# Lipid modification of proteins in Archaea: attachment of a mevalonic acid-based lipid moiety to the surface-layer glycoprotein of *Haloferax volcanii* follows protein translocation

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Once the newly synthesized surface (S)-layer glycoprotein of the halophilic archaeaon *Haloferax volcanii* has traversed the plasma membrane, the protein undergoes a membrane-related, $Mg^{2+}$-dependent maturation event, revealed as an increase in the apparent molecular mass and hydrophobicity of the protein. To test whether lipid modification of the S-layer glycoprotein could explain these observations, *H. volcanii* cells were incubated with a radiolabelled precursor of isoprene, [$^3$H]mevalonic acid. In Archaea, isoprenoids serve as the major hydrophobic component of archaeal membrane lipids and have been shown to modify other haloarchaeal S-layer glycoproteins, although little is known of the mechanism, site or purpose of such modification. In the present study we report that the *H. volcanii* S-layer glycoprotein is modified by a derivative of mevalonic acid and that maturation of the protein was prevented upon treatment with mevinolin (lovastatin), an inhibitor of mevalonic acid biosynthesis. These findings suggest that lipid modification of S-layer glycoproteins is a general property of halophilic archaea and, like S-layer glycoprotein glycosylation, lipid-modification of the S-layer glycoproteins takes place on the external cell surface, i.e. following protein translocation across the membrane.

Key words: isoprenylation, halophilic archaea, plasma membrane, post-translational modification, protein biosynthesis.

## INTRODUCTION

Post-translational modification of nascent polypeptide chains includes a variety of events such as removal of protein targeting and other pre-sequences, homo- and hetero-oligomerization, attachment of lipid moieties as well as glycosylation. Long thought to be uniquely a eukaryotic phenomenon, it is now established that prokaryotes are also capable of protein glycosylation [1,2]. The first prokaryotic glycoprotein to be described in detail was the surface (S)-layer glycoprotein of the halophilic archaeon *Halobacterium salinarum* [3]. Since that report, the presence of S-layer glycoproteins has been demonstrated in the cell walls of numerous archaeal species [4]. In the haloarchaeon *Haloferax volcanii*, the S-layer glycoprotein is the sole component of the proteinaceous shell which surrounds the cell [5,6]. The *H. volcanii* S-layer glycoprotein gene has been cloned, sequenced and shown to encode a 794-amino-acid-residue protein preceeded by a cleavable 34-residue signal sequence [6]. In addition to this sequence information, the chemical composition and attachment sites of the glycan moieties of the protein have also been described [6,7], as have aspects of the glycosylation process itself [8,9]. Thus, given the various modifications experienced from the time of its translation until its eventual integration into the S-layer surrounding the cell, the *H. volcanii* S-layer glycoprotein represents an excellent reporter of archaeal protein processing.

Recent studies have begun to elucidate the steps that take place during the biogenesis of the *H. volcanii* S-layer glycoprotein [10]. In *H. volcanii*, the S-layer glycoprotein is first synthesized as an immature precursor, possessing a lower apparent molecular mass than the final version of the protein. Post-translational conversion into the mature form of the protein is largely completed within the first 10 min following the appearance of the full-length polypeptide. Subcellular fractionation revealed that the modification responsible for the maturation effect only takes place once the S-layer glycoprotein has translocated across the plasma membrane. Indeed, S-layer glycoprotein modification requires $Mg^{2+}$-dependent membrane association of the protein, as no maturation was detected in the absence of $Mg^{2+}$, which is known to be involved in attachment of the S-layer glycoprotein to the membrane [5,6]. The maturation event is apparently not related to protein glycosylation, a process believed to occur on the external cell surface of haloarchaea [11], yet does result in an increased hydrophobicity of the protein, as detected by detergent-based two-dimensional gel electrophoresis.

Lipid modification of the *H. volcanii* S-layer glycoprotein offers a plausible explanation for these observations. Lipid-modified archaeal proteins, including the isoprenylated proteins of *H. salinarum*, have been reported [12–15]. In particular, the *H. salinarum* S-layer glycoprotein is modified with a diphytanylglyceryl phosphate entity at a region near the C-terminal membrane-spanning domain of the protein [15]. In what follows, we report that the *H. volcanii* S-layer glycoprotein is also modified by a mevalonic acid-based lipid moiety and that such modification is responsible for the maturation of the *H. volcanii* S-layer glycoprotein that occurs early in the biosynthesis of the protein, following protein translocation across the plasma membrane.

