 for 4 × 10 min, the membrane was incubated with ¹²⁵I-labeled TAFI (2 × 10⁶ cpm/ml) in VBS with 0.1% (w/v) gelatin overnight at 4 °C. The membrane was then washed in PBS 0.25%
were added to a premix of thrombin (16 nm) and thrombomodulin (32 nm) in the presence of CaCl₂ (5 mM), in 20 mM Hepes, pH 8.0/0.1%, bovine serum albumin. This mixture was incubated for 1 min at 37 °C before the thrombin activity was stopped by adding H-D-Phe-Pro-Arg-chloromethylketone, while the mixture remained at 37 °C to allow spontaneous TAFIa inactivation. At several time points, 20-μl samples were withdrawn and added to 50 μl of the substrate hippuryl-arginine (21.4 mM). After 30 min of incubation with the substrate at 37 °C, substrate conversion was stopped by adding 50 μl of 1 M HCl. Then, o-methyl hippuric acid (1.7 μM) was added as an internal standard, both o-methyl hippuric acid and hippuric acid were extracted using a Solid Phase Extraction unit according to the supplier’s recommendation (Waters Oasis, Wexford, Ireland), and samples were analyzed by high pressure liquid chromatography as described (25). The TAFIa activity is expressed in units/liter. One unit of enzyme activity was defined as the amount of enzyme required to hydrolyze 1 μmol of substrate/min at 37 °C under the conditions described (25).

Influence of Scls on TAFI Activation—The assay was done as described (26) with minor modifications. In a microtiter plate, 40 μl of reaction mixture, 20 μl of TAFI (100 nm), and 20 μl of rScIA/AP/M41 or rScIB/AP/M41 (0–0.45 μM) were mixed and prewarmed to 37 °C. The reaction mixture was composed of P-enolpyruvate (2 mM) (all concentrations are final concentrations in the assay), ATP (2.7 mM), NADH (0.5 mM), arginine kinase (21 units/liter), pyruvate kinase/dehydrogenase, and hippuryl-arginine (5 mM). The activation mixture contained thrombin (8 nm), thrombomodulin (16 nm), and CaCl₂ (5 mM) or plasmin (0.36 units/ml). Volumes were adjusted with 20 mM Hepes, pH 7.4, 0.01% Tween 20. The reactions were started by adding 20 μl of TAFI (100 nm). TAFI activation was followed over time as a loss of NADH absorbance at 340 nm in a Thermomax microplate reader (Molecular Devices Corp., Menlo Park, CA).

Clot-Lysis Assay—The clot-lysis assay was performed essentially as described previously (27). Briefly, 47 μl of citrated, pooled, human plasma (pooled normal plasma) was mixed with various concentrations of TAFI and rScIA/AP/M41 or rScIB/AP/M41 (0–0.45 μM) (all concentrations are final concentrations in the assay) in a 96-well microtiter plate. The experiments were done in the presence or absence of carboxypeptidase inhibitor (12 μM). The volumes were adjusted to 65 μl with HBS (25 mM Hepes, 137 mM NaCl, 3.5 mM KCl, pH 7.4) containing 0.1% (w/v) bovine serum albumin. A mixture (35 μl) of a thousand-fold dilution of Innovin, CaCl₂ (17 mM), and recombinant tissue-type plasminogen activator (actilysé, 0.3 ng/ml) in HBS/0.1% (w/v) bovine serum albumin, was added to the plasma and turbidity was measured in time at 37 °C at 405 nm in a Thermomax microplate reader (Molecular Devices). The clot-lysis time was defined as the time difference between half-maximal lysis and half-maximal clotting.

RESULTS

TAFI Binds to Collagen-like Surface Proteins from S. pyogenes—To investigate the interaction between TAFI and streptococci, the binding of 125I-TAFI to twelve different streptococcal sero-
Binding of TAFI to SclA and SclB from S. pyogenes

FIGURE 1. Binding of TAFI to S. pyogenes. Twelve different strains of S. pyogenes, 2 × 10⁷ CFU/ml, were incubated with ¹²⁵I-TAFI for 1 h at room temperature. Cells were washed, and the amount of radiolabeled protein that remained bound to the bacteria was determined in a γ counter.

FIGURE 2. Isolation of a TAFI-binding protein from S. pyogenes. A, surface proteins from the AP41 strain of S. pyogenes were solubilized with CNBr as described under “Experimental Procedures” and incubated with TAFI-coupled Sepharose. After washing the Sepharose with PBS, bound proteins were eluted and analyzed on SDS-PAGE. The figure shows a Coomassie-stained SDS-PAGE gel with solubilized surface proteins from AP41 (lane 1) and eluted proteins from the TAFI-coupled Sepharose (lane 2). The arrow indicates the protein band that was subjected to internal sequencing. B, SclA and SclB from the AP41 strain were cloned and expressed in E. coli and analyzed by SDS-PAGE followed by Coomassie staining.

