Glyceraldehyde-3-Phosphate Dehydrogenase of *Streptococcus pneumoniae* Is a Surface-Displayed Plasminogen-Binding Protein

Simone Bergmann,1 Manfred Rohde,2 and Sven Hammerschmidt1*

Research Center for Infectious Diseases, University of Würzburg, Würzburg,1 and GBF-German Research Centre for Biotechnology, Braunschweig,2 Germany

Received 18 August 2003/Returned for modification 7 November 2003/Accepted 22 December 2003

The recruitment of plasminogen endows the bacterial cell surface of *Streptococcus pneumoniae* with proteolytic activity. In this study we demonstrate specific plasmin- and plasminogen-binding activity for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is located in the cytoplasm as well as on the surface of pneumococci. GAPDH exhibits a high affinity for plasmin and a significantly lower affinity for plasminogen.

A prerequisite for the invasiveness of a pathogen is the pathogen’s ability to breach epithelial as well as endothelial barriers in order to gain access to the submucosa and blood. A successful strategy used by pathogenic bacteria to degrade the extracellular matrix and to promote invasiveness is the recruitment of proteolytic activity to the bacterial cell surface (19, 21). *Streptococcus pneumoniae*, a common etiologic agent of respiratory tract diseases and life-threatening invasive diseases, is able to capture plasminogen on the bacterial cell surface. Subsequent activation by tissue-type and urokinase-type plasminogen activators allows the bacteria to acquire surface-associated proteolytic activity (10, 17). Plasminogen, a glycoprotein, is the zymogen of the serine protease plasmin, which is a key enzyme of the fibrinolytic pathway (7). The recruitment of plasminogen endows the bacterial cell surface of *Streptococcus pneumoniae* with proteolytic activity. In this study we demonstrate specific plasmin- and plasminogen-binding activity for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is located in the cytoplasm as well as on the surface of pneumococci. GAPDH exhibits a high affinity for plasmin and a significantly lower affinity for plasminogen.

A prerequisite for the invasiveness of a pathogen is the pathogen’s ability to breach epithelial as well as endothelial barriers in order to gain access to the submucosa and blood. A successful strategy used by pathogenic bacteria to degrade the extracellular matrix and to promote invasiveness is the recruitment of proteolytic activity to the bacterial cell surface (19, 21). *Streptococcus pneumoniae*, a common etiologic agent of respiratory tract diseases and life-threatening invasive diseases, is able to capture plasminogen on the bacterial cell surface. Subsequent activation by tissue-type and urokinase-type plasminogen activators allows the bacteria to acquire surface-associated proteolytic activity (10, 17). Plasminogen, a glycoprotein, is the zymogen of the serine protease plasmin, which is a key enzyme of the fibrinolytic pathway (7). The recruitment of plasminogen endows the bacterial cell surface of *Streptococcus pneumoniae* with proteolytic activity. In this study we demonstrate specific plasmin- and plasminogen-binding activity for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is located in the cytoplasm as well as on the surface of pneumococci. GAPDH exhibits a high affinity for plasmin and a significantly lower affinity for plasminogen.
Streptococcus dysgalactiae, and Streptococcus mutans (Table 1). Interestingly, the GAPDHs of streptococci of groups A, C, and G were recently identified as extracellular targets for a broad spectrum of extracellular matrix proteins and especially for plasmin and plasminogen (20, 22).

In order to demonstrate the plasminogen-binding activity of the pneumococcal GAPDH, total proteins of the serotype 2 strain and the serotype 2 enol internal deletion (eno<sup>int/del</sup>) mutant were incubated with immobilized plasminogen. The mutant expresses an α-enolase with a deletion of the C-terminal lysyl residues and amino acid substitutions in the internal plasminogen-binding motif of Enol. The Enol<sup>int/del</sup> mutant exhibited substantially reduced plasminogen-binding activity (3) and was used in order to avoid binding of the α-enolase to plasminogen. Plasminogen (obtained from Sigma or provided by K. Preissner, Giessen, Germany) was immobilized on a polyvinylidene fluoride membrane, and GAPDH binding was detected with polyclonal anti-GAPDH antisera (21) followed by horseradish peroxidase-conjugated anti-rabbit antibody. Results demonstrated a concentration-dependent GAPDH-plasminogen interaction, in which the protein-protein interaction depends on both the amount of immobilized plasminogen and the amount of soluble GAPDH (Fig. 2).

In an attempt to visualize the subcellular localization of GAPDH and the binding of plasminogen to GAPDH protein, preembedding, labeling studies were carried out as recently demonstrated for Enol (2). The unencapsulated pneumococcal strain R6x and the encapsulated pneumococcal strains of serotypes 2 (ATCC 11733) and 35A (NCTC 10319) were prelabeled with polyclonal protein A-purified anti-GAPDH immunoglobulin G and 15-nm-diameter gold particles coupled to protein A before embedding. Ultrathin sections revealed that the GAPDH of S. pneumoniae resembles the α-enolase located on the bacterial cell surface of unencapsulated (Fig. 3B) and encapsulated (Fig. 3A and C) strains. These results indicate that the common surface disposition of GAPDH is independent of the state of capsular polysaccharide expression. The surface-located glycolytic enzymes of the Embden-Meyerhof-Parnas pathway and other surface-displayed proteins like PavA of S. pneumoniae (15) and FBP54 of Streptococcus pyogenes (8, 9) lack the classical secretion and anchoring mechanisms (11, 12, 27, 29) and thus constitute a novel class of exported proteins of gram-positive bacteria (5). The conditions and factors required for protein secretion, as well as the mechanism of anchoring these proteins, are not known. How plasminogen is bound to GAPDH or Enol through the capsule also remains unknown.