## EXPERIMENTAL

### Materials

Periodic acid–Schiff (PAS) reagent, trichloroacetic acid and Tween 20 were from Sigma (St. Louis, MO, U.S.A.). Mevinolin (lovastatin) came from Merck, Sharpe and Dohme (Petah Tikva, Israel) in the form of Lovalip 40 tablets. The mevinolin was extracted in ethanol and stored at −20 °C. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibodies and pre-

Abbreviations used: ECL®, (Amersham) enhanced chemiluminescence; HRP, horseradish peroxidase; PAS, periodic acid–Schiff; S-layer, surface layer.

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stained SDS/PAGE molecular mass standards came from Bio-Rad (Hercules, CA, U.S.A.). Endoproteinase Glu-C from *Staphylococcus aureus* V8 came from Boehringher-Mannheim (Mannheim, Germany). Nitrocellulose (0.45 $\mu$m pore size) came from Schleicher and Schuell (Dassel, Germany). $^{35}$S-radiolabelling mixture (> 1000 Ci/mmol) and an enhanced chemiluminescence (ECL®) kit were from Amersham International (Little Chalfont, Bucks., U.K.). [5-$^{3}$H]Mevalonic acid (60 Ci/mmol) came from American Radiolabelled Chemicals (St. Louis, MO, U.S.A.).

### Culture and radiolabelling conditions

*H. volcanii* DS2 was obtained from the American Type Culture Collection (Manassas, VA, U.S.A.) and grown aerobically at 40 °C in minimal medium [16]. For [$^{3}$H]mevalonic acid labelling, cells were incubated with radiolabel (1 $\mu$Ci/ml) for 72 h at 40 °C. The cell protein content was precipitated with 15% trichloroacetic acid (30 min on ice) and collected by centrifugation (Eppendorf microcentrifuge, 10500 ***g***, 15 min, 4 °C). Non-specifically bound lipids were removed as previously described [15]. Pulse–chase $^{35}$S-radiolabelling and subcellular fractionation were also performed as previously described [10].

### Release of the [$^{3}$H]mevalonic acid-derived moiety

To release the [$^{3}$H]mevalonic acid-derived moiety from the labelled S-layer glycoprotein, non-specifically bound lipids were first removed, as above. The delipidated radiolabelled protein was incubated with 0.5 M HCl for 2 h at either 37 or 95 °C. Following treatment, the samples were extracted with chloroform/methanol (2:1, v/v) and the level of released radioactivity was determined by scintillation counting.

### Other methods

Samples containing unlabelled or [$^{3}$H]mevalonic acid-labelled proteins were separated by SDS/PAGE (7.5% gel) and, after Coomassie Blue staining, the S-layer glycoprotein was subjected to one-dimensional peptide mapping essentially as described previously [17]. PAS reagent staining was performed as previously described [18]. For immunoblotting, SDS/PAGE-separated proteins were transferred to nitrocellulose and probed with antibodies (1:10000 in PBS containing 5% milk powder and 0.5% Tween-20) raised against a peptide corresponding to the S-layer glycoprotein N-terminal 13 amino acid residues [6,19]. Antibody binding was revealed by HRP-conjugated goat anti-rabbit antibodies and ECL®. Densitometry was performed using IPLab Gel software (Signal Analytics, Vienna, VA, U.S.A.).

## RESULTS

### The S-layer glycoprotein is modified by a derivative of mevalonic acid

During its biosynthesis, the *H. volcanii* S-layer glycoprotein undergoes a post-translational modification revealed as an increase in the molecular mass of the protein upon SDS/PAGE following pulse–chase $^{35}$S metabolic radiolabelling (Figure 1, upper panel, and [10]). To determine whether this maturation event took place before or after the S-layer glycoprotein had crossed the plasma membrane, the membrane fraction of aliquots removed at various points of the chase-phase of the reaction was isolated. As observed above (when aliquots of cell suspension were addressed), examination of the isolated membranes also revealed the presence of an immature version of the S-layer glycoprotein eventually being transformed into a slower-migrating, mature form of the protein (Figure 1, lower panel, and [10]). The S-layer glycoprotein is anchored to the membrane via a membrane-spanning domain located very close to the C-terminus of the protein [6]. As such, the presence of full-length immature S-layer glycoprotein in membranes isolated from the earliest points of the chase phase of the reaction suggests that translocation of the protein up to at least the C-terminal membrane-spanning domain had occurred during this interval. The subsequent maturation of the membrane-associated protein (as reflected by an increase in molecular mass) suggests, therefore, that S-layer glycoprotein maturation occurs only after the protein has translocated across the plasma membrane. Although the basis for the maturation event is not known, it has been previously shown that the increase in the apparent molecular mass of the S-layer glycoprotein maturation is not the result of protein glycosylation, but is associated with increased hydrophobicity of the protein [10]. Thus, in the present study, experiments aimed at better understanding the molecular basis of the maturation event were undertaken.

