Proteolytic Post-Translational Processing of Adhesins in a Pathogenic Bacterium

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Abstract

Mollicutes, including mycoplasmas and spiroplasmas, have been considered as good representatives of the « minimal cell » concept: these wall-less bacteria are small in size and possess a minimal genome and restricted metabolic capacities. However, the recent discovery of the presence of post-translational modifications unknown so far, such as the targeted processing of membrane proteins of mycoplasma pathogens for human and swine, revealed a part of the hidden complexity of these microorganisms. In this study, we show that in the phytopathogen, insect-vectored *Spiroplasma citri* GII-3 adhesion-related protein (ScARP) adhesins are post-translationally processed through an ATP-dependent targeted cleavage. The cleavage efficiency could be enhanced *in vitro* when decreasing the extracellular pH or upon the addition of polyclonal antibodies directed against ScARP repeated units, suggesting that modification of the surface charge and/or ScARP conformational changes could initiate the cleavage. The two major sites for primary cleavage are localized within predicted disordered regions and do not fit any previously reported cleavage motif; in addition, the inhibition profile and the metal ion requirements indicate that this post-translational modification involves at least one non-conventional protease. Such a proteolytic process may play a role in *S. citri* colonization of cells of the host insect. Furthermore, our work indicates that post-translational cleavage of adhesins represents a common feature to mollicutes colonizing distinct hosts and that processing of surface antigens could represent a way to make the most out of a minimal genome.

Introduction

Mollicutes are wall-less bacteria including many species pathogens for humans, animals, or plants. Despite their minimal genome, mollicutes have evolved different means to adapt and survive within very distinct hosts. Their plasma membrane contains a large number of proteins exposed at the bacterial surface and thus localized at the pathogen/host interface. The adaptive and survival capacities within the host and various aspects of pathogenesis are thought to partially rely on the variation of surface proteins [1]. Indeed, in many pathogenic species, the dynamic use of variable surface antigens can help evade the immune system and establish chronic infections in diverse human and animal hosts. In many mollicutes, genetic switches altering the expression, size, or structure of surface-exposed proteins are major means used to modify the surface topography [2–8]. In *Mycoplasma hyopneumoniae*, a mollicute responsible for the porcine enzootic pneumonia, the targeted proteolysis of surface antigens coupled with variable cleavage efficiency has been identified as another mechanism participating in the diversification of surface-exposed antigens [9–12]. More generally, in mycoplasmas, cell surface protein proteolysis appears to be involved at different levels of the host invasion process, as illustrated by proteolytic events occurring at the surface of *Mycoplasma fermentans*. Indeed, this human mycoplasma binds plasminogen

and activates it into plasmin [13], a protease with a broad specificity thought to be involved in proteolysis of the mycoplasma cell surface proteins and to facilitate bacterial internalization [14]. Also in *M. fermentans*, the endogenous cleavage of the lipoprotein MALP-404 results in the cell-bound lipopeptide MALP-2 [15], a Toll-like receptor 2-mediated macrophage-activating lipopeptide [16]. Although cell surface protein endoproteolysis appears to support virulence and/or pathogenesis in several mollicute species [17], the protease(s) involved and the underlying proteolytic mechanism have not been identified in most cases, with the exception of plasmin-endoproteolytic activation in *M. fermentans*. In non-mammalian mollicutes, like in spiroplasma species, mechanisms of adhesin proteolysis have not been identified. Therefore, it is an open question whether this targeted protein processing corresponds to a general mechanism involved in the host invasion process in the class Mollicutes or is restricted to a few mammalian-infecting species.