Table 1: Internal sequence analysis of TAFI-binding protein

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>Data base match*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal peptide 1</td>
<td>DTGAQGPVGPQ</td>
</tr>
<tr>
<td>SclA from S. pyogenes (serotype M41)</td>
<td>ETGAQGPVGPQ (positions 159–169)</td>
</tr>
<tr>
<td>Internal peptide 2</td>
<td>G3GLPLGLPG</td>
</tr>
<tr>
<td>SclA from S. pyogenes (serotype M41)</td>
<td>GLTGGLPLPG (positions 134–143)</td>
</tr>
</tbody>
</table>

* Solubilized surface proteins from AP41 bacteria were incubated with TAFI-coupled Sepharose, followed by extensive washing and elution of bound proteins. A 35-kDa protein was identified and analyzed by internal sequencing. Two peptide sequences were generated and matched against streptococcal proteins in a BLAST search.

shown that the extracellular domains of the two proteins differ significantly from each other and also vary within serotypes, we decided to sequence both genes from the AP41 strain. As expected, the results show that the deduced nucleotide sequences of the two peptides (Table 1) were found in SclA, but not in SclB.

To study the interaction between Scls and TAFI at the protein level, the extracellular part of SclA (rSclA/AP/M41) and SclB (rSclB/AP/M41) from the AP41 strain were recombinantly expressed in E. coli and purified as described under “Experimental Procedures.” Fig. 2B shows that the apparent molecular masses of the two proteins were slightly higher than expected, which was also reported when recombinantly expressed Scls from other serotypes were examined by SDS electrophoresis. Binding of TAFI to purified Scls was studied using the BIAcore biosensor system. Analysis of the binding curves (Fig. 3, A and B) showed that the interactions did not follow a one-to-one stoichiometry model. The $K_D$ for steady state binding of TAFI to SclA was 215 ± 31 nM and to SclB 253 ± 37 nM. For further binding studies, rSclA/AP/M41 and rSclB/AP/M41 were immobilized on a polyvinylidene difluoride membrane and probed with $^{125}$I-TAFI. Moreover, SclA from the M41 strain 6183 (rSclA/176/M41) and the M28 strain 6274 (rSclA/161/M28) as well as SclB from that M28 strain (rSclB/163/M28) were also applied to the membrane. After a washing step, bound $^{125}$I-TAFI was detected by exposing the membrane to an x-ray film. Fig. 3C shows that rSclA/AP/M41 binds more efficiently to TAFI than rSclB/AP/M41. Interestingly, rSclA/176/M41 derived from another M41 strain bound TAFI to a similar degree as rSclA/AP/M41, whereas rSclA/161/M28 and rSclB/163/M28 showed no interaction. The latter observation is in accordance with the finding that streptococci of the AP28 strain displayed only poor TAFI binding properties (Fig. 1). To further establish the role of Scls in the binding of TAFI to S. pyogenes, AP41 bacteria were incubated with $^{125}$I-TAFI in the absence or presence of recombinant Scls. As a control, the surface protein PAB from Finegildia magna was used. PAB had no influence on TAFI binding, whereas rSclA/AP/M41 and rSclB/AP/M41 reduced TAFI binding 84 and 42%, respectively (Fig. 3D). No additional effect was seen with both proteins in combination (data not shown).

To visualize the interaction of rSclA/AP/M41 and rSclB/AP/M41 with TAFI, negative staining and transmission electron microscopy were employed. Fig. 4, A and C, shows that rSclA/AP/M41 displayed a characteristic lollipop-like structure with a globular head and a tail as has also been described for other Scls (9, 23), whereas TAFI had a horseshoe-like shape (Fig. 4,
and E). When incubating TAFI with rSclA/AP/M41, the carboxypeptidase was frequently (>95%) localized close to the heads of SclA (Fig. 4, D and F), and this was also found when the interaction between rSclB/AP/M41 and TAFI was investigated (data not shown). Interestingly, similar findings were recently reported when the binding of ApoB100 to SclA was analyzed (9).