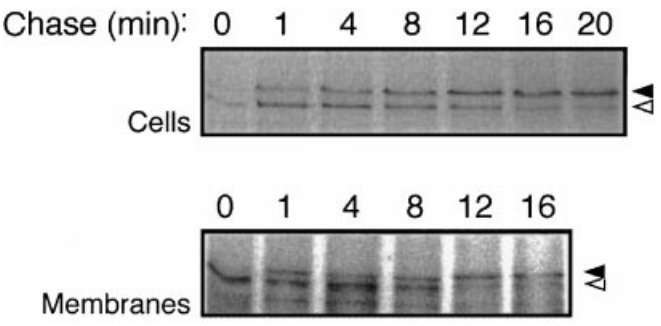


**Figure 1 *H. volcanii* S-layer glycoprotein undergoes a maturation event following its transport across the plasma membrane**

Cells (upper panel): cells were $^{35}$S-metabolically radiolabelled and then chased with an excess of unlabelled methionine. Aliquots were removed immediately prior to addition of unlabelled methionine and then at intervals following onset of the chase. The samples were then examined by SDS/7.5% PAGE and fluorography. Membranes (lower panel): membranes from pulse–chase radiolabelled cells were separated and their S-layer glycoprotein content was examined. In each panel, the solid arrowhead reflects the position of the mature S-layer, while the open arrowhead reflects the position of the immature version of the protein.

To determine whether a lipid modification event could explain the post-translational post-translocational membrane-associated maturation of the *H. volcanii* S-layer glycoprotein, preliminary experiments aimed at discerning whether the S-layer glycoprotein incorporates an isoprene-based lipid moiety were conducted. In Archaea such as *H. volcanii*, isoprene is a major component of membrane lipids [20], while isoprenylation of haloarchaeal S-layer glycoproteins has been previously reported [15]. Accordingly, *H. volcanii* cells were incubated with a $^{3}$H-radiolabelled version of the isoprene precursor, mevalonic acid. Following a 72 h incubation, the cellular protein content was precipitated and analysed by SDS/PAGE (7.5% gel) and fluorography (Figure 2). In such experiments, a 190 kDa protein band was most prominently radiolabelled (Figure 2, arrow, lane 1), although other proteins, such as the ~ 60 kDa band shown in Figure 2, also incorporated the radiolabel. Assignment of the 190 kDa radiolabelled band as the S-layer glycoprotein was confirmed by immunoblotting with anti-(S-layer glycoprotein) antibodies (Figure 2, lane 2) and PAS staining (Figure 2, lane 3), which exploits the fact that the S-layer glycoprotein is the prominent glycoprotein species in *H. volcanii* [19].

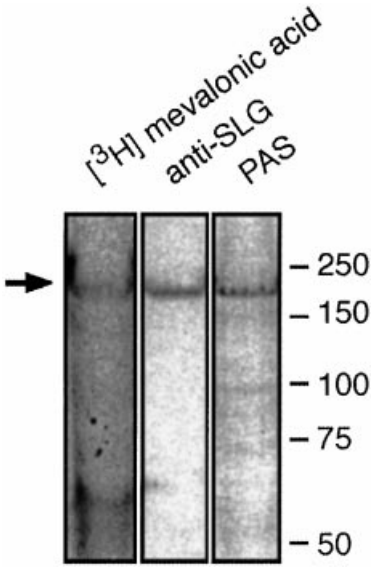


**Figure 2 *H. volcanii* S-layer glycoprotein can be labelled by [$^3$H]mevalonic acid**

*H. volcanii* cells (5 ml) were grown for 72 h in the presence of 5 $\mu$Ci of [$^3$H]mevalonic acid for 72 h. Aliquots were then removed and processed for SDS/PAGE and examined by fluorography (lane 1), immunoblotting with anti-(S-layer glycoprotein) antibodies (lane 2) or PAS staining (lane 3). The arrow on the left depicts the position of the S-layer glycoprotein, whereas the positions of molecular-mass markers (kDa) are shown on the right.

