Bacterial plasminogen activators and receptors

Kaarina Lähteenmäki a, Pentti Kuusela b,c, Timo K. Korhonen a,*

a Division of General Microbiology, Department of Biosciences, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland
b Department of Bacteriology and Immunology, The Haartman Institute, University of Helsinki, P.O. Box 21, FIN-00014 Helsinki, Finland
c HUCH Laboratory Diagnostics, Division of Clinical Microbiology, Helsinki University Central Hospital, FIN-00029 Helsinki, Finland

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Abstract

Invasive bacterial pathogens intervene at various stages and by various mechanisms with the mammalian plasminogen/plasmin system. A vast number of pathogens express plasmin(ogen) receptors that immobilize plasmin(ogen) on the bacterial surface, an event that enhances activation of plasminogen by mammalian plasminogen activators. Bacteria also influence secretion of plasminogen activators and their inhibitors from mammalian cells. The prokaryotic plasminogen activators streptokinase and staphylokinase form a complex with plasmin(ogen) and thus enhance plasminogen activation. The Pla surface protease of Yersinia pestis resembles mammalian activators in function and converts plasminogen to plasmin by limited proteolysis. In essence, plasminogen receptors and activators turn bacteria into proteolytic organisms using a host-derived system. In Gram-negative bacteria, the filamentous surface appendages fimbriae and flagella form a major group of plasminogen receptors. In Gram-positive bacteria, surface-bound enzyme molecules as well as M-protein-related structures have been identified as plasminogen receptors, the former receptor type also occurs on mammalian cells. Plasmin is a broad-spectrum serine protease that degrades fibrin and noncollagenous proteins of extracellular matrices and activates latent procollagenases. Consequently, plasmin generated on or activated by Haemophilus influenzae, Salmonella typhimurium, Streptococcus pneumoniae, Y. pestis, and Borrelia burgdorferi has been shown to degrade mammalian extracellular matrices. In a few instances plasminogen activation has been shown to enhance bacterial metastasis in vitro through reconstituted basement membrane or epithelial cell monolayers. In vivo evidence for a role of plasminogen activation in pathogenesis is limited to Y. pestis, Borrelia, and group A streptococci. Bacterial proteases may also directly activate latent procollagenases or inactivate protease inhibitors of human plasma, and thus contribute to tissue damage and bacterial spread across tissue barriers. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Plasminogen; Plasmin; Plasminogen activation; Plasminogen receptor; Bacterial metastasis

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* Corresponding author. Tel.: +358 (9) 19159260; Fax: +358 (9) 19159262.
E-mail address: timo.korhonen@helsinki.¢ (T.K. Korhonen).

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1. Introduction

Proteolytic activity is an important factor in the pathogenesis of bacterial infections, both in the tissue damage associated with these infections and in bacterial invasiveness into secondary infection sites within the host body. Proteolysis associated with bacterial infections may serve several functions. Bacteria such as *Proteus vulgaris*, *Porphyromonas gingivalis*, and *Pseudomonas aeruginosa* secrete proteinases with broad substrate specificity and utilize these for nutritional demands by releasing amino acids or peptides from mammalian tissues, or by increasing vascular permeability, which ensures a supply of nutrients to the site of infection (for reviews on bacterial proteases, see [1,2]).

Proteolysis at the infection site may also provide a suitable environment for growth and multiplication of anaerobic bacteria. Bacterial proteases may be targeted against circulating proteins that are important for our immunological defence, such as antibodies or complement proteins, or for the control of proteolytic activity in our body, i.e. protease inhibitors. Finally, the bacterial protease can activate a precursor of mammalian protease, such as procollagenase. The latter two cases can lead to uncontrolled proteolytic activity at the infection site and to massive tissue damage, and degradation of tissue structures by bacterial proteases may potentiate bacterial spread through tissue barriers.

Within the mammalian body, tissue barriers are mainly formed by extracellular matrices (ECM) and basement membranes (BM), which invasive bacteria must penetrate in order to reach the circulation. ECMs and BMs contain collagens, laminins, fibronectins, proteoglycans and elastin as major constituents. These molecules interact with each other and other tissue and cell components (reviewed in [3]). In general, ECM collagens constitute ca. one-third of the total protein in mammalian organisms. Consequently, degradation of and penetration through the insoluble collagen fibers or networks in the tissues represents a major problem in migration of bacterial or eukaryotic cells. Production of ECM-degrading proteases and of collagenases in particular is, however, limited to a restricted number of bacterial pathogens and infectious diseases (for a recent review on bacterial collagenases, see [4]). Degradation of collagens, elastin, and fibronectin by secreted bacterial proteases leads to massive tissue destruction seen in diseases like corneal keratitis (caused by *Serratia marcescens* and *Pseudomonas aeruginosa*), periodontitis (*Porphyromonas gingivalis*), cystic fibrosis (*P. aeruginosa*), and gangrene (*Clostridium perfringens*) [1].

A number of severe bacterial pathogens produce low levels of proteinases and, in particular, have not been reported to produce collagenases. Such bacteria include most enteric bacteria as well as major agents causing bacterial meningitis: *Neisseria meningitidis, Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli* K1. Some of these bacteria are intracellular pathogens, and their penetration through cellular layers apparently involves an intracellular phase. However, a number of invasive bacteria are extracellular pathogens that obviously must rely on other mechanisms for invasiveness. Pathogenic bacteria are known to interact with proteinase-dependent cascade systems of their hosts, including coagulation, fibrinolysis, complement activation, phagocytosis, and the kallikrein–kinin cascade [1,5,6]. These pathways are tightly regulated by host proteinase activators or inhibitors. Bacteria may activate or inactivate these cascades directly through their proteases or other surface components, or indirectly by causing release of effector molecules from epithelial or endothelial cells or of proteolytic enzymes or their precursors from phagocytic cells. Some of these protease-dependent pathways can be utilized by bacteria to ensure growth or spread within the host.

Due to the high concentration of the serine proteinase precursor plasminogen (Plg) in plasma and the broad proteolytic activity of the enzymatic form plasmin, the mammalian Plg system offers a highly potential proteolytic system that could be utilized by pathogenic bacteria. Plasmin has been proposed to play a role in several physiological processes in mammals (Fig. 1): it is a key enzyme in fibrinolysis, degrades various ECM components, and is involved in activation of certain prohormones and growth factors as well as in tumor cell metastasis (reviewed in [7–9]). Bacteria interact with the Plg system in various ways: they have been found to produce Plg activators (PA) and Plg receptors (PlgR), influence the production of host PAs and their inhibitors, and have an effect on the host plasmin inhibitors. In recent years, understanding of the mechanisms and the role of the Plg system in bacterial infections has increased significantly, and this review summarizes our current knowledge on the molecular mechanisms and pathogenetic functions of bacteria–Plg interactions.

2. The mammalian plasminogen system

2.1. Plasminogen and plasmin

Plg is a 90-kDa plasma proenzyme that is converted to the active serine protease plasmin by proteolytic activation
The mammalian PAs cleave the peptide bond between Arg 560 and Val 561 in the Plg molecule, leaving the heavy and light chains of the formed plasmin molecule joined via two disulfide bonds (Fig. 2). The 65-kDa heavy chain contains five disulfide-bonded triple-loop kringle structures, and the 25-kDa light chain harbors the active site with His 602, Asp 645 and Ser 740 forming the characteristic catalytic triad of serine proteases. Plasmin is a broad-spectrum protease that preferably cleaves peptide bonds next to lysine or arginine residues [10,11]. The native form of Plg contains 791 amino acids and is called Glu-Plg because it has an amino-terminal glutamic acid residue. Glu-Plg can be modified by digestion with plasmin between the residues Arg67-Met68, Lys 76-Lys77, or Lys 77-Val78, thereby releasing an 8-kDa activation peptide (see Fig. 2). The truncated forms of the zymogen are designated Lys-Plg. The conformation of Lys-Plg enables stronger interaction with receptor and target molecules of Plg and also facilitates conversion to plasmin [12]. The three-dimensional structure of the full-length Plg has not been reported yet, but the crystal structures of the kringles as well as of microplasminogen and microplasmin, truncated forms that consist of a 20 amino acid long fragment of the heavy chain connected to the light chain by two disulfide bridges, have been resolved ([13–18], reviewed in [11]; see Fig. 2). The catalytic domain of plasmin is a compact module that can recruit adapter molecules, or cofactors, such as the bacterial PAs streptokinase and staphylokinase, which modify the substrate presentation to the enzyme and modulate its specificity [11]. The structural changes in the catalytic domain that are associated with the activation of Plg have recently been resolved by crystallography [18].

