Biochimica et Biophysica Acta 1828 (2013) 938–943

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Lipid modification gives rise to two distinct *Haloferax volcanii* S-layer glycoprotein populations

Lina Kandiba [a], Ziqiang Guan [b], Jerry Eichler [a,*]

[a] Department of Life Sciences, Ben Gurion University of the Negev, Beersheva 84105, Israel
[b] Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form 15 November 2012
Accepted 19 November 2012
Available online 29 November 2012

Keywords:
Archaea
Haloferax volcanii
Lipid modification
Membrane protein
S-layer glycoprotein

ABSTRACT

The S-layer glycoprotein is the sole component of the protein shell surrounding *Haloferax volcanii* cells. The deduced amino acid sequence of the S-layer glycoprotein predicts the presence of a C-terminal membrane-spanning domain. However, several earlier observations, including the ability of EDTA to selectively solubilize the protein, are inconsistent with the presence of a trans-membrane sequence. In the present report, sequential solubilization of the S-layer glycoprotein by EDTA and then with detergent revealed the existence of two distinct populations of the S-layer glycoprotein. Whereas both S-layer glycoprotein populations underwent signal peptide cleavage and N-glycosylation, base hydrolysis followed by mass spectrometry revealed that a lipid, likely archaetidic acid, modified only the EDTA-solubilized version of the protein. These observations are consistent with the S-layer glycoprotein being initially synthesized as an integral membrane protein and subsequently undergoing a processing event in which the extracellular portion of the protein is separated from the membrane-spanning domain and transferred to a waiting lipid moiety.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

In the years when 16S rRNA sequence analysis was first employed to distinguish Archaea from Bacteria [1,2], other characteristic traits of Archaea were identified, including a unique cell wall composition (for reviews, see [3,4]). Lacking peptidoglycan, a major component of the bacterial cell wall [4,5], many Archaea are instead surrounded by a cell envelope composed solely of a protein-based surface (S)-layer, a self-assembling two-dimensional crystalline array found in intimate association with the plasma membrane [6–9]. In the case of the haloarchaea *Haloferax volcanii*, the S-layer glycoprotein is the sole component of this structure [10]. Synthesized as an 827 amino acid precursor that includes a 34 amino acid residue N-terminal signal peptide, the *Hfx. volcanii* S-layer glycoprotein also includes a stretch of 20 hydrophobic residues near the C-terminus (amino acid residues 804–823) that is thought to serve as a trans-membrane domain, anchoring the protein to the membrane [11].

With a broad range of genetic tools available for working with *Hfx. volcanii*, the species has become an important model for addressing molecular questions in Archaea. In a widely employed protocol for the transformation of *Hfx. volcanii* cells, spheroplasts are generated upon incubation with 0.5 M EDTA-containing solution [12]. Such EDTA treatment releases the S-layer glycoprotein into the surrounding growth medium [11]. A similar effect is observed in cells grown in medium lacking magnesium [13]. EDTA-generated spheroplasts, however, regain the cup-shaped morphology of the native cells when magnesium is once again provided, presumably due to a restoration of the S-layer [14]. Although the precise requirement for magnesium in S-layer biogenesis is unclear, it has been shown that in the absence of magnesium, the S-layer glycoprotein fails to experience a maturation event that transpires on the external cell surface, possibly the addition of a lipid moiety attached to the mature protein [13,15].

Based on current understanding, it is difficult to envisage how the S-layer glycoprotein can be associated with the plasma membrane simultaneously in an EDTA-sensitive, magnesium-dependent manner and via a trans-membrane domain. With the aim of clarifying this seeming paradox, the present study more closely examined the *Hfx. volcanii* S-layer glycoprotein and its mode of membrane attachment. Such efforts reveal that two S-layer glycoprotein populations co-exist, with one requiring detergent for solubilization, presumably corresponding to S-layer glycoprotein anchored to the membrane via the C-terminal trans-membrane domain, and the other being lipid-modified and associated with the membrane in an EDTA-sensitive manner.