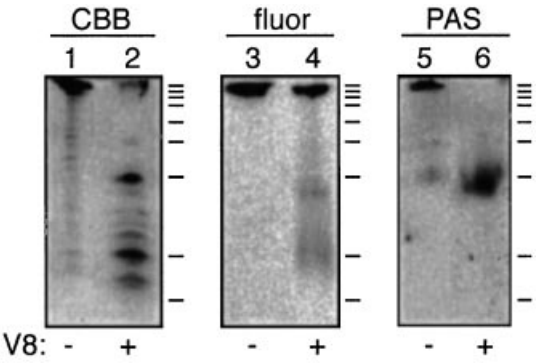


**Figure 3 One-dimensional peptide mapping of the *H. volcanii* S-layer glycoprotein**

The S-layer glycoprotein from [$^3$H]mevalonic acid-treated *H. volcanii* cells was subjected to one-dimensional peptide mapping upon addition of 0.05 $\mu$g of *S. aureus* V8 endoproteinase Glu-C (lanes 2, 4 and 5). After SDS/PAGE, gel lanes were Coomassie Brilliant Blue stained (CBB; lanes 1 and 2), processed for fluorography (fluor; lanes 3 and 4) or PAS stained (PAS; lanes 5 and 6). The marks on the right of each panel correspond (from the top) to 250, 150, 100, 75, 50, 37, 25, 15 and 10 kDa molecular-mass markers.

To address the site of the mevalonic acid-based modification, the $^3$H-labelled S-layer glycoprotein was digested with *S. aureus* V8 endoproteinase Glu-C. Such treatment led to the appearance of radiolabelled 24 and 15 kDa protein bands (Figure 3, lane 4). To determine whether these fragments contained glycan moieties of the S-layer glycoprotein, the V8-endoproteinase-generated peptide fragments were PAS-stained. The glycan stain clearly labelled the 24 kDa degradation product (Figure 3, lane 5). Similarly, proteolytic digestion of the *H. salinarum* S-layer

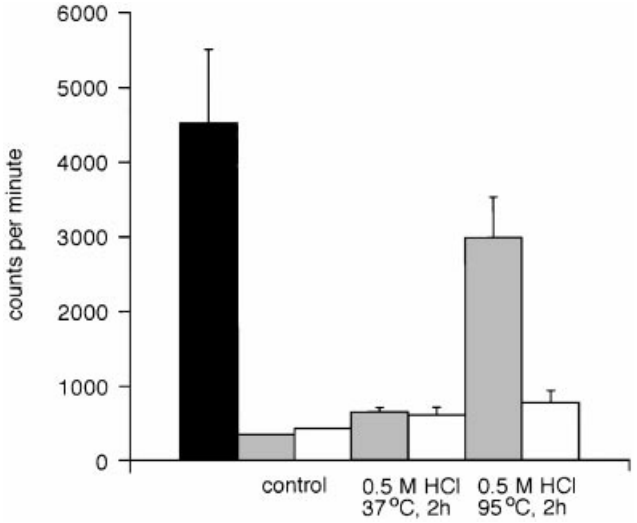


**Figure 4 The [$^3$H]mevalonic acid-derived radiolabel can be removed from the S-layer glycoprotein by harsh acid treatment**

Delipidated [$^3$H]mevalonic acid-labelled S-layer glycoprotein was incubated in water or in 0.5 M HCl at 37 or 95 °C for 2 h. The samples were then extracted in chloroform/methanol (2:1, v/v) and the released radioactivity was counted. The radioactivity released in both the aqueous and organic phases, as well as the total radioactivity present in the samples, are shown as the average for two to three experiments ± S.D. Black bar, total radioactivity (c.p.m.); grey bars, radioactivity in the aqueous phase; white bars, radioactivity in the organic phase.

glycoprotein from cells incubated with radioactive isoprenoid precursors also yielded a PAS-stainable radiolabelled peptide fragment of similar size [15]. In *H. salinarum*, the stained 28 kDa peptide fragment was localized to a region near the C-terminus of the protein. As the C-terminal regions of the *H. volcanii* and *H. salinarum* proteins are not only similar in terms of amino acid sequence, but also in terms of their pronounced glycosylation [3,6], it is possible that the sites of modification in the S-layer glycoproteins of the two halophilic archaeal strains are similar [15].

### Release of the S-layer glycoprotein-derived [$^3$H]mevalonic acid-based moiety

As the [$^3$H]mevalonic acid label remains associated with an S-layer glycoprotein peptide fragment after delipidation, proteolytic digestion and SDS/PAGE, it is likely that the mevalonic acid-derived moiety is covalently linked to the protein, rather than simply being loosely associated with it. To test this hypothesis, [$^3$H]mevalonic acid-labelled *H. volcanii* S-layer glycoprotein was incubated with 0.5 M HCl for 2 h at either 37 °C or 95 °C. Following these treatments, the extent of release of the radiolabelled moiety was assessed by chloroform/methanol extraction (Figure 4). In control experiments, negligible amounts of the radiolabel were released into either the aqueous or organic phases of the extraction solution, with the bulk of the radioactivity lying in the interface between the two. Similary, the mild-acid treatment failed to release significant amounts of radioactivity from the labelled *H. volcanii* S-layer glycoprotein. In contrast, the harsher acid treatment resulted in the release of a substantially higher level (66 %) of the radioactivity associated with the delipidated protein into the aqueous phase of the reaction. In contrast, only control levels of radioactivity were released into the organic phase following such treatment. These results suggest that a phosphoester- or phosphodiester-type