2.2. Control of the plasminogen system: activators and inhibitors

Plg circulates in the body in large amounts: in adult human plasma the concentration is 180–200 μg ml⁻¹ (ca. 2 μM) [8]. It is therefore understandable that Plg activation must be tightly regulated. This is achieved by specific PAs, by inhibitors that control the Plg system both at the level of Plg activation and plasmin (Fig. 1), and by immobilization of Plg to cellular receptors or to target molecules. Binding of Plg to PlgRs is usually inhibited by lysine or lysine analogs, such as ε-aminocaproic acid and tranexamic acid, indicating that the binding is mediated by lysine-binding sites in Plg [19]. Binding to a lysine analog leads to a dramatic conformational change in the Plg molecule: the closed molecule opens to an extended flexible conformation, which renders it more susceptible to cleavage by PAs [20].

Mammalians have two PAs, tissue-type plasminogen activator (tPA) and urokinase (uPA), which both are serine proteases that are secreted in a single-chain form (sctPA; scuPA or pro-uPA) and processed proteolytically...
into a fully active two-chain form (tctPA; tcuPA) (Table 1; for reviews, see [21–23]). The processed molecules consist of an A-chain and a B-chain held together by disulfide bonds. The His, Asp, and Ser residues forming the active site are located in the B-chain in both PAs. Crystal structures of the catalytic domains of tPA and uPA have been resolved [24,25]; their overall structures exhibit the typical serine proteinase fold, with insertion loops around the active site cleft determining their specificity for Plg.

In uPA, the A-chain consists of one kringle structure and a growth factor-like domain, which contains the receptor-binding amino acid sequence [26]. tPA contains two kringle structures, a growth factor-like domain, and an amino-terminal domain that is homologous to the α-brin-binding finger domain in fibronectin. The finger-like domain, together with the second kringle domain, provides tPA with high affinity for fibrin [27,28]. For uPA a specific, high-affinity 55-kDa glycoprotein receptor (uPAR) has been identified in various cell types [29,30].

The main inhibitors of the Plg system belong to the serine protease inhibitor (serpin) group of antiproteases that specifically inhibit catalytic activity of serine proteinases (Table 2; for a review, see [31]). The mode of action of serpins relies on formation of a stable complex between the substrate-like region of the serpin and the active site substrate-binding region of the target enzyme. Consequently, the serpin reactive center loop is cleaved by the protease, resulting in formation of a covalent acyl-enzyme intermediate and distortion of the active site of the enzyme in a way that prevents deacylation but rather traps the inhibitor–enzyme complex [32,33].

The primary PA inhibitors are PAI-1 and PAI-2 (Table 2), and other proteins able to inhibit PAs include protease nexin, a broad-spectrum serpin found in tissues, and PAI-3, which has been isolated from urine. PAI-3 inhibits both tPA and uPA, but with a slower rate than PAI-1 and PAI-2 [23,34]. The main physiological inhibitor of plasmin is the serpin K2-antiplasmin, which forms a complex with plasmin by binding to the lysine-binding kringles 1–3 of plasmin in a rapid, reversible reaction. This is followed by a slower, irreversible reaction in which a covalent bond is formed between the Ser residue in the active site of plasmin and the reactive site of K2-antiplasmin [9,31,35,36]. As the binding of Plg to receptor structures is mediated via the same lysine-binding sites as the binding to K2-antiplasmin, receptor-bound plasmin(ogen) is protected from inhibition by K2-antiplasmin. The most abundant plasmin inhibitor in human plasma is the broad-spectrum proteinase inhibitor α2-macroglobulin (Table 2). However, inhibition

### Table 1
Plasminogen activators

<table>
<thead>
<tr>
<th>Activator</th>
<th>Molecular mass</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA</td>
<td>70 kDa</td>
<td>Produced mainly by endothelial cells. Concentration in plasma 5–10 ng ml⁻¹. Activates Plg by limited proteolysis at the Arg560-Val561 bond. Major circulating activator; main activator in fibrinolysis.</td>
</tr>
<tr>
<td>uPA</td>
<td>50 kDa</td>
<td>Produced by various normal and malignant cells. Concentration in plasma ca. 3.5 ng ml⁻¹ and in urine 200–300 ng ml⁻¹. Activates Plg by limited proteolysis at the Arg560-Val561 bond. Major cell-bound activator (binds to cell surface uPAR). Functions in cell-mediated degradation of extracellular matrix during inflammation, wound healing, tissue remodeling, and tumor metastasis.</td>
</tr>
<tr>
<td>Bacterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptokinase</td>
<td>47 kDa</td>
<td>Secreted by group A, C and G streptococci. Forms a 1:1 complex with Plg, which acts as a PA.</td>
</tr>
<tr>
<td>Staphylokinase</td>
<td>16 kDa</td>
<td>Secreted by lysogenic Staphylococcus aureus. Forms a 1:1 complex with plasmin(ogen). Only the complex with plasmin acts as a PA. Activates preferably fibrin-bound Plg.</td>
</tr>
<tr>
<td>Pla</td>
<td>35 kDa</td>
<td>Surface-bound protease of Yersinia pestis. Activates Plg by cleaving the same Arg560-Val561 bond in Plg as do tPA and uPA.</td>
</tr>
<tr>
<td>PauA</td>
<td>Dimer of 29-kDa subunits</td>
<td>Secreted by Streptococcus uberis. Mechanism for Plg activation not known yet.</td>
</tr>
</tbody>
</table>

### Table 2
Main inhibitors of the Plg system

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Molecular mass</th>
<th>Principal target</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1</td>
<td>52 kDa</td>
<td>tPA, uPA</td>
<td>Produced by endothelial and various other cells.</td>
</tr>
<tr>
<td>PAI-2</td>
<td>60 kDa</td>
<td>uPA</td>
<td>Normal plasma concentration 10–30 ng ml⁻¹. Found in monocytes and macrophages, detected in plasma only during pregnancy.</td>
</tr>
<tr>
<td>α2-Antiplasmin</td>
<td>70 kDa</td>
<td>Soluble plasmin (receptor-bound plasmin is protected from inhibition).</td>
<td>Concentration in plasma 60–70 μg ml⁻¹.</td>
</tr>
<tr>
<td>α2-Macroglobulin</td>
<td>725 kDa</td>
<td>Plasmin and various other serine proteases, various thiol, carboxyl, and metalloproteinases.</td>
<td>Concentration in plasma 2.5 mg ml⁻¹.</td>
</tr>
</tbody>
</table>
of plasmin by α2-macroglobulin begins only if the local or systemic concentration of α2-antiplasmin is significantly decreased [7,31].