Abbreviations: liquid chromatography-electrospray ionization mass spectrometry, LC-ESI/MS; methanol, MeOH; surface layer, S-layer; trichloroacetic acid, TCA

* Corresponding author at: Dept. of Life Sciences, Ben Gurion University of the Negev, P.O. Box 653, Beersheva 84105, Israel. Tel.: +972 8646 1343; fax: +972 8647 9175.
E-mail address: jeichler@bgu.ac.il (J. Eichler).

0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2012.11.023

2. Materials and methods

2.1. Growth conditions

Haloferax volcanii strain H53 was grown in complete medium containing 3.4 M NaCl, 0.15 M $MgSO_4 * 7H_2 0$, 1 mM $MnCl_2$, 4 mM KCl, 3 mM $CaCl_2$, 0.3% (w/v) yeast extract, 0.5% (w/v) tryptone, 50 mM Tris–HCl, pH 7.2, at 37 °C [16].

2.2. S-layer glycoprotein solubilization

Hfx. volcanii cells were grown to stationary phase ($OD_{600}=3.0$) and harvested by centrifugation (10,900×*g*, 10 min). The cell pellet was washed with 2 M NaCl, 50 mM Tris–HCl, pH 7.2 and resuspended in minimal medium to which EDTA was added at a final concentration of 50, 100, 150, 200, 300 or 500 mM, or not at all. The cells were incubated for 3–4 h at 37 °C and harvested by centrifugation. The supernatant was dialyzed against 50 mM Tris–HCl, pH 7.2, and precipitated with 15% trichloroacetic acid (TCA). The pellet was washed with 2 M NaCl, 50 mM Tris–HCl, pH 7.2, and resuspended in the same buffer to which Triton X-100 was added to a final concentration of 1%. After a 20 min incubation at room temperature, the samples were subjected to centrifugation, the supernatant was precipitated with 15% TCA. The TCA-precipitated samples were acetone-washed, incubated with sample buffer, separated on 7.5% SDS-PAGE and Coomassie-stained.

2.3. Native gel electrophoresis

S-layer glycoprotein solubilized by either Triton X-100 or EDTA treatment was examined by native gel electrophoresis performed using 7.5% Tris–glycine gels (pH 8.9) containing 0.5% Triton X-100 [17]. The gels were run at 70 V overnight at 4 °C and the S-layer glycoprotein was visualized by Coomassie staining.

2.4. Liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS)

LC-ESI/MS analysis of tryptic fragments of the S-layer glycoprotein was performed as described previously [18]. Triton X-100- and EDTA-solubilized *Hfx. volcanii* proteins were separated on 7.5% polyacrylamide gels and stained with Coomassie R-250 (Fluka, St. Louis MO). For in-gel digestion of each version of the protein, the relevant bands were excised, destained in 400 μl of 50% (vol/vol) acetonitrile (Sigma, St Louis, MO) in 40 mM NH_4HCO_3, pH 8.4, dehydrated with 100% acetonitrile, and dried using a SpeedVac drying apparatus. The S-layer glycoprotein was reduced with 10 mM dithiothreitol (Sigma) in 40 mM NH_4HCO_3 at 56 °C for 60 min and then alkylated for 45 min at room temperature with 55 mM iodoacetamide in 40 mM NH_4HCO_3. The gel pieces were washed with 40 mM NH_4HCO_3 for 15 min, dehydrated with 100% acetonitrile, and SpeedVac-dried. The gel slices were rehydrated with 12.5 ng/μl of mass spectrometry (MS)-grade Trypsin Gold (Promega, Madison, WI) in 40 mM NH_4HCO_3. The protease-generated peptides were extracted with 0.1% (*v*/*v*) formic acid in 20 mM NH_4HCO_3, followed by sonication for 20 min at room temperature, dehydration with 50% (*v*/*v*) acetonitrile, and additional sonication. After three rounds of extraction, the gel pieces were dehydrated with 100% acetonitrile, dried completely with a SpeedVac, resuspended in 5% (v/v) acetonitrile containing 1% formic acid (v/v). The protein digests, infused using static nanospray Econotips (New Objective, Woburn, MA), were separated on-line by nano-flow reverse-phase liquid chromatography (LC) by loading onto a 150-mm by 75-μm (internal diameter) by 365-μm (external diameter) Jupifer pre-packed fused silica 5-μm C_{18} 300 Å reverse-phase column (Thermo Fisher Scientific, Bremen, Germany). The sample was eluted into the LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) using a 60-min linear gradient of 0.1% formic acid (v/v) in acetonitrile/0.1% formic acid (1:19, by volume) to 0.1% formic acid in acetonitrile/0.1% formic acid (4:1, by volume) at a flow rate of 300 nl/min.