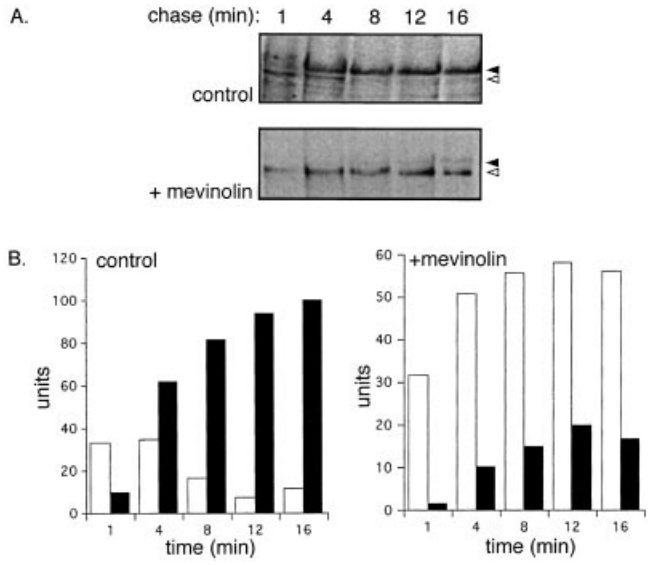


**Figure 5 Mevinolin interferes with *H. volcanii* S-layer glycoprotein maturation**

(**A**) *H. volcanii* cells, grown in minimal medium, were incubated in the absence (upper panel, control) or presence (lower panel, + mevinolin) of 1.4 $\mu$g/ml mevinolin for 14 h. The cultures were then labelled with [$^{35}$S]methionine for 3 min, after which an excess of unlabelled methionine was added. Aliquots were removed at various time points and processed for SDS/PAGE and fluorography. In each panel, the solid arrowhead reflects the position of the mature S-layer, while the open arrowhead reflects the position of the immature version of the protein. (**B**) Densitometric analysis of the data presented in (**A**) is shown. In the control (left) and mevinolin-treated (right) samples, empty bars correspond to the intensity of the immature form of the S-layer glycoprotein, while the full bars correspond to the intensity of the mature form of the protein at each time point.

linkage is involved in attachment of the mevalonic acid-based moiety to the S-layer glycoprotein. Thus, the manner of linkage between haloarchaeal S-layer glycoproteins and their isoprenoid moieties may be conserved, since similar behaviour was reported during attempts at releasing the lipid moiety from the *H. salinarum* S-layer glycoprotein [15].

### Mevalonic acid-based modification is responsible for maturation of the *H. volcanii* S-layer glycoprotein

To determine whether modification by a mevalonic acid-derived compound accounts for the post-translational maturation of the *H. volcanii* S-layer glycoprotein that occurs after translocation of the protein to the external cell surface (Figure 1 and [10]), the effect of pretreatment with mevinolin on S-layer glycoprotein maturation was addressed. Mevinolin inhibits the function of 3-hydroxy-3-methylglutaryl-CoA reductase, the enzyme responsible for the conversion of acetyl-CoA into mevalonic acid, thereby blocking an important step on the mevalonic acid-dependent isoprene-synthetic pathway [21]. Moreover, the drug has been previously shown to interfere with isoprene synthesis in *H. volcanii* [22]. Accordingly, *H. volcanii* cells were subjected to [$^{35}$S]methionine pulse–chase radiolabelling in the absence or presence of a sub-lethal concentration of mevinolin. In control experiments, an immature form of the radiolabelled S-layer glycoprotein was first detected following a 3 min pulse label with [$^{35}$S]methionine. However, within 8 min of the onset of a chase with an excess of unlabelled methionine, this precursor was converted into the mature version of the protein (Figure 5A, upper panel), as previously shown (Figure 1 and [10]). Pretreatment of *H. volcanii* cells with 1.4 $\mu$g/ml mevinolin for 14 h at 40 °C, however, led to a drastic reduction in S-layer glycoprotein maturation, even after 16 min of chase with unlabelled methionine (Figure 5A, lower panel) or even as long as 30 min post-chase (results not shown). Densitometric quantification of the levels of immature and mature S-layer glycoprotein confirmed the decrease in the amount of the immature version of the protein, concomitant with an increase in the level of the mature S-layer glycoprotein in the control cells. Such analysis also revealed the relatively constant level of immature S-layer glycoprotein in the treated cells, as well as a minor increase in mature protein levels (Figure 5B). To confirm that the mevinolin-mediated prevention of S-layer glycoprotein maturation was not the result of a general inhibitory effect of the drug on cell growth, the cell density of both cultures was measured. Exposure of the cells to this level of mevinolin for 14 h under the growth conditions employed only decreased culture density by 35% (wild-type cells, $D_{550} = 0.59 \pm 0.05$, $n = 3$; treated cells, $D_{550} = 0.38 \pm 0.02$, $n = 3$; $D$ is attenuance). This, together with the similar levels of total radiolabel-incorporation in the two versions of the S-layer glycoprotein (Figure 5B), imply that the mevinolin-mediated prevention of S-layer glycoprotein maturation was not the result of a non-specific inhibitory effect on overall cellular function, but rather reflects an S-layer glycoprotein maturation-specific event.