2.2.1. Mammalian plasminogen receptors and plasmin target molecules

Activators and inhibitors precisely control plasmin formation in the body, and via PlgRs plasmin activity is directed to locations where proteolytic activity is required. Various mammalian cells have receptors for Plg on their surface (reviewed in [19,37]). The best-characterized cellular PlgRs are the glycolytic enzyme α-enolase [38,39], the calcium- and phospholipid-binding protein annexin II [40,41], and amphoterin, which was originally characterized by its affinity to heparin [42,43]. Amphoterin is found in large amounts in malignant cells, and it contributes effectively to their migration [44]. The lysine-binding sites of Plg, which reside in the kringle structures, recognize particularly well carboxy-terminal lysine residues [19]. In α-enolase and annexin II, carboxy-terminal lysine residues mediate the interaction with Plg [38,41], whereas amphoterin seems to represent a PlgR without a carboxy-terminal lysine residue [42,43].

Fibrin is a target for binding of both Plg and tPA, as well as for subsequent plasmin-mediated degradation [9]. Upon binding of Plg and tPA to fibrin, Plg activation is accelerated roughly 1000-fold [45]. Likewise, binding of Plg to ECM enhances tPA-mediated Plg activation [46]. Various ECM proteins, including laminin [47], fibronectin [48], vitronectin [49,50], and heparan sulfate proteoglycan [51], bind Plg and are targets for plasmin-mediated degradation. ECM degradation is required during cellular migration in various physiological and pathological processes. Plasmin-mediated degradation of matrix components has been proposed to play a role in migration of inflammatory cells to the site of inflammation [52], in several processes connected to reproduction (reviewed in [7,21,34]), and in neuronal cell death [53]. Production of PAs, as well as expression of PlgRs and uPAR, is characteristic for malignant cells, and abundant evidence suggests that the Plg system is important in tumor cell invasion (for a review, see [54]). Several studies suggest that uPA, in particular, is associated with metastasis [55–57], although certain malignant cell lines have been reported to secrete tPA [58,59]. Development of knock-out mice deficient in Plg, tPA, uPA, uPAR, or PAI-1 [60–64] has provided new tools to analyze the function of each component of the Plg system. In studies of inflammatory responses, wound healing, and tumor invasion, Plg-deficient mice have exhibited reduced cell migration compared to normal mice (reviewed in [65]).

Degradation of the complex ECM structure also requires collagenolytic activity. Type IV collagen is known to immobilize Plg and thus enhance tPA-mediated Plg activation [66], but plasmin is relatively inefficient in the breakdown of collagens [67]. In addition to PAs, invasive cells produce proteinases that can directly degrade collagens and other ECM components [54]. Matrix metalloproteinases (MMPs) are a large group of enzymes, of which interstitial collagenses and type IV collagenases degrade different types of collagens, and stromelysins degrade fibronectin, laminin, elastin, and proteoglycans [54]. The collagenases are secreted in a proenzyme form and require proteolytic cleavage for activation. Plasmin can activate procollagens [68–70] as well as a latent macrophage elastase [71], and thus acts in concert with other proteinase systems in ECM breakdown.

3. Interaction of pathogenic bacteria with the plasminogen system

3.1. Bacterial effects on mammalian plasminogen activators and inhibitors

The concentrations of PAs in plasma are normally small, and the amount of tPA varies considerably in response to various physiological and pathological conditions [23]. Several mammalian cell types secrete tPA or uPA (Table 1). There is limited evidence that their production is affected by bacterial components, and that Plg activation may take place at an early phase of bacterial infection. Production of uPA has been found to be enhanced in human monocytes infected with *Borrelia burgdorferi* [72], and in bovine mammary epithelial cells infected with *Staphylococcus aureus* [73]. A 35-kDa protease of *Bacteroides gingivalis* stimulates PA activity in gingival fibroblasts [74]. In meningococcal septicemia, formation of plasmin is detected in patient plasma at an early phase, but at a later phase the amount of PAI-1 rises suggesting that Plg activation is inhibited [75]. In healthy human volunteers, injection of endotoxin resulted in a rapid rise of plasma tPA level and in formation of plasmin–α2-antiplasmin complexes [76]. This was followed by a slower increase in plasma PAI-1 level with a concomitant decline in tPA activity [76]. Endotoxin also upregulates uPAR: lipopolysaccharide administration in mice increased uPAR mRNA levels in various tissues [77], and in experimental human endotoxemia the expression of uPAR in monocytes is increased [78]. Monocyte uPAR has also been found to be upregulated by intact *B. burgdorferi* cells, by purified *Borrelia* outer surface protein A, and by group A streptococcal lipoteichoic acid [79].

It is not yet known whether the observed changes in PA and PAI levels are directly induced by contact of bacterial components with host cells or whether they are mediated by other host factors that are produced during inflammatory reactions. Bacteria are known to induce secretion of cytokines from epithelial and endothelial cells [80,81]. Some cytokines, such as interleukin-1 (IL-1), increase the permeability of cellular layers, which enables accumulation of fluid and migration of inflammatory cells to the infec-
tion site. It is possible that these inflammatory processes act together with the proteolytic systems. The fimbral filament curli of *E. coli* functions as an ECM-specific adhesin as well as a PlgR (discussed in Section 3.3.3 below) and was found to enhance secretion of IL-6, IL-8 and tumor necrosis factor-α (TNF-α) from human macrophages [82] as well as to bind proinflamatory contact phase proteins and fibrinogen onto the bacterial surface [6]. Plasmin has been reported to release IL-1 from endotoxin-induced monocytes [83]; on the other hand, IL-1 has been proposed to upregulate the Plg system by increasing tPA synthesis and decreasing PAI-1 synthesis in human mesangial cells [84]. IL-1 has also been reported to activate MMPs and thereby promote cartilage degradation in a rabbit model of *Haemophilus influenza* arthritis [85]. These findings indicate a possible interplay between various host responses associated with invasive bacterial infections.

3.2. *Bacterial plasminogen activators*

Several invasive human pathogens have evolved PAs that are either secreted or surface-bound proteins (Table 1). Functionally, the bacterial PAs fall into two groups. Streptokinase (SK) and staphylokinase (SAK) are not enzymes themselves but form 1:1 complexes with Plg and plasmin, leading to changes in conformation and specificity of plasminogen(ogen). In contrast to plasmin alone, the SK–plasmin and SAK–plasmin complexes acquire a remarkable efficiency to activate Plg (for a review, see [11]). SK and SAK share little sequence homology but resemble the mammalian PAs in functional aspects, such as the fibrin dependence of activation by SAK and SK is basically similar but differs in some essential aspects, such as the fibrin dependence of the activation in human plasma. The Pla surface protease of *Yersinia pestis* resembles the mammalian PAs in function and activates Plg by limited proteolysis at the same Arg560-Val561 bond as do tPA and uPA.

3.2.1. *Streptokinase*

SK is a single-chain 414-amino acid protein secreted by β-hemolytic group A, C, and G streptococci. It is an efficient PA which has been associated with the pathogenesis of post-streptococcal glomerulonephritis (PSGN). The overall amino acid sequence identity between the SKs produced by human group A, C and G streptococci is 80–98%. The variable amino acid residues are clustered in two regions designated V1 and V2 (residues 147–218 and 244–264 of the mature protein). Sequence analysis of the V1 coding region in a number of streptococcal isolates has enabled division of the V1 region into seven classes, V1L–V1II, of which classes V1I, V1II and V1V1 are found in SKs of PSGN-associated isolates and the others in non-nephritogenic ones [88]. The structure–function relationships in SK have been reviewed recently [88, 89].

