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Interactions of surface-displayed glycolytic enzymes of *Mycoplasma pneumoniae* with components of the human extracellular matrix

Anne Gründel, Enno Jacobs, Roger Dumke*

Technische Universität Dresden, Medizinische Fakultät Carl Gustav Carus, Institut für Medizinische Mikrobiologie und Hygiene, Fetscherstrasse 74, 01307 Dresden, Germany

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ABSTRACT

Mycoplasma pneumoniae is a major cause of community-acquired respiratory infections worldwide. Due to the strongly reduced genome, the number of virulence factors expressed by this cell wall-less pathogen is limited. To further understand the processes during host colonization, we investigated the interactions of the previously confirmed surface-located glycolytic enzymes of *M. pneumoniae* (pyruvate dehydrogenase A-C [PdhA-C], glyceraldehyde-3-phosphate dehydrogenase [GapA], lactate dehydrogenase [Ldh], phosphoglycerate mutase [Pgm], pyruvate kinase [Pyk] and transketolase [Tkt]) to the human extracellular matrix (ECM) proteins fibrinogen (Fn), fibronectin (Fc), lactoferrin (Lf), laminin (Ln) and vitronectin (Vc), respectively. Concentration-dependent interactions between Fn and Vc and all eight recombinant proteins derived from glycolytic enzymes, between Ln and PdhB-C, GapA, Ldh, Pgm, Pyk and Tkt, between Lf and PdhA-C, GapA and Pyk, and between Fc and PdhC and GapA were demonstrated. In most cases, these associations are significantly influenced by ionic forces and by polyclonal sera against recombinant proteins. In immunoblotting, the complex of human plasminogen, activator (tissue-type or urokinase plasminogen activator) and glycolytic enzyme was not able to degrade Fc, Lf and Ln, respectively. In contrast, degradation of Vc was confirmed in the presence of all eight enzymes tested. Our data suggest that the multifaceted associations of surface-localized glycolytic enzymes play a potential role in the adhesion and invasion processes during infection of human respiratory mucosa by *M. pneumoniae*.

1. Introduction

Mycoplasma pneumoniae is a common cause of a broad spectrum of infections of the human respiratory tract ranging from mild, often undiagnosed forms of tracheobronchitis to severe interstitial pneumonia. Infections due to *M. pneumoniae* are transmitted by aerosols and have been described in all age groups but older children and young adults are more frequently affected (Atkinson et al., 2008). Besides small-scale endemic transmission in populations with close person-to-person contact, nation- or even world-wide incidence peaks every 5–7 years have been reported, in which up to 40% of all cases of community-acquired pneumonia are attributed to this pathogen (Dumke et al., 2015). Extra-pulmonary manifestations, mainly of the central nervous system and of the skin, further complicate the clinical signs of infections (Narita, 2016).

Members of the class *Mollicutes* are cell wall-less bacteria characterized by a strong reduction of genetic resources during evolutionary interaction with their hosts. In consequence, *M. pneumoniae* is not only limited in its metabolic capabilities (Kühner et al., 2009), requiring uptake of many metabolites from the environment, but also in the repertoire of factors determining pathogenicity and the clinical symptoms of infected humans as the only known natural host. To date, the complex adhesion apparatus of the bacteria (Hasselbring et al., 2006), the expression of the CARDS toxin (Kannan and Baseman, 2006) and the release of cell-damaging substances such as hydrogen peroxide (Hames et al., 2009) and hydrogen sulfide (Großhennig et al., 2016) are confirmed or potential virulence factors.

First described in streptococci (Pancholi and Fischetti, 1992), bacterial proteins with a primary function in cytosol-localized metabolic processes can be transported by an unknown mechanism to the surface of the cells. These proteins also include glycolytic enzymes essential for glycolysis. Surface-displayed glycolytic enzymes have now been demonstrated in many different bacterial species (Henderson and Martin, 2011; Wang et al., 2013; Kainulainen and Korhonen, 2014) indicating a common and multifaceted mechanism among prokaryotes. In addition to the typical representatives of this class of proteins, such as enolase (Eno) and

* Corresponding author.
E-mail address: roger.dumke@tu-dresden.de (R. Dumke).

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glyceraldehyde-3-phosphate dehydrogenase (GapA), the number of further enzymes with a dual role in the cytosol and at the cell surface has increased in recent years (Henderson, 2014). Because of interaction(s) with host factors, mainly with components of the extracellular matrix (ECM), it has been suggested that surface-displayed proteins increase the efficiency of micro-organisms in colonizing the host.

Especially in mycoplasmas, multi-function of proteins might be a way to compensate for the limited genetic resources. In this context, further investigation of moonlighting proteins is also important for a general understanding of the organisation of the successful parasitic lifestyle of many members of the *Mollicutes* group. Regarding the pathogenic species *M. pneumoniae*, the characterization of glycolytic enzymes that associate with human factors is an aspect of host-pathogen interaction that also may contribute to pathogenesis. Recently, we confirmed the surface localization of the following eight glycolytic enzymes of *M. pneumoniae*: GapA, lactate dehydrogenase (Ldh), phosphoglycerate mutase (Pgm), pyruvate kinase (Pyk), pyruvate dehydrogenase A to C (PdhA-C) and transketolase (TkT) and demonstrated that all these proteins bind to human plasminogen (Plg; Gründel et al., 2016), an important factor of the fibrinolysis system (Sanderson-Smith et al., 2012). In addition, proteolytically active plasmin was generated in the presence of all glycolytic enzymes, Plg and the urokinase plasminogen activator (uPA). However, only the complexes between Plg and the enzymes PdhB as well as Pgm are able to induce the *in vitro* degradation of human fibrinogen (Fn).