*S. pyogenes Strain AP41 Binds Plasmin and Thrombin to its Surface*—Under physiological conditions, TAFI can be activated by plasmin or the thrombin-thrombomodulin complex (12, 13). The binding of plasmin/ogen to various streptococcal strains, including the AP41 strain (28), is well established and has been described earlier by many other groups (29–31). However, the interaction between Streptococci and (pro)thrombin has been less investigated, and to our knowledge all binding studies conducted so far showed that (pro)thrombin had no affinity for the streptococcal strains tested (31, 32). Thus, to investigate whether activation of TAFI at the bacterial surface can be induced by recruited plasmin or thrombin-thrombomodulin, we studied the interaction of 125I-plasmin or 125I-thrombin with AP41 bacteria. Fig. 5A shows that 125I-plasmin binds avidly to the bacteria. In addition, an interaction with 125I-thrombin was observed. In both cases the binding was displaceable by the addition of unlabeled plasmin and thrombin, respectively (Fig. 5, B and C). 50% displacement occurred at concentrations around 30 μg/ml for plasmin and 300 μg/ml for thrombin, which would correlate to $K_D$ values of ~0.3 and 8 μM, respectively. Taken together, the results demonstrate that not only TAFI, but also two TAFI activators, namely plasmin and thrombin, bind to the surface of AP41 bacteria.

*TAFI Is Cleaved by Plasmin and Thrombin-Thrombomodulin at the Bacterial Surface*—TAFI activation by plasmin or the thrombin-thrombomodulin complex occurs by the removal of a 19-kDa activation peptide from the amino-terminal part of the protein. The resulting 36-kDa active carboxypeptidase (TAFIa) has a very short half-life (~5 min) because of a conformational reorganization that drives the enzyme into its inactive form (TAFIai). Further processing by plasmin or the thrombin-thrombomodulin complex leads then to the generation of smaller inactive fragments (13, 20). To analyze whether streptococcal-bound TAFI can be cleaved by a similar mechanism as described above, AP41 bacteria were incubated with 125I-TAFI in the presence or absence of 40 μg/ml SclA, SclB, or protein PAB. Statistical analysis (n = 3) was performed using Student’s t-test (un-paired, two-tailed). ***p < 0.001.
or thrombin-thrombomodulin in the absence of bacteria. Fig. 6, A and B, shows that treatment of bacteria-bound TAFI with plasmin or thrombin-thrombomodulin leads to the generation of a 36-kDa fragment that was also seen when TAFI was cleaved in solution. To verify that cleavage occurs at the bacterial surface, AP41 bacteria were preincubated with plasmin or thrombin-thrombomodulin. Unbound proteins were removed by a washing step, and bacteria were incubated with 125I-TAFI for 30 min (plasmin) and 15 min (thrombin-thrombomodulin). Thereafter, 125I-TAFI that was recovered from the bacterial surface or remained in solution was analyzed by SDS-PAGE. Fig. 6C shows that TAFI processing occurred at the bacterial surface, but not in solution. These findings implicate that bacteria-bound TAFI is susceptible to cleavage by its two most important activators.

The Physiologic Activity of TAFI Is Not Inhibited by SclA or SclB — To investigate the influence of rScls on the activation profile of TAFI, TAFI activation by thrombin-thrombomodulin or plasmin was followed over time. At the concentrations tested (0–0.45 μM), neither rSclA/AP/M41 nor rSclB/AP/M41 had any influence on the activation profile (data not shown). In addition, the TAFIa half-life was not changed by the presence of rScls (0–0.18 μM) (data not shown). Based on these findings we next wanted to investigate whether the rScls affected the fibrinolytic action of TAFI. To this end, the effect of rScls was studied in a clot-lysis test. In these assays, clotting of normal pooled plasma supplemented with various concentrations of rSclA/AP/M41 or rSclB/AP/M41 was initiated by tissue factor, and fibrinolysis was initiated by recombinant tissue plasminogen activator. Clot-lysis times were determined in the presence or absence of a carboxypeptidase inhibitor to visualize the TAFI-dependent prolongation. The results show that the clot-lysis times of plasma were not influenced by the presence of rScls (Fig. 7). Taken together, these experiments demonstrate that binding of TAFI to rSclA/AP/M41 and rSclB/AP/M41 does not affect TAFI activation, TAFIa stability, or the anti-fibrinolytic potential of TAFI, which may point to an important function of bacteria-bound TAFI in the regulation of the inflammatory host response.