2.5. Base hydrolysis of the S-layer glycoprotein

For base hydrolysis of the S-layer glycoprotein, comparable amounts of the protein, as determined by SDS-PAGE and Coomassie staining, were combined with 0.6 ml PBS, 1 ml $CHCl_3$ and 2 ml methanol (MeOH) in a 15 ml glass tube with a teflon-lined cap to yield a single phase ($CHCl_3$:MeOH:PBS = 1:2:0.8, v/v) solution. Following addition of 200 μl (15 N) KOH and intermittent mixing by vortex for 5 min, the mixture was sonicated in a water bath for 15 min at room temperature. The sample was centrifuged (3000 rpm) for 5 min at room temperature and the supernatant was transferred to a fresh 15 ml glass tube with a Teflon-lined cap. One ml $CHCl_3$ and 1 ml PBS were added to yield a two-phase Bligh-Dyer mixture ($CHCl_3$:MeOH:PBS = 2:2:1.8, v/v). After mixing, the sample was centrifuged (3000 rpm) for 5 min at room temperature to separate the phases. The upper phase was removed and the lower $CHCl_3$ phase was dried under a stream of nitrogen. The dried lipid samples were then dissolved in 120 μL $CHCl_3$:MeOH (1:1; v/v) and analyzed by LC-ESI/MS.

For LC separation, an Ascentis Si HPLC column (5 μm, 25 cm × 2.1 mm) was used. Mobile phase A consisted of chloroform/methanol/aqueous ammonium hydroxide (800:195:5, v/v/v). Mobile phase B consisted of chloroform/methanol/water/aqueous ammonium hydroxide (600:340:50:5, v/v/v/v). Mobile phase C consisted of chloroform/methanol/water/aqueous ammonium hydroxide (450:450:95:5, v/v/v/v). The elution program consisted of the following: 100% mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 11 min. The LC gradient was then changed to 100% mobile phase C over 3 min and held at 100% C for 3 min, and finally returned to 100% A over 0.5 min and held at 100% A for 5 min. The total LC flow rate was 300 μl/min.

3. Results

3.1. Hfx. volcanii contains two distinct versions of the S-layer glycoprotein

The *Hfx. volcanii* S-layer glycoprotein can be released from the cell surface by either treatment with Triton X-100 or following chelation with EDTA (Fig. 1A). Towards determining whether both treatments solubilized the same S-layer glycoprotein population, *Hfx. volcanii* cells were grown in minimal medium lacking tryptone or yeast extract [11], treated with increasing amounts of EDTA (0–500 mM) for 3–4 h, and collected by centrifugation. Whereas the resulting supernatant was subjected to dialysis prior to TCA-precipitation and SDS-PAGE, the cell pellet was washed to remove the chelator and challenged with 1% Triton X-100. After centrifugation and TCA-precipitation, any detergent-solubilized proteins were also separated by SDS-PAGE. The minimal medium in which the *Hfx. volcanii* cells were grown contains 150 mM Mg^{2+} (as well as 3 mM Ca^{2+} and 1 mM Mn^{2+}) [16]. Yet, even following a challenge with 500 mM EDTA (i.e. a 3.3-fold excess of chelator over the concentration of divalent ions), the subsequent detergent challenge still led to the release of S-layer glycoprotein (Fig. 1B). These results thus point to the existence of distinct EDTA- and detergent-sensitive forms of the *Hfx. volcanii* S-layer glycoprotein.

Further support for the existence of sub-populations of the S-layer glycoprotein was obtained when the EDTA- and Triton X-100-solubilized versions of the protein were examined by native gel electrophoresis, using gels containing 0.5% Triton X-100. In such gels, the embedded detergent molecules bind to exposed

Download English Version:

https://daneshyari.com/en/article/10797166

Download Persian Version:

https://daneshyari.com/article/10797166

Daneshyari.com