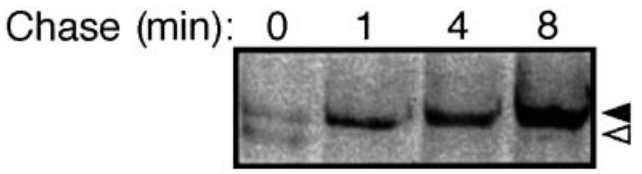


**Figure 6 Nascent *H. salinarum* S-layer glycoprotein also undergoes a maturation step**

Cells were $^{35}$S-metabolically radiolabelled for 3 min followed by addition of an excess of unlabelled methionine. Aliquots were removed immediately prior to addition of unlabelled methionine and then 1, 4 and 8 min following onset of a chase with an excess of unlabelled methionine. The samples were then examined by SDS/7.5% PAGE and fluorography.

### The isoprenylated S-layer glycoprotein of *H. salinarum* also undergoes maturation

Like its *H. volcanii* counterpart, the S-layer glycoprotein of *H. salinarum* also experiences various processing steps. To determine whether a maturation event, such as that described above, also occurs for newly synthesized S-layer glycoprotein in *H. salinarum*, $^{35}$S-pulse–chase radiolabelling was performed on *H. salinarum* cells. As observed in the case of *H. volcanii*, the *H. salinarum* S-layer glycoprotein also undergoes an increase in apparent molecular mass soon after its biosynthesis (Figure 6). In *H. salinarum*, however, modification was largely completed by the fourth minute of the chase phase of the experiment, i.e. much faster than modification observed in *H. volcanii*. The finding that a known isoprenylated haloarchaeal S-layer glycoprotein also experiences a maturation step offers indirect support for the concept that a post-translocational isoprenylation event forms the basis for the maturation of the *H. volcanii* S-layer glycoprotein [15]. Indeed, it has been previously shown that the *H. salinarum* S-layer glycoprotein undergoes glycosylation on the external cell surface [23], revealing the presence of protein-modifying enzymes on the outer surface of the haloarchaeal plasma membrane.

## DISCUSSION

In showing modification of the *H. salinarum* S-layer glycoprotein by a diphytanylglyceryl phosphate moiety near the membrane-spanning C-terminal region of the protein, it was suggested that the C-terminal region of the *H. volcanii* S-layer glycoprotein may also be lipid-modified, given the similarities in the amino acid sequences of these portions of the proteins [6,15]. Accordingly, the present study offers support to the concept that isoprenoid modification of S-layer glycoproteins is not restricted to *H. salinarum*, but rather may be a general trait in halophilic Archaea. Moreover, it appears that such modification is involved in the post-translational post-translocational maturation of the S-layer glycoprotein in *H. volcanii*. The claim is based on the ability of sub-lethal concentrations of mevinolin to interfere with the maturation event, together with the results of earlier experiments correlating *H. volcanii* S-layer glycoprotein maturation with an increase in the hydrophobicity of the protein and the observed requirement for membrane association of the glycoprotein for its maturation to occur [10]. Additionally, the isoprenylated S-layer glycoprotein of *H. salinarium* also undergoes a maturation process similar to that experienced by its *H. volcanii* counterpart, suggesting that the maturation process may be widespread in the haloarchaea.