The current view of how the SK-mediated (as well as SAK-mediated) Plg activation takes place is largely based on recent findings from crystallography and complexing of microplasminogen and kringles with SK fragments [11,87,90]. Plg activation is initiated by formation of a binary Plg–SK complex involving interactions between the COOH-terminal domain of SK and the catalytic domain of Plg as well as between another site in SK and the kringle domains of Plg. In the binary complex, the active center of Plg is exposed and functional without hydrolysis of the Arg560-Val561 peptide bond. Formation of the binary Plg–SK complex is not inhibited by lysine, and the proteolytic activity is not regulated by α2-antiplasmin [91,92]. In the next step, another, the so-called substrate Plg molecule binds to the SKα domain in the binary complex to form a ternary Plg–SK–Plg complex and is then converted to plasmin. Formation of the ternary complex is inhibited by lysine analogs, indicating that the kringle residues are involved in the binding of the substrate Plg molecule [90–92]. An 8-kDa fragment is cleaved from the NH2-terminus of SK and the altered SK remains associated with the complex [93]. The activation model [90] depicts that the formed plasmin is released from the ternary complex and does not directly explain how SK activates Plg bound to streptococcal PlgRs via its kringle domains. There is evidence that SK also activates receptor-bound Plg [94,95] and that SK-producing streptococci can acquire surface-bound plasmin after cultivation in human plasma [96,97].

The regions in SK involved in formation of the binary SK–Plg and the ternary Plg–SK–Plg complex have been identified. Deletion analysis revealed that the peptide Ser560-Lys583 in SK is the minimal structure required for formation of the binary complex and that the COOH-terminal portion Ala134-Lys387 is also required for high-affinity binding of SK to Plg [90,98]. Random mutagenesis of SK indicated that the NH2-terminal residues Asp51-His58, as well as Asp220 provide interaction sites for the substrate Plg molecule in formation of the ternary complex [99], which is in agreement with the crystallographic data suggesting that the SK residues Arg55-Gly59 interact with the kringle 5 of the substrate Plg [87].

SKs produced by strains of human and non-human origin differ structurally from each other and activate Plg in a species-specific manner. SKs of equine and porcine group C streptococci (*S. equisimilis*) are 380 and 374 amino acids long, respectively, and display 25% and 35% sequence identity with SK from *S. equisimilis* of human origin [100]. These SKs form the binary Plg–SK complex with Plg from any host studied, and the species specificity in Plg activation seems to depend on the formation of the ternary Plg–SK–Plg complex [100]. The cleavage site in both
human and porcine Plg is the Arg<sub>560</sub>-Val<sub>561</sub> bond; in equine Plg cleavage takes place at the corresponding Arg-Ile bond [100]. In accordance with the species-specific function of the SKs, the non-human streptococci are able to generate surface-associated plasmin activity after cultivation in plasma originating from the homologous host only [94].

3.2.2. Staphylokinkase

SAK is a 136-amino acid protein produced by strains of <i>S. aureus</i> carrying one of the three prophages which contain the sak gene and mediate lyogenic conversion of staphylococci (for a review, see [101]). SAK belongs to the group of staphylococcal proteins whose synthesis takes place during the late exponential growth phase and is positively regulated by <i>agr</i>, the accessory gene regulator [102]. Four SAK variants differing in sequence at four amino acid positions have been identified [103–107]. A few coagulase-negative staphylococci, such as <i>S. lentus</i> and <i>S. lugdunensis</i>, alternatively express either β-hemolysin or SAK. In these strains the sak gene is carried by a converting phage which inactivates the β-hemolysin gene during lysogeny [108].

Plg activation mediated by SAK is complex and differs from SK-mediated Plg activation on some major aspects. (i) The SAK–Plg complex is enzymatically inactive and requires conversion of Plg to plasmin. (ii) Active SAK–plasmin complex is efficiently inactivated by α<sub>2</sub>-antiplasmin. (iii) Binding of α<sub>2</sub>-antiplasmin to the SAK–plasmin complex releases SAK from the complex and makes it ready to interact with other plasmin(o)gen molecules again [109–111]. (iv) SAK primarily activates fibrin-bound Plg [101]. The initial step in the activation involves association of SAK with the trace amounts of plasmin formed as a result of weak spontaneous Plg activation. SAK–plasmin complex formation is favored by the 160-fold higher affinity of SAK to plasmin than to Plg [112]. The observation that SAK primarily activates Plg bound to fibrin without causing systemic Plg activation has raised great enthusiasm to use SAK as a thrombolytic agent to dissolve the fibrin of blood clots (reviewed in [101]).

SAK-producing <i>S. aureus</i> is able to generate surface-associated, inhibitor-protected plasmin activity after cultivation in plasma containing Plg and α<sub>2</sub>-antiplasmin [115]. However, no evidence of SAK as a staphylococcal virulence factor exists so far. No differences were found in the capacity to produce SAK or in the amount of secreted SAK among <i>S. aureus</i> isolates from nasal carriers and from patients suffering from invasive infections such as multiple abscesses or bacteremia (Mölkänen, T. and Kuusela, P., unpublished data).

3.2.3. Y. pestis Pla and the omptin family of surface proteases

<i>Y. pestis</i> Pla is a surface protease responsible for the invasive character of plague [116]. Pla is an outer membrane protein encoded by the 9.5-kb virulence plasmid termed pPCP1, which also contains genes encoding the bacteriocin pesticin as well as the pesticin immunity protein [117]. A <i>pla</i> homolog gene is lacking in <i>Yersinia enterocolitica</i> and <i>Yersinia pseudotuberculosis</i>, whereas <i>E. coli</i> and <i>Salmonella typhimurium</i> carry chromosomally homologs termed <i>ompT</i> and <i>pgtE</i> (also called gene <i>E</i>) [118,119]. Whereas the PA function of Pla is well established, a similar activity in OmpT and PgtE has remained less clear. Originally, <i>E. coli</i> outer membrane preparations were found to express PA activity [120] associated with expression of OmpT. However, at least under laboratory conditions, PA activity is rarely detected in <i>E. coli</i> cells. The majority of the 282 clinical <i>E. coli</i> isolates screened by Lundrigan and Webb [121] harbored the <i>ompT</i> gene, but only one isolate expressed PA activity in a fibrin lysis assay. Similarly, other groups have failed to detect PA activity in <i>E. coli</i> or <i>S. typhimurium</i> cells [117,122,123]. A recombinant <i>E. coli</i> expressing <i>S. typhimurium</i> <i>pgtE</i> in a multicopy plasmid exhibited 1000-fold lower PA activity than did a Pla-positive <i>Y. pestis</i> strain [118], and we detected only very weak PA activity in <i>E. coli</i> cells expressing cloned <i>ompT</i> [124]. Taken together, these results indicate that Pla has much higher PA activity than OmpT. On the other hand, OmpT is likely to have other functions. OmpT
has been shown to be active under extreme denaturing conditions and to have affinity for denatured substrates, and it may have a role in turnover or degradation of membrane proteins in E. coli [125]. OmpT has also been proposed to have a role in complicated urinary tract infections [126] and to degrade antimicrobial peptides in the urine [127]. Recently, Salmonella was reported to gain resistance against antimicrobial peptides by PgtE-mediated cleavage [119].

The predicted amino acid sequences of Pla, OmpT and PgtE are highly similar. They share a total of 41% conserved residues and together with SopA, a homologous protein in Shigella flexneri [128], have been proposed to form a family of closely related serine proteases termed omphtins [129]. The sizes of the mature omphtins vary from 280 to 300 amino acid residues. A β-barrel topology model with 10 transmembrane β-chains and five surface loops was recently proposed for OmpT [130,131]. Kramer and co-workers [131] identified by substitution mutagenesis the surface-exposed residues Ser99 and His212 as active site residues in OmpT. We have found that the corresponding residues in Pla are needed for PA activity, and that the specificity of Pla and OmpT towards different peptide or polypeptide substrates is influenced by residues in the last three predicted surface loops that are somewhat different in Pla and OmpT [124]. Mature Pla (αPla with an apparent molecular mass of 37 kDa) is slowly processed into smaller membrane-bound forms termed βPla (35 kDa) and γPla (31 kDa) [117,132]. βPla is formed by an autoprocessing event at the COOH-terminal surface loop of the Pla molecule [124]. Somewhat surprisingly, prevention of the formation of the β form by mutagenesis does not have a detectable effect on the PA activity of Pla, and the biological significance of the processing remains unknown [124].