DISCUSSION

Over the years, a number of host-pathogen interactions have been described to be triggered by proteolytic-driven cascades (33, 34). Based on the intensity of activation, these systems can either clear an infection or promote pathological inflammatory reactions (35). Typical examples are complement, coagulation, and fibrinolysis, which are normally involved in host defense during infection but can also cause deleterious and sometimes life-threatening complications once they are activated in a systemic manner. Thus, the study of these systems in relation to their activation by invading pathogens may lead to a better understanding of how a normally harmless infection can evoke serious conditions in the human host. For instance, many bacterial pathogens have been shown to utilize the proteolytic systems of the host to establish an infection. Probably the best studied human proteinase in this respect is plasminogen, which apart from S. pyogenes also interacts with many other bacterial
species, including *Borrelia burgdorferi*, *E. coli*, *Salmonella enteritidis*, *Yersinia pestis*, and the *Neisseria* species gonorrhoeae and meningitides (34). Activation of plasminogen is a critical step in the progression of an infection, and several modes of actions for different bacteria have been described.

**FIGURE 5.** AP41 bacteria bind plasmin and thrombin. A, serial dilutions of AP41 bacteria were incubated with $^{125}$I-plasmin (black line) or $^{125}$I-thrombin (gray line) for 1 h, followed by a washing step to remove unbound ligand. Bacteria were centrifuged, and the amount of radiolabeled protein in the cell pellet was determined with a $\gamma$ counter. The figure shows the means and S.D. of three independently performed experiments. B and C, AP41 bacteria were incubated for 1 h with $^{125}$I-plasmin (B) or $^{125}$I-thrombin (C) in the presence of varying concentrations of the corresponding unlabelled ligand. Cells were washed, and the radioactivity of the cell pellets was thereafter determined as described above. The figure shows a representative experiment out of four, each done in duplicate.
While species such as *S. pyogenes* and *Y. pestis* express plasminogen-activating proteins (streptokinase and protein Pla, respectively), others such as *B. burgdorferi*, *Salmonella enteritidis*, and *E. coli* recruit tissue plasminogen activator or urokinase-type plasminogen activator from the host for this purpose (34). Importantly, studies with *S. pyogenes*, *B. burgdorferi*, and *S. aureus* have demonstrated that plasm, mobilized to the bacterial surface, is protected from inhibition by host proteinase inhibitors, in particular α₂-antiplasmin, resulting in a long-lasting surface-associated enzymatic activity (29, 36, 37). The binding of plasminogen to the streptococcal surface is well documented and involves M and M-like proteins (30, 38), α-enolase (39), and glyceraldehyde-3-phosphate dehydrogenase (40). Whereas some M proteins bind plasminogen indirectly via fibrinogen (38), other strains express an M-like protein called PAM (plasminogen-binding group A streptococcal M-like protein) that binds plasminogen directly with high affinity (30). Notably, the strains that displayed the highest TAFI binding (M type 41 and 53) were also PAM-expressing strains (28). Bacterial interactions with pro/thrombin, on the other hand, are less investigated, but it is well established that *S. aureus* secretes a staphylococcalase that interacts with thrombin without proteolysis (41). Both plasmin and thrombin have in common that they are important physiologic activators of TAFI and, of note for the present study, they assemble in their active forms at the surface of AP41 bacteria. In the present study, we described for the first time that TAFI binds to *S. pyogenes* by interacting with the streptococcal collagen-like surface proteins ScIA and ScIB. Importantly, bacteria do not have the machinery to activate surface-bound TAFI, and they therefore have to recruit natural activators of TAFI. The finding that TAFI is assembled with its activators plasmin and thrombin at the streptococcal surface implies that potentially high concentrations of activated TAFI may be obtained locally at the site of infection. They also suggest that TAFI activation can be regulated by bacteria via, for instance, streptokinase-activated, bacteria-bound plasmin. Activated TAFI is mostly known as an inhibitor of fibrinolysis. Notably, previous studies have shown that *S. pyogenes* bacteria adhering to epithelial cells are coated with a fibrin network (32). It can therefore be speculated that the blood clot forms a shield that protects the bacterium from the immune defense systems of the host and that the recruitment and activation of TAFI on the bacterial surface would help stabilize the clot. Moreover, activation of complement, coagulation, and fibrinolysis occurs by limited proteolysis and often involves the cleavage of an Arg-Ile or a Lys-Ile bond. Thus, many peptides involved in these processes, such as the anaphylatoxins C3a and C5a, bradykinin and kallidin, as well as fibrinopeptide B have an arginine at their carboxyl terminus. As the generation of these peptides is induced in response to infection, it is tempting to speculate that bacteria-bound TAFI is used as a tool to modulate the host response. Taken together, the present study shows that TAFI is bound to AP41 bacteria via ScIA and ScIB and can be converted into its active fragment by recruited plasmin and thrombin.

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Binding of TAFI to SclA and SclB from S. pyogenes