Although isoprene-based compounds serve a variety of biological functions, ranging from involvement in electron-transport chains to protein regulation and intracellular protein traffic to plant defence [24,25], it remains unclear why halophilic archaea attach isoprene-based moieties to S-layer glycoproteins already anchored to the membrane by a transmembrane domain. One possibility would be to offer physical support to the S-layer itself, thereby creating a periplasmic-like space [26]. Alternatively, lipid modification of membrane-inserted proteins in Archaea may reflect a primitive version of a mode of protein membrane association widely used in Eukarya. At the experimental level, lipid modification was proposed as the basis for the aberrant migration of haloarchaeal S-layer glycoproteins [15]. Although the molecular masses of the *H. volcanii* and *H. salinarum* S-layer glycoproteins, as predicted by their deduced amino acid sequences, are 86.5 and 81.7 kDa respectively [3,6], both proteins migrate as $\approx$200 kDa proteins on SDS/PAGE. Similarly, the predicted 87.1 kDa *Haloarcula japonica* S-layer glycoprotein migrates as a 170 kDa protein [27]. In the case of the *H. salinarum* protein, it was shown that this retarded migration was not related to protein glycosylation [28]. The present study would, however, discount the lipid moiety as being responsible for the unexpected electrophoretic behaviour of haloarchaeal S-layer glycoproteins, given that the mevinolin-treated *H. volcanii* S-layer glycoprotein also behaves as a $\approx$200 kDa species upon gel electrophoresis. Instead, it is likely that an abundance of acidic amino acid residues is responsible for the retarded SDS/PAGE migration of the S-layer glycoproteins [3,6,27].

In addition to revealing the lipidation of a second haloarchaeal S-layer glycoprotein, the present study shows that, like protein glycosylation [11,23] and (most likely) signal sequence cleavage [29], lipid modification of proteins in Archaea represents another post-translational modification that takes place on the external surface of the plasma membrane. Such protein processing is apparently not restricted to *H. volcanii*, as maturation of the S-layer glycoprotein also occurs in the case of the isoprenylated S-layer glycoprotein of *H. salinarum*, similarly detected as an increase in apparent molecular mass soon after protein biosynthesis. Isoprenylation may not, however, be the sole form of lipid modification experienced by exported archaeal proteins, as glycosylphosphatidylinositol-anchored proteins have also been detected in Archaea. Indeed, one of these, a 185 kDa species, could be released from the membrane by a bacterial phosphatidylinositol-specific phospholipase C, suggesting an external orientation of the lipid-anchored protein [13]. These observations present a scenario reminiscent of the situation in Eukarya, where selected proteins experience a series of post-translational modifications (including the covalent attachment of lipid moieties) at the luminal face of the endoplasmic-reticulum membrane, i.e. once they have translocated across the membrane [30–32]. Hence, the external archaeal cell surface not only corresponds to the topological homologue of the endoplasmic-reticulum membrane, but, in several instances, may represent its functional homologue.

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## REFERENCES

1 Messner, P. (1997) Bacterial glycoproteins. Glycoconj. J. **14**, 3–11
2 Moens, S. and Vanderleyden, J. (1997) Glycoproteins in prokaryotes. Arch. Microbiol. **168**, 169–175
3 Lechner, J. and Sumper, M. (1987) The primary structure of a procaryotic glycoprotein. Cloning and sequencing of the cell surface glycoprotein gene of halobacteria. J. Biol. Chem. **262**, 9724–9729
4 Messner, P. and Sleytr, U. B. (1992) Crystalline bacterial cell-surface layers. Adv. Microb. Physiol. **33**, 213–275
5 Charlebois, R. L., Lam, W. L., Cline, S. W. and Doolittle, W. F. (1987) Characterization of pHV2 from *Halobacterium volcanii* and its use in demonstrating transformation of an archaebacterium. Proc. Natl. Acad. Sci. U.S.A. **84**, 8350–8354
6 Sumper, M., Berg, E., Mengele, R. and Strobel, I. (1990) Primary structure and glycosylation of the S-layer protein of *Haloferax volcanii*. J. Bacteriol. **172**, 7111–7118
7 Mengele, R. and Sumper, M. (1992) Drastic differences in glycosylation of related S-layer glycoproteins from moderate and extreme halophiles. J. Biol. Chem. **267**, 8182–8185
8 Zhu, C. R., Drake, R. R., Schweingruber, H. and Laine, R. A. (1995) Inhibition of glycosylation by amphomycin and sugar nucleotide analogs PP36 and PP55 indicates that *Haloferax volcanii* $\beta$ glycosylates both glycoproteins and glycolipids through lipid-linked sugar intermediates. Arch. Biochem. Biophys. **319**, 355–364
9 Kuntz, C., Sonnenbichler, J., Sonnenbichler, I., Sumper, M. and Zeitler, R. (1997) Isolation and characterization of dolichol-liinked oligosaccharides from *Haloferax volcanii*. Glycobiology **7**, 897–904
10 Eichler, J. (2001) Post-translational modification unrelated to protein glycosylation follows translocation of the S-layer glycoprotein across the plasma membrane of the haloarchaeon *Haloferax volcanii*. Eur. J. Biochem. **268**, 4366–4373
11 Sumper, M. (1987) Halobacterial glycoprotein biosynthesis. Biochim. Biophys. Acta **906**, 69–79
12 Pugh, E. L. and Kates, M. (1994) Acylation of proteins of the archaebacteria *Halobacterium cutirubrum* and *Methanobacterium thermoautotrophicum*. Biochim. Biophys. Acta **1196**, 38–44
13 Kobayashi, T., Nishizaki, R. and Ikezawa, H. (1997) The presence of GPI-linked protein(s) in an archaeobacterium, *Sulfolobus acidocaldarius*, closely related to eukaryotes. Biochim. Biophys. Acta **1334**, 1–4
14 Sagami, H., Kikuchi, A. and Ogura, D. (1995) A novel type of protein modification by isoprenoid derived materials. Diphytanylglycerylated proteins in Halobacteria. J. Biol. Chem. **270**, 14851–14854
15 Kikuchi, A., Sagami, H. and Ogura, K. (1999) Evidence for covalent attachment of diphytanylglyceryl phosphate to the cell-surface glycoprotein of *Halobacterium halobium*. J. Biol. Chem. **274**, 18011–18016
16 Mevarech, M. and Werczberger, R. (1985) Genetic transfer in *Halobacterium volcanii*. J. Bacteriol. **162**, 461–462
17 Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**, 1102–1106
18 Dubray, G. and Bezard, G. (1982) A highly sensitive periodic acid-silver stain for 1,2-diol groups of glycoproteins and polysaccharides in polyacrylamide gels. Anal. Biochem. **119**, 325–329
19 Eichler, J. (2000) Novel glycoproteins of the halophilic archaeon *Haloferax volcanii*. Arch. Microbiol. **173**, 445–448
20 Kates, M. (1993) Biology of halophilic bacteria, Part II. Membrane lipids of extreme halophiles: biosynthesis, function and evolutionary significance. Experientia **9**, 1027–1036