3.2.3.1. Biological functions of Pla. Plague is transmitted by fleas that infect both rodents and humans. Y. pestis exhibits remarkably efficient organ invasion during plague infection. It spreads from the subcutaneous site of the flea bite to lymph nodes, where it multiplies to large numbers and causes extensive swelling of the lymph nodes (bubonic plague). Bacteria then invade the circulation, travel to the liver and the spleen, and finally colonize the lungs, causing the most severe form of the disease, pneumatic plague. During the pneumatic phase, direct transmission from human to human may also occur. For a recent review of the multiple virulence factors of Y. pestis, the reader is referred to [133].

The importance of Pla for Y. pestis virulence has been convincingly demonstrated in vivo [116]. When bacteria were injected subcutaneously into mice, the LD50 value of Pla-negative mutant strains was close to 107 bacteria, as compared to an LD50 value of less than 50 bacteria for the isogenic Pla-positive strain. In contrast, when bacteria were injected intravenously, there was no difference between the virulence of the strains, suggesting that Pla specifically enables the dissemination of Y. pestis from the peripheral infection site into the circulation [116]. Although the virulence function of Pla in plague is well established, the mechanisms by which it contributes to the infection have remained elusive. Pla may have multiple functions during infection; the following hypotheses about the role of Pla have been proposed.

1. Kienle and co-workers proposed that Pla mediates adhesion to eukaryotic cells, to glycolipid extracts, and to murine type IV collagen [134]. Later studies showed that Pla binds strongly to laminin and with a weak affinity to heparan sulfate proteoglycan [135]. These adhesive characteristics may serve to localize Y. pestis to ECMs and BMs.

2. Pla-mediated adhesion to ECM and Plg activation may be of great importance in potentiating spread of Y. pestis through tissue barriers. Pla-mediated plasmin formation results in degradation of ECM produced by lung epithelial cells as well as radiolabeled laminin in vitro [135]. These structures are not degraded by Pla directly, indicating that ECM degradation is dependent on the PA activity of Pla [135]. Plasmin generated by Pla may also clear fibrin deposits that could hinder bacterial migration in the circulation [136]. Goguen and co-workers have found in experimental Y. pestis infection that Plg-deficient mice have an approximately 100-fold increase in LD50 compared to normal mice [137], indicating that Plg activation is indeed involved in the pathogenesis of plague.

3. Pla may interfere with the complement system. Soeinde and co-workers [116] found that Pla cleaves the complement component C3. They also found that infectious lesions in mice infected with a wild-type Y. pestis contained fewer inflammatory cells than the lesions in mice infected with an isogenic Pla-negative mutant. They suggested that Pla-mediated cleavage of C3 or another component of the complement system may reduce the production of the chemotactant C5a, and thereby suppress the migration of inflammatory cells to the infection site [116].

4. Pla possesses a weak coagulase activity [117,136]. This activity is hardly detected with human plasma and is normally tested with rabbit plasma [136,138]; it is not known whether it is important in human infection. However, it has been suggested that the coagulase activity may have an effect in the invertebrate host [139].

5. Pla has been reported to degrade Yops [140], Yersinia outer proteins that are essential virulence factors in Yersinia species. The Pla-mediated degradation seems to be specifically addressed to Yops, as other Yersinia proteins are not significantly degraded [140] and E. coli outer membrane proteins are not degraded by expression of Pla [117,135]. However, the function of the degradation is not yet known.
6. We have found that Pla, but not OmpT, inactivates $\alpha_2$-antiplasmin [124]. Inactivation apparently results from cleavage of a single peptide bond in the inhibitor, and is critically affected by the residue Arg211 in Pla. Such inactivation of the major plasmin inhibitor is likely to enhance uncontrolled proteolysis during infection.

3.2.4. Plasminogen activators in other bacteria

*Streptococcus uberis* is an important etiological agent of bovine mastitis, infection of the udder. The bacterium activates bovine, ovine and equine Plg, but does not activate human Plg [141–143]. Plg activation is mediated by PauA, a 251-amino acid protein which shows only low sequence homology to SK [144]. Bovine milk contains Plg, and peptides released from bovine casein by plasmin have been shown to satisfy amino acid requirements of *S. uberis* [145], which suggests a mechanism of how PauA may enhance colonization of the lactating gland.

*Mycobacterium tuberculosis* was recently demonstrated to express several PlgRs as well as a yet unidentified PA [146]. PAs have also been detected in bacteria associated with periodontal disease, which involves destruction of tooth-supporting tissues and reduction of gingival collagen fibers. An 80-kDa trypsin-like protease of *P. gingivalis* has been demonstrated to activate Plg [147]. Interestingly, the protease was also found to degrade several antiproteases, which, together with the plasmin formation, may result in an uncontrolled degradation of periodontal tissue and progression of the disease.

3.3. Bacterial plasminogen receptors

3.3.1. What does a bacterial plasminogen receptor do?

Bacterial PlgRs immobilize Plg on the bacterial surface and thus enhance Plg activation. In essence, bacterial PlgRs function to turn bacteria into proteolytic organisms.

<table>
<thead>
<tr>
<th>Bacterial species reported to express plasmin(ogen) receptors</th>
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<tbody>
<tr>
<td><strong>Bacterial species</strong></td>
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<tr>
<td>Group A, C, and G streptococci</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td><em>Salmonella enteritidis</em></td>
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<tr>
<td><em>Salmonella typhimurium</em></td>
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<tr>
<td><em>Haemophilus influenzae</em></td>
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<tr>
<td><em>Brachysella catarrhalis</em></td>
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<tr>
<td><em>Proteus mirabilis</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td><em>Neisseria meningitidis, N. gonorrhoeae</em></td>
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<tr>
<td><em>N. gonorrhoeae</em></td>
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<tr>
<td><em>Borrelia burgdorferi</em></td>
</tr>
<tr>
<td><em>Borrelia coriaceae, B. garinii, B. parkeri, B. anserina, B. turicatae, B. hermsii</em></td>
</tr>
<tr>
<td><em>Treponema denticola</em></td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
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<tr>
<td><em>Mycoplasma fermentans</em></td>
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<tr>
<td><em>Fusobacterium nucleatum</em></td>
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<tr>
<td><em>Mycobacterium tuberculosis</em></td>
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</table>

An example of Plg activation by tPA on the surface of *S. typhimurium* is shown in Fig. 3. It can be seen that formation of active plasmin (Fig. 3A) takes place on the cell surface but not in solution. Type 1 fimbria is one of the PlgR molecules on the *Salmonella* surface [148], and incu-
Table 4
Bacterial Plasmin(ogen) receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Bacterial species</th>
<th>Other functions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, SDH, Plr)</td>
<td>Group A and C streptococci</td>
<td>Glycolytic enzyme. Adhesion to fibronectin, lysozyme, myosin and actin.</td>
<td>[179–184]</td>
</tr>
<tr>
<td>α-Enolase (SEN)</td>
<td>Group A streptococci</td>
<td>Glycolytic enzyme.</td>
<td>[190]</td>
</tr>
<tr>
<td>Flagella</td>
<td>Escherichia coli, Salmonella</td>
<td>Motility organelle.</td>
<td>[166], Lähteenmäki, K. and Korhonen, T.K., unpublished data</td>
</tr>
<tr>
<td>Aspartase</td>
<td>Haemophilus influenzae</td>
<td>Catabolic enzyme.</td>
<td>[196]</td>
</tr>
<tr>
<td>Outer surface protein A (OspA)</td>
<td>Borrelia burgdorferi</td>
<td>Homology to periplasmic oligopeptide-binding proteins.</td>
<td>[172,198]</td>
</tr>
<tr>
<td>70-kDa surface protein (OppA)</td>
<td>Treponema denticola</td>
<td></td>
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</tr>
</tbody>
</table>

GAPDH was first identified as a streptococcal receptor for plasmin and found to bind Glu-Plg very weakly [179–181]. The localization of GAPDH on the cell surface of group A streptococci has been demonstrated by immunological and enzymatic methods, hence the name SDH (streptococcal surface dehydrogenase) [184]. The COOH-terminal lysine residue in SDH seems to be important for the interaction with plasmin, as substitution of this residue with leucine abolishes binding [182]. SDH also displays auto-ADP-ribosylating and phosphorylation activities in vitro, which suggests a role for the enzyme in signal transduction between streptococci and pharyngeal cells [199,200]. Replacement of SDH with a surface GAPDH variant lacking plasmin-binding capacity had no effect on plasmin(ogen) binding by the recombinant bacteria, which indicates that group A streptococci probably express multiple PlgRs [182].