With the recombinant proteins derived from genes coding for the confirmed surface-localized enzymes and their corresponding monospecific antisera, experimental tools to investigate possible interactions with other factors of the human ECM are now available. These host factors include Fn, vitronectin (Vc), fibronectin (Fc), lactoferrin (Lf), laminin (Ln), collagen and elastin which are regarded as important targets of bacterial proteins to promote the ability of microorganisms to escape the host response, to adhere effectively and/or to invade tissues (Singh et al., 2010, 2012). The complex of ECM proteins comprises diverse components mediating and influencing many essential processes in the host. For example, via binding to different factors multidomain Fn and Fc are involved in matrix physiology, cell migration and cell signalling (Henderson et al., 2011; Yamaguchi et al., 2013; Halper and Kjaer, 2014) whereas Vc is a complement regulator and promotes many processes such as cell adhesion and differentiation (Singh et al., 2010; Preissner and Reuning, 2011). Laminins play an important role in structuring the ECM matrix by interactions with other proteins not least to protect tissues from pathogens (Chagnot et al., 2012; Singh et al., 2012). The iron-binding glycoprotein Lf contributes to iron homeostasis in the host and is a protective component of the innate immune system (Gonzalez-Chavez et al., 2009). Binding of Lf to bacterial proteins is discussed as important for iron supply of micro-organisms (Morgenthau et al., 2013). Binding between individual glycolytic enzymes of *M. pneumoniae* and selected host components such as Fc or Fn has been reported previously (Dallo et al., 2002; Dumke et al., 2011). Nevertheless, overall knowledge of the interactions of surface-localized glycolytic enzymes with ECM factors is far from well-documented and not only for *M. pneumoniae*. In this study, we investigated these associations to better understand the network of interactions between host ECM factors and a class of primary metabolic bacterial proteins in a common pathogen.

2. Materials and methods

2.1. Bacteria and human cells

The *M. pneumoniae* strains M129 (ATCC 29342), FH (ATCC 15531), the *Escherichia coli* strain BL21(DE3) (Novagen, Darmstadt, Germany) and human A549 cells (human lung carcinoma cell line ATCC CCL-185) were grown as described previously (Schurwanz et al., 2009). Protein concentration was measured using the BCA protein assay kit (Pierce, Rockford, USA) as recommended by the manufacturer.

2.2. Recombinant production of surface-localized glycolytic enzymes of M. pneumoniae and polyclonal antibodies

The *E. coli* strains containing the pET vectors with the cloned full-length genes coding for the glycolytic enzymes of *M. pneumoniae* (Table 1) were produced as described (Gründel et al., 2016). Expression of the N-terminal 6 x His-tagged proteins was induced with 1 mM isopropylthiogalactoside (Sigma, St. Louis, USA) for 5 h at 37 °C. The proteins were purified under denaturing conditions by using Ni-nitrilotriacetic acid columns (Qiagen, Hilden, Germany) as recommended by the manufacturer. The eluates were concentrated with ultrafiltration spin columns (Vivascience, Hannover, Germany) and the protein concentration was measured as described. The quality of the expression results was checked after separation of recombinant proteins by SDS-PAGE, using Coomassie blue staining and standard immunoblotting procedures as reported (Gründel et al., 2016).

Polyclonal antisera were produced in guinea pigs (Charles River, Sulzfeld, Germany). The animal experiments were conducted in accordance with the recommendations of the Federation of Laboratory Animal Science Associations (FELASA) and approved by the ethical board of Landesdirektion Sachsen, Dresden, Germany (permit no. 24-9168.25-1/2011/1). Primary subcutaneous immunization of guinea pigs with total proteins of *M. pneumoniae* (positive control serum) or the recombinant proteins, booster

Table 1
Summary of binding characteristics of surface-located glycolytic enzymes of *M. pneumoniae* to selected components of human ECM.

Glycolytic enzyme	ECM component														
	Lactoferrin			Laminin			Vitronectin			Fibrinogen			Fibronectin		
	Binding[a]	NaCl[b]	Anti-serum[c]	Binding	NaCl	Anti-serum	Binding	NaCl	Anti-serum	Binding	NaCl	Anti-serum	Binding	NaCl	Anti-serum
PdhA	±	+	±	–			±	±	+	±	–	+	–		
PdhB	+	+	±	+	+	±	+	+	+	±	–	+	–		
PdhC	+	+	–	±	+	±	+	–	±	±	±	±	±	–	+
GapA	+	+	±	+	+	+	+	±	+	+	±	+	+	±	+
Pgm	–			+	+	+	+	–	+	±	–	+	–		
Pyk	+	+	±	+	+	+	+	±	+	+	±	+	–		
Tkt	–			+	+	+	+	±	±	+	±	+	–		
Ldh	–			+	+	±	+	±	+	+	–	±	–		

[a] Strength of binding (**+**: OD values after comparative binding of highest concentration of ECM component >0.4; ±: OD values ≥ 0.2 − ≤ 0.4; **-**: OD values < 0.2).
[b] Influence of ionic interactions on binding (**+**: reduction of 50–100%; ±: 10–50%; **-**: <10% in comparison with negative control).
[c] Influence of antiserum to glycolytic enzyme on binding (**+**: reduction of 50–100%; ±: 10 − ≤ 50%; **-**: <10% in comparison with negative control).