Among non-mammalian mollicutes, spiroplasmas form a large group of pathogens infecting mostly arthropods, either as final or intermediary hosts. *Spiroplasma citri* is by far the most studied *Spiroplasma* species and represents a well-established model for studies dealing with spiroplasmal biology. *S. citri* is the causal agent of citrus stubborn disease, transmitted to its host plant by a phloem sap-feeding insect of the genus *Circulifer*. The bacteria tightly interact with cells of different tissues of their vector, notably the salivary gland cells and mid-intestine cells [18]. In *S. citri*, the presence of genome-inserted prophages encoding membrane proteins and the abundance of lipoproteins and of transmembrane proteins belonging to multigenic families [19] are at least in part responsible for the antigenic diversity at the spiroplasma surface. Among surface-exposed proteins, *S. citri* GII-3 adhesion-related proteins (ScARPs) belong to a multigenic protein family, which includes eight members (ScARP2a-b, ScARP3a-d, ScARP4a, and ScARP5a) encoded by plasmids pSci1 to 5. In addition, pSci6 harbors two C-terminal *arp* truncated genes. At least one member of this family, ScARP3d, is involved in the pathogen adhesion process to insect cells [20]. A schematic representation of full-length *arp* genes products (encoded on pSci1–5) is given Fig. 1. The difference in sizes between the different *scarp* gene products can be accounted for by the number (up to seven) of internal repeats (Rep domain) within the N-terminal domain. Repeats are followed by a central conserved region of about 340 aa with 64% identity among ScARPs (Conv domain) and a variable region (VR) of 110 aa. All eight putatively expressed full-length ScARPs possess a predicted N-terminal signal peptide, allowing their membrane targeting, and a transmembrane segment that anchors the protein in the lipid bilayer of the plasma membrane [21]. Thus, a large ScARP part including repeats and the conserved and variable parts is exposed at the bacterial surface, while the short C-tail is cytoplasmic. In this work, we aimed at demonstrating that ScARPs are the targets of a specific endoproteolytic post-translational cleavage. To get clues on the underlying mechanisms, we have identified the major primary cleavage regions and the conditions, under which ScARPs undergo *in vitro* endoproteolysis.

Results

Expression of ScARPs in *S. citri* strains GII-3 and G/6 in broth culture

The predicted molecular masses of ScARPs encoded by plasmids pSci1 to 5 range from 74.6 (ScARP4a) to 92.9 kDa (ScARP5a; Table S1). Using liquid chromatography (LC) with tandem mass spectrometry (LC-MS/MS) after SDS-PAGE and slicing of migration lanes into 16 bands, all 8 ScARPs could be detected in membrane extracts of *S. citri* strain GII-3 in broth cultures. In addition, peptides derived from ScARPs sequences were recovered in all 16 bands of the gel slice, suggesting that these adhesins were extensively endoproteolyzed *in vitro*.

No ScARP-derived peptide was detected in a total cell extract from G/6 strain, lacking pSci1–5 but still having pSci6 [21] carrying the two truncated *scarp* sequences. This result confirmed the lack of expression of full-length ScARPs encoded by pSci-1-5 in strain G/6 and strongly suggested that the truncated genes carried on pSci6 were not expressed in broth culture in this strain (Table S1). Consequently, in further experiments, all fragments resulting from

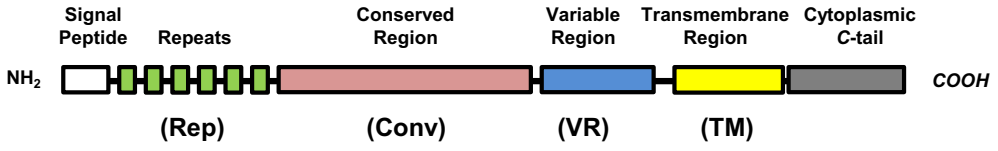

Fig. 1. Schematic representation of ScARPs encoded on plasmids pSci1–5 in *S. citri* GII-3. ScARPs possess an N-terminal and cleavable sequence, a Rep domain containing up to seven repeated motifs, a conserved central region (Conv) followed by a variable segment (VR), a transmembrane segment (TM), and a cytoplasmic C-terminal domain. ScARP4a possesses all domains, except Rep domain. Adapted from Ref. [21].