The surface localization of SEN, another glycolytic enzyme, has been demonstrated for most streptococcal groups and serotypes [190]. Removal of the COOH-terminal lysine of SEN by carboxypeptidase B treatment diminished binding of Plg only partially, suggesting that multiple lysine residues in the molecule are involved in the interaction with Plg [190]. Comparison of the affinities of SDH and SEN to Plg suggests that SEN is the principal PlgR in streptococci [190]. It is noteworthy that α-enolase has been identified as a Plg-binding molecule also on the surface of several eukaryotic cells [38,201,202]. Antibodies to α-enolase can be detected in sera of patients suffering from PSGN, suggesting that the protein is expressed in vivo [203]. Human and streptococcal α-enolases share immunological cross-reactivity; these findings suggest that streptococcal α-enolase is involved in consequences of the diseases caused by group A streptococci [204].

The best-characterized PlgRs on Gram-positive bacteria are those identified on group A and C streptococci isolated from humans. These PlgRs include the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH; also called SDH and Plr) and α-enolase (called SEN), as well as the streptococcal M-like protein PAM [161, 179,190].

bation of purified fimbriae, Plg and tPA together results in plasmin formation (Fig. 3B).

PlgR activity has been detected in a number of bacterial pathogens (Table 3), and several PlgR molecules have been characterized (Table 4). It is noteworthy that most of the identified bacterial PlgRs have other important functions as well, such as adhesion, movement, enzymatic activity, nutrient uptake, or interaction with the immunological system. Furthermore, it is obvious that a single bacterial species can express multiple PlgR types. These facts complicate the analysis of the functions and possible virulence association of PlgRs by mutant construction or epidemiological analysis, and may in part explain the observed lack of correlation of virulence and PlgR function in isolates of Borrelia burgdorferi [197].

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3.3.2. Identified plasminogen receptors on Gram-positive bacteria

The best-characterized PlgRs on Gram-positive bacteria are those identified on group A and C streptococci isolated from humans. These PlgRs include the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH; also called SDH and Plr) and α-enolase (called SEN), as well as the streptococcal M-like protein PAM [161, 179,190].
together, SDH and SEN form a novel group of enzymes that are found on both eukaryotic and prokaryotic cells and known to be located both in the cytoplasm and on the cell surface. The mechanism of their surface expression remains to be resolved.

M-proteins are antiphagocytic fibrillar, helical surface proteins that contain abundant repeat regions (reviewed in [188]). Within the PAM (plasminogen-binding group M streptococcal protein) molecule, plasminogen binding has been localized to the NH$_2$-terminal portion that contains two 13-amino acid repeats termed a1 and a2. Both repeats contain a single central lysine residue (for a recent review, see [95]). The a1 repeat contains the sequence DAELQRLKNERHE, where substitution of the importance of this adhesion property is not yet fully understood.

Table 4 also exhibit PlgR activity.

The flagellar filaments of *E. coli* and *S. typhimurium* bind Plg and enhance its activation by tPA ([166]; Lähteenmäki, K., and Korhonen, T.K., unpublished data), and the PlgR activity does not seem to be related to a particular H serotype. Also, in the case of the flagellar filaments, the subunit(s) interacting with Plg has not been identified. The function of type 1 fimbriae (which are expressed by ca. 80% or more of *E. coli* isolates), curli and flagella as PlgRs indicates that the vast majority of *E. coli* strains have the capacity to immobilize Plg.

Trepomonas denticola and *B. burgdorferi* express 70-kDa surface proteins that bind Plg and show homology to the oligopeptide-binding components of ABC transporter proteins [172,198]. The permease functions of these proteins have not been characterized, but the homologies suggest an interesting interplay of plasmin proteolysis and bacterial peptide transport.

3.4. Biological functions of bacteria-plasminogen interactions

That Plg activation is involved in systemic infection was inferred from the observations that meningococcal meningitis is associated with enhanced fibrinolytic activity [75]. The finding of PlgR or PA function in several invasive bacterial pathogens has led to suggestions that plasmin formation has a role in tissue damage associated with or biogenesis, see [208,209]). A major function identified for fimbriae is adhesion to epithelial receptors which promotes bacterial colonization and diminishes mechanical removal of bacteria from tissue sites. We have found that many fimbrial types of pathogenic *E. coli* also bind to ECM receptors [210]. Fimbrial binding to ECM or BM proteins seems to be a true tissue adhesion mechanism as it is seen both in vitro on frozen tissue sections or with extracted ECM preparations and in vivo in rats injected with purified type IV collagen-binding fimbriae [211,212]. Overall, a surprisingly large number of intracellular and extracellular human pathogens have been found to adhere to the mammalian ECM (reviewed in [210,213]), and the pathogenetic importance of this adhesion property is not yet fully understood.

Purified type 1, S, and G fimbriae, and curli of *E. coli* express PlgR activity [148,164,167]; tPA has also been found to bind to fimbriae and curli in a lysine-sensitive manner. The fimbrial subunit that is recognized by Plg has not been identified for any of the identified fimbrial PlgRs.

Fimbrial filaments which have been genetically deleted for the lectin subunit or made deficient in carbohydrate binding are not affected in PlgR function [148,165], which indicates that the interaction is not dependent on the lectin activity of the fimbriae. Other remaining questions on fimbrial PlgRs include the significance of structural variability within a fimbrial type [214] on PlgR function, as well as whether fimbrial types other than the ones described in Table 4 also exhibit PlgR activity.

The flagellar filaments of *E. coli* and *S. typhimurium* bind Plg and enhance its activation by tPA ([166]; Lähteenmäki, K., and Korhonen, T.K., unpublished data), and the PlgR activity does not seem to be related to a particular H serotype. Also, in the case of the flagellar filaments, the subunit(s) interacting with Plg has not been identified. The function of type 1 fimbriae (which are expressed by ca. 80% or more of *E. coli* isolates), curli and flagella as PlgRs indicates that the vast majority of *E. coli* strains have the capacity to immobilize Plg.
needed for invasive infections, including meningitis [215–218]. Although the number of bacterial species found to express PlgRs is appreciable (see Table 3), the pathogenic function of Plg activation has been addressed with a limited group of invasive bacteria. These include *Y. pestis* [116] and other enteric bacteria [123,215], *Borrelia* [171,219–221], *H. influenzae* [222], staphylococci [73], group A streptococci [223], and *Fusobacterium nucleatum* [178]. The studies have mainly focused on testing whether bacterium-associated plasin activity degrades proteins of the ECM or BM and whether plasin formation potentiates bacterial penetration through these tissue barriers. Other proposed functions for plasin in invasive bacterial infections include fibrin degradation [136,224], degradation of protease inhibitors in tissues [178], as well as release of peptides for nutrition of bacteria [145]. Demonstration of SK- and PlgR-related structures of group A streptococcus in kidney lesions suggests that localized Plg activation plays a role in the pathogenesis of PSGN [225].