The dynamics of GAPDH-plasminogen and GAPDH-plasmin complex formation and their dissociation were analyzed by surface plasmon resonance (SPR) technique. GAPDH was covalently immobilized on a BIAcore CM5 sensor chip as previously described (3, 23). The association and dissociation kinetics of Glu-plasminogen and plasmin (Sigma) to GAPDH were evaluated according to the heterogeneous ligand model (A + B1 ↔ AB1; A + B2 ↔ AB2). This revealed two equilibrium constants; for plasminogen, K<sub>D1</sub> was equal to 4.3 × 10<sup>−7</sup> M and K<sub>D2</sub> was equal to 1.6 × 10<sup>−10</sup> M, and for plasmin, K<sub>D1</sub> was equal to 2.8 × 10<sup>−8</sup> M and K<sub>D2</sub> was equal to 5.2 × 10<sup>−8</sup> M. Studies using SPR showed that the pneumococcal GAPDH had a higher affinity for the serine protease plasmin than for its zymogen plasminogen (Fig. 4), as has also been shown previously for the Prl/streptococcal surface dehydrogenase of group A streptococci (20). The differences between

![Image](http://iai.asm.org/)

**FIG. 2.** Binding of pneumococcal GAPDH to plasminogen. Human plasminogen was immobilized in the different amounts indicated. GAPDH was purified under native conditions from the cytosolic compartment of the serotype 2 enol<sup>int/del</sup> mutant pneumococci (ATCC 11733). Rows 1 to 3, binding of GAPDH applied in serial dilutions (1:2).

---

**TABLE 1. Comparison of GAPDH sequences and C-terminus identities among different streptococcal species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>% Identity</th>
<th>C-terminal sequence</th>
<th>Accession no.</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>GapA</td>
<td>100</td>
<td>VRTLEYFAKIAK</td>
<td>AJ505822</td>
<td>This paper</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>Plr/SDH</td>
<td>89.0</td>
<td>VRTLEYFAKIAK</td>
<td>M95569</td>
<td>20, 24</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>GapC</td>
<td>86.4</td>
<td>VRTLEYFAKIAK</td>
<td>AF321899</td>
<td>13</td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>GapC</td>
<td>89.6</td>
<td>VRTLEYFAKIAK</td>
<td>AF375662</td>
<td>EMBL</td>
</tr>
<tr>
<td>S. equisimilis</td>
<td>GapC</td>
<td>85.2</td>
<td>VRTLEYFAKIAK</td>
<td>X97788</td>
<td>14</td>
</tr>
<tr>
<td>S. mutans</td>
<td>GapC</td>
<td>88.7</td>
<td>VRTLEYFAKIAK</td>
<td>AE014883</td>
<td>1</td>
</tr>
</tbody>
</table>

**FIG. 3.** Immunoelectron microscopic visualization of GAPDH on the surface of S. pneumoniae ATCC 11733 (type 2) (A), unencapsulated R6x (B), and S. pneumoniae NCTC 10319 (type 35A) (C). (A to C) GAPDH was visualized on the bacterial cell surfaces of ultrathin sections of preembedding labeled samples by using anti-GAPDH antibodies and protein A–15-nm-diameter gold particle studies. (D) Visualization of unspecific binding of protein A–15-nm-diameter gold particles to type 2 pneumococci.
equilibrium constants are probably the result of conformationally dependent structure recognition by Plr or plasminogen molecule (4). The interaction of GAPDH and plasminogen produced lower equilibrium constants than the Enol-plasminogen dissociation and complex formation did ($K_{D1} = 8.6 \times 10^{-8}$ M; $K_{D2} = 5.5 \times 10^{-10}$ M) in S. pneumoniae (3), indicating a lower affinity of GAPDH for plasminogen. Pathogenic bacteria acquire proteolytic activity by the recruitment and subsequent activation of plasminogen. A recent study indicated that the nonglycolytic property of $\alpha$-enolase contributes to the pathogenesis of pneumococci (3). The concerted action of both $\alpha$-enolase and GAPDH on the bacterial cell surface might, therefore, result in an enhancement of the pathogen’s ability to degrade the extracellular matrix and to invade host tissues.

We are grateful to U. Hentschel and J. Reidl for critical readings of the manuscript. This work was partially supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 587/479, Teilprojekt A6/A7 to S. H.) and the Bundesministerium für Bildung und Forschung (CAPNetz to S. H.).

REFERENCES