21 Cabrera, J. A., Bolds, J., Shields, P. E., Havel, C. M. and Watson, J. A. (1986) Isoprenoid synthesis in *Halobacterium halobium*. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A concentration in response to mevalonate availability. J. Biol. Chem. **261**, 3578–3583
22 Lam, W. L. and Doolittle, W. F. (1992) Mevinolin-resistant mutations identify a promoter and the gene for a eukaryote-like 3-hydroxy-3-methylglutaryl-coenzyme A reductase in the archaebacterium *Haloferax volcanii*. J. Biol. Chem. **267**, 5829–5834
23 Lechner, J., Wieland, F. and Sumper, M. (1985) Transient methylation of dolichyl oligosaccharides is an obligatory step in halobacterial sulfated glycoprotein biosynthesis. J. Biol. Chem. **260**, 8984–8989
24 Sinensky, M. (2000) Functional aspects of polyisoprenoid protein substituents: roles in protein-protein interaction and trafficking. Biochim. Biophys. Acta **1484**, 93–106
25 Edwards, P. A. and Ericsson, J. (1999) Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. Annu. Rev. Biochem. **68**, 157–185
26 Kessel, M., Wildehaber, I., Cohen, S. and Baumeiser, W. (1988) Three-dimensional structure of the regular surface glycoprotein layer of *Halobacterium volcanii* from the Dead Sea. EMBO J. **7**, 1549–1554
27 Wakai, H., Nakamura, S., Kawasaki, H., Takada, K., Mizutani, S., Aono, R. and Horikoshi, K. (1997) Cloning and sequencing of the gene encoding the cell surface glycoprotein of *Haloarcula japonica* strain TR-1. Extremophiles **1**, 29–35
28 Mescher, M. F. and Strominger, J. L. (1976) Structural (shape-maintaining) role of the cell surface glycoprotein of *Halobacterium salinarium*. Proc. Natl. Acad. Sci. U.S.A. **73**, 2687–2691
29 Eichler, J. (2002) Archaeal signal peptidases from the genus *Thermoplasma*: structural and mechanistic hybrids of the bacterial and eukaryal enzymes. J. Mol. Evol. **54**, 411–415
30 Shelness, G. S., Lin, L. and Nicchitta, C. V. (1993) Membrane topology and biogenesis of eukaryotic signal peptidase. J. Biol. Chem. **268**, 5201–5208
31 Wang, J., Maziarz, K. and Ratnam, M. (1999) Recognition of the carboxyl-terminal signal for GPI modification requires translocation of its hydrophobic domain across the ER membrane. J. Mol. Biol. **286**, 1303–1310
32 Schenk, B., Fernandez, F. and Waechter, C. J. (2001) The ins(ide) and outs(ide) of dolichyl phosphate biosynthesis and recycling in the endoplasmic reticulum. Glycobiology **11**, 61R–70R

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