The evidence for a pathogenic role of plasin in bacterial infections has largely resulted from in vitro studies. In the cases of *Y. pestis, Borrelia*, and group A streptococci, in vivo challenge tests have also been reported [116,137,219,221,223].

3.4.1. Involvement of plasminogen activation in bacterial metastasis through tissue barriers – in vitro studies

Many of the bacterial species causing systemic infections and expressing PlgR or PA activity (see Tables 1, 3, and 4) are known to adhere to BM [210,213], which could target bacteria into an environment favorable for Plg activation and plasin activity. Adherence to BM and Plg activation are involved in tumor cell metastasis (reviewed in [226]). In order to emphasize the similar behavior of tumor cells and invasive bacteria and to distinguish the phenomenon from bacterial invasion into epithelial or phagocytic cells, we have proposed the term bacterial metastasis for the process of bacterial penetration through BM [215]. The term bacterial metastasis was actually used decades earlier to describe bacterial translocation into secondary infection sites [227]. The methodology used to demonstrate ECM degradation by bacterium-bound plasin, i.e. degradation of radio labelled ECM proteins or metabolically labeled ECM from cultured epithelial cells, as well as penetration of bacteria into or through BMs, has been adapted from the technology used in tumor cell metastasis studies (see [54] for a critical review of the methods and [228] for applications in bacterial infection studies). It appears that under in vitro conditions, plasin-coated bacteria have been found to display all characteristics that probably are sufficient for bacterial penetration through the BM or ECM: degradation of individual plasin-sensitive proteins of BM/ECM, degradation of BM/ECM, and activation of procollagenases. The current evidence for ECM degradation by bacterium-bound plasin can be summarized as follows.

1. Plasin formed on the bacterial surface degrades radio labeled noncollagenous ECM proteins laminin, fibronectin, and vitronectin. Fig. 4 shows degradation of fibronectin and laminin immobilized on glass by plasin-coated *H. influenzae* cells [222]. Degradation is seen with plasin-coated cells but not with plain bacterial cells, and aprotinin (an inhibitor of plasin) prevents degradation. Similar findings have been reported for plasin-coated cells of *S. typhimurium* [123], *B. burgdorferi* [220], and *F. nucleatum* [178], as well as for Pla-expressing cells of recombinant *E. coli* incubated with Plg [135].

2. Plasin generated on the surface of bacteria degrades a more complex ECM structure, such as ECM secreted by human endothelial or epithelial cells (Fig. 4). Again, in the case of *S. typhimurium* [123], *H. influenzae* [222], and *B. burgdorferi* [220] it has been shown that ECM degradation is exhibited by bacterium-bound plasin.

3. Surface-bound plasin enhances penetration of *S. typhimurium, E. coli, H. influenzae* and *S. pneumoniae* [123,215,222,229] through the reconstructed basement membrane Matrigel. Matrigel is secreted by mouse tumor cells and contains type IV collagen and laminin as major components [230,231], and at room temperature it polymerizes into a BM-like gel resembling in structure the laminin and type IV collagen networks visualized in tissues [232]. Matrigel has been frequently used in eukaryotic cell migration research, in particular tumor metastasis studies [233,234] and, more recently, also in bacterial adherence and penetration studies [123,191,222,235]. Matrigel also contains PAs, as well as procollagenases and Plg that can be activated and remain active, and, in general, BMs are considered reservoirs of Plg system components [236,237].

4. Plasin-coated bacteria have an enhanced potential to penetrate through cultured cell monolayers. Plasin-coated cells of *B. burgdorferi* penetrated human endothelial monolayers grown on connective tissue substrate, and the penetration was inhibited by aprotinin but not by α2-antiplasmin [171]. Transcytosis of *S. aureus* through a bovine mammary epithelial cell monolayer was enhanced in the presence of Plg and inhibited by addition of aprotinin [73]. Interestingly, the staphylococci preferentially penetrated the cell layer in the basal-to-apical direction, i.e. from the side where the BM proteins are expected to be accessible for bacterial adhesins.

5. Bacterium-bound plasin activates procollagenases which are needed for breakdown of the collagen network. Plasin bound on the *S. aureus* surface cleaves the intestinal procollagenase pro-MMP-1 at the same peptide bond as does soluble plasin, and the MMP-1 thus formed is proteolytically active [238]. Recently, *borreliae* were shown to upregulate the release of pro-
MMPs in human monocytes and, when coated with plasmin, to convert pro-MMP-9 to active MMP-9 [239]. Human monocytes were also found to enhance penetration of *B. burgdorferi* across layers of ECM proteins. The penetration was partially inhibited by an MMP inhibitor, indicating that it was at least partly mediated by MMPs produced by the monocytes [239].

### 3.4.2. In vivo evidence for the role of the plasminogen system in bacterial infections

In vivo data about the role of the Plg system in bacterial infections are limited so far and have resulted mainly from studies using Plg-deficient mice as infectious disease models. Coleman and co-workers [219] compared the dissemination of *B. burgdorferi* in Plg-deficient and normal mice after intradermal inoculation and found that the frequency of positive blood cultures was reduced in the Plg-deficient mice. However, similar amounts of bacteria were found in the spleen, heart, and bladder of both normal and Plg-deficient mice. As borreliosis is transmitted via ticks, it is of interest that acquisition of Plg and PA from murine blood was found to enhance the subsequent spread of *B. burgdorferi* also in the tick [219]. In another study, a *Borrelia* sp. causing relapsing fever was detected in similar amounts in the blood of Plg-positive and Plg-negative animals, but bacterial invasion into the brain and heart of normal mice was three to five times more efficient than invasion into the organs of the Plg-deficient mice [221]. Thus, Plg seems to enhance migration of borreliae in the host.

Acquisition of a SK–Plg–fibrinogen complex on the bacterial surface increases the virulence of group A streptococci in normal mice, but has no such effect in Plg-deficient mice [223]. Plg-deficient mice have also been found to be more resistant to *Y. pestis* infection than normal mice, although the difference in survival is less evident than between normal mice infected with either Pla-negative or Pla-positive bacteria [116,137]. This underscores the role of Pla-mediated Plg activation, but also indicates that other functions of Pla are likely to be active as well.

Antibodies recognizing PA may give protection against invasive infection. Immunization of dairy cows with bacterial culture fluid containing PauA of *S. uberis* raised protection against streptococcal mastitis [240]. An immune response to PauA was detected and the protection was concluded to result from inhibition of PauA activity [240].

### 4. Variations on the theme: activation of matrix metalloproteinases by bacterial proteases and bacterium-induced cytokines

Degradation or loss of integrity of collagen fibers or network has significant consequences for the tissue dam-

---

**Fig. 4.** Degradation of 

**S-ECM**

<table>
<thead>
<tr>
<th>cpm / sample</th>
</tr>
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<tbody>
<tr>
<td>1000</td>
</tr>
<tr>
<td>500</td>
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<td>0</td>
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**125I-Laminin**

<table>
<thead>
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<tr>
<td>3000</td>
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<td>1000</td>
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<td>0</td>
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**125I-Fibronectin**

<table>
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<th>cpm / sample</th>
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<td>4000</td>
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<td>2000</td>
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<td>1000</td>
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**Time (h)**

- **Bacteria-plasminogen + tPA**
- **Bacteria-plasminogen + tPA + aprotinin**
- **Bacteria**

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Fig. 5. Hypothesis on bacterial migration through the extracellular matrix. The models are based on the current knowledge of bacterial interaction with the plasminogen system and the extracellular matrix. Thick arrows indicate the direction of bacterial migration which is here depicted to take place via pericellular route. Red arrows indicate conversion of plasminogen (Plg) to plasmin. Green arrows indicate inhibition by the plasmin inhibitor α2-antiplasmin (α2AP): plain arrow indicates susceptibility to inhibition by α2AP, whereas the arrow with a bar indicates that α2AP cannot inhibit the target molecule. A. Migration of bacteria through an epithelial layer by using host-produced Plg and plasminogen activator (PA). Bacterial contact with epithelial cells stimulates secretion of PA from the cells. Bacteria bind Plg and PA, and this enhances the PA-mediated conversion of Plg to active plasmin on bacterial surface. In contrast to free plasmin, bacterium-bound plasmin is protected from inhibition by α2AP. After passing through the cell layer, bacteria adhere to the extracellular matrix (ECM), and, in order to penetrate into underlying tissues, degrade ECM by using the surface-bound plasmin. B. Streptokinase (SK) produced by group A, C, and G streptococci and staphylokinase (SAK) produced by Staphylococcus aureus form a complex with Plg. This complex activates other Plg molecules to plasmin (see text for details). The free SAK-plasmin complex is inhibited by α2AP, whereas the SK-Ptg complex is not inhibited. Streptococci also express various mechanisms for binding Ptg, the SK-Ptg-complex or plasmin, and the plasmin thus generated on bacterial surface enhances bacterial migration through the ECM. C. Migration of Yersinia pestis from the subcutaneous infection site into lymphatic vessels and circulation. The Pla surface protease mediates bacterial adherence to laminin on the ECM and also activates Plg. The formed plasmin then degrades ECM and thereby enhances bacterial invasion. Plasmin is here illustrated as cell-bound, however, no Plg receptor in Y. pestis has yet been reported. Pla also proteolytically inactivates α2AP (black arrow). This renders α2AP unable to inhibit plasmin, and thus may potentiate systemic proteolysis.

Figures A, B, and C are used to illustrate the hypothesis on bacterial migration through the extracellular matrix. They are based on the current knowledge of bacterial interaction with the plasminogen system and the extracellular matrix. Thick arrows indicate the direction of bacterial migration which is here depicted to take place via pericellular route. Red arrows indicate conversion of plasminogen (Plg) to plasmin. Green arrows indicate inhibition by the plasmin inhibitor α2-antiplasmin (α2AP): plain arrow indicates susceptibility to inhibition by α2AP, whereas the arrow with a bar indicates that α2AP cannot inhibit the target molecule. A. Migration of bacteria through an epithelial layer by using host-produced Plg and plasminogen activator (PA). Bacterial contact with epithelial cells stimulates secretion of PA from the cells. Bacteria bind Plg and PA, and this enhances the PA-mediated conversion of Plg to active plasmin on bacterial surface. In contrast to free plasmin, bacterium-bound plasmin is protected from inhibition by α2AP. After passing through the cell layer, bacteria adhere to the extracellular matrix (ECM), and, in order to penetrate into underlying tissues, degrade ECM by using the surface-bound plasmin. B. Streptokinase (SK) produced by group A, C, and G streptococci and staphylokinase (SAK) produced by Staphylococcus aureus form a complex with Plg. This complex activates other Plg molecules to plasmin (see text for details). The free SAK-plasmin complex is inhibited by α2AP, whereas the SK-Ptg complex is not inhibited. Streptococci also express various mechanisms for binding Ptg, the SK-Ptg-complex or plasmin, and the plasmin thus generated on bacterial surface enhances bacterial migration through the ECM. C. Migration of Yersinia pestis from the subcutaneous infection site into lymphatic vessels and circulation. The Pla surface protease mediates bacterial adherence to laminin on the ECM and also activates Plg. The formed plasmin then degrades ECM and thereby enhances bacterial invasion. Plasmin is here illustrated as cell-bound, however, no Plg receptor in Y. pestis has yet been reported. Pla also proteolytically inactivates α2AP (black arrow). This renders α2AP unable to inhibit plasmin, and thus may potentiate systemic proteolysis.

age and outcome of bacterial infections, and the pathogenetic role of plasmin formation described above most likely results partly from activation of MMPs. Pathogenic bacteria also appear to have alternative mechanisms to activate metalloproteases. Direct activation of MMPs by bacterial proteases has been studied in relation to periodontal disease and P. gingivalis ([74,241,242], see also the review [4]). P. gingivalis and other bacterial species commonly associated with periodontitis were found to activate latent human fibroblast-type and neutrophil interstitial procollagenases. Bacterial proteases were found to cleave collagen fragments generated by the activated host collagens, and it was concluded that the host and bacterial proteases act in concert to cause periodontal tissue destruction. It is interesting to note that P. gingivalis also expresses protease activity that degrades various protease inhibitors [147,243].

Increased expression of type IV collagenases on endothelial or monocytic cells is noted in vitro after treatment with pathogenic bacteria [239,244,245]. Collagenases have recently been shown to contribute to brain damage and collapse of the blood–brain barrier in experimental bacterial meningitis [246–248]. It appears that in this disease the upregulation of MMP-9 involves the cytokine TNF-α [248]. Concentrations of MMP-9 and TNF-α in cerebrospinal fluid were increased in rats with bacterial meningitis compared to uninfected animals, and treatment with a hydroxamic acid-type MMP inhibitor reduced the MMP-9 and TNF-α levels as well as neuronal damage. It seems logical that bacterial adherence to endothelial and epithelial cells in choroid plexus and meninges contributes to the observed release of TNF-α.

5. Conclusions

The experimental evidence discussed above clearly demonstrates that a vast number of severe bacterial pathogens interact with the Plg system by expressing PlgRs or PAs, or both, or by interacting with the system in other ways. That these interactions enhance bacterial virulence is suggested by in vitro evidence showing enhanced proteolytic activity, tissue damage as well as spread through reconstituted tissue barriers by bacteria with surface-bound plasmin. These studies have led to the hypothesis (Fig. 5) that metastatic bacteria utilize similar principles for migration through tissue barriers as do metastatic tumor cells. Given the large number of bacteria observed to interact with the Plg system in vitro, it is somewhat disturbing that the in vivo evidence from experimental infections is not abundant. This may result from difficulties encountered in the experimental approaches that are available. It is difficult to interfere experimentally with Plg activation in vivo; Plg is very abundant in the circulation and inhibitors or anticataytic antibodies should be present in large amounts and be targeted against the correct conformation of Plg or PAs. The conventional approach in bacterial pathogenesis – site-specific mutagenesis – is also problematic, particularly in the case of bacterial PlgRs. The identified bacterial PlgRs seem as a rule to be multifunctional surface pro-
teins, such as metabolically important enzymes, adhesins, motility organelles, or proteins affecting immunological reactivity against the invading pathogen. These functions influence the survival or infectivity of the bacterium in the host. Very detailed information on the structure–function relationships in bacterial PlgRs/PAAs will be needed to construct mutants specifically impaired in the PlgR/PA function only. Such structural information exists at present for the activators SK and SAK (discussed in [18]), but our information on the structural details of bacterial PlgRs, in particular, is still limited. The multifunctional nature as well as multiplicity of PlgRs on pathogenic bacteria probably reflects affinity of Plg for a range of terminal and internal Lys residues. It is noteworthy, however, that a class of bacterial PlgRs is composed of fibrillar surface proteins (PAM, fimbriae, flagella) that have morphological similarity to fibrin. The other major class of bacterial PlgRs is surface-bound enzyme molecules, a theme also seen in eukaryotic organisms.

Plg-negative as well as PA-negative knock-out mice have been constructed [60–65] and tested in experimental infections (reviewed in [137]). While it is obvious that these animal models will give valuable and definite answers on the significance of bacteria–Plg interactions, they also have certain limitations in this respect. Some bacterial PAs exhibit marked host specificity in their interaction with Plg [94,224], which makes them unsuited to a murine infection model. It also appears that Plg-negative animals demonstrate increased thrombus formation and problems in growth and inflammatory responses, which may enhance bacterial migration in the body. Hopefully, future research on these mechanisms will provide new tools to prevent systemic bacterial infections.

Acknowledgements

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