Add salt, add sugar: N-glycosylation in \textit{Haloferax volcanii}

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Abstract

Although performed by members of all three domains of life, the archaeal version of N-glycosylation remains the least understood. Studies on \textit{Haloferax volcanii} have, however, begun to correct this situation. A combination of bioinformatics, molecular biology, biochemical and mass spectrometry approaches have served to delineate the Agl pathway responsible for N-glycosylation of the S-layer glycoprotein, a reporter of this post-translational modification in \textit{Hfx. volcanii}. More recently, differential N-glycosylation of the S-layer glycoprotein as a function of environmental salinity was demonstrated, showing that this post-translational modification serves an adaptive role in \textit{Hfx. volcanii}. Furthermore, manipulation of the Agl pathway, together with the capability of \textit{Hfx. volcanii} to N-glycosylate non-native proteins, forms the basis for establishing this species as a glyco-engineering platform. In the present review, these and other recent findings are addressed.

Introduction

Across evolution, analysis of the proteome reveals additional levels of complexity not predicted at the genome level. Post-translational modifications are a major source of this proteomic diversity. Insight into how such protein-processing events transpire in Archaea has come from studies on \textit{Halofex volcanii}, where numerous and varied examples of post-translational modifications have been reported [1]. N-glycosylation, the covalent linkage of glycan moieties to select asparagine residues of a target protein, was among the first post-translational modifications to be described in \textit{Hfx. volcanii}.

In \textit{Hfx. volcanii}, the surface (S)-layer glycoprotein, comprising the sole component of the S-layer surrounding the cell, contains seven putative N-glycosylation sites, namely the Asn-Xaa-Ser/Thr sequence motif, where Xaa is any residue but proline [2]. Early studies had reported modification of the S-layer glycoprotein by linear strings of glucose residues, as well as by a second glycan containing glucose, galactose and idose subunits [2,3]. The recent use of more sophisticated MS tools has, however, served to revise the composition of the N-linked glycans decorating the S-layer glycoprotein [4–6]. At the same time, studies combining gene deletion with lipid- and protein-linked glycan analysis have served to delineate a pathway of N-glycosylation in \textit{Hfx. volcanii} [7]. In the present review, recent discoveries regarding \textit{Hfx. volcanii} N-glycosylation are discussed. In particular, the adaptive role of \textit{Hfx. volcanii} N-glycosylation to changes in the environment and the manipulation of the \textit{Hfx. volcanii} N-glycosylation pathway for glyco-engineering purposes are addressed.

Although beyond the scope of the present review, it should be stressed that our present understanding of archaeal N-glycosylation is not solely based on results obtained using \textit{Hfx. volcanii} as a model system. Indeed, recent studies of the methanogens \textit{Methanococcus voltae} and \textit{Methanococcus maripaludis}, and the thermophiles \textit{Sulfolobus acidocaldarius}, \textit{Pyrococcus furiosus} and \textit{Archaeoglobus fulgidus} have also provided considerable insight into the process of N-glycosylation in Archaea [8–13].

The \textit{Hfx. volcanii} Agl pathway

Over the last few years, substantial progress in deciphering the pathway of \textit{Hfx. volcanii} N-glycosylation has been made stemming from the identification of a series of \textit{agl} (archaeal glycosylation) genes encoding proteins involved in the assembly and attachment of a pentasaccharide comprising a hexose, two hexuronic acids, the methyl ester of a hexuronic acid and a mannose to select asparagine residues of the S-layer glycoprotein. The \textit{agl} genes were originally identified on the basis of the homology of their protein products with components of the better-defined eukaryal and bacterial N-glycosylation pathways [14]. Additional \textit{agl} genes were later identified on the basis of their genomic proximities to the first set of \textit{agl} genes [15]. Indeed, all but one of the \textit{agl} genes (i.e. \textit{aglD}) are clustered into a single gene island. Moreover, as the major protein species in \textit{Hfx. volcanii}, the S-layer glycoprotein represents a convenient reporter for studying the roles of the \textit{agl} gene products in N-glycosylation.

Acting at the cytoplasmic face of the plasma membrane, AglJ, AglG, AglI and AglE are glycosyltransferases that sequentially add the first four pentasaccharide residues on to a common DolP (dolichol phosphate) carrier, while AglD adds the final pentasaccharide residue, mannose, to a distinct DolP [4–6,16–19]. The use of DolP as the
lipid carrier during N-linked glycan assembly is also the case in eukaryal N-glycosylation [20]; bacterial N-linked glycans are instead assembled on a different polyisoprenoid, undecaprenol pyrophosphate [21,22]. In *Hfx. volcanii*, N-glycosylation-related roles have also been assigned to AglF, a glucose-1-phosphate uridyltransferase [19], AglM, a UDP-glucose dehydrogenase [19], and AglP, a methyltransferase [5]. Indeed, AglF and AglM were shown to act in a sequential and co-ordinated manner in vitro, transforming glucose 1-phosphate into UDP-glucuronic acid [19]. In a reaction requiring the archaeal oligosaccharyltransferase, AglB [8,9,14], the lipid-linked tetrasaccharide and its precursors are delivered to select asparagine residues of the S-layer glycoprotein. The final mannose residue is subsequently transferred from its DolP carrier to the protein-bound tetrasaccharide [6]. Current understanding of the *Hfx. volcanii* Agl pathway is depicted in Figure 1.

**Hfx. volcanii as a glyco-engineering platform**

Although N-glycosylation has only been addressed in a limited number of species, genomic analysis predicts this post-translational modification to be common in Archaea [23]. At the same time, the glycans N-linked to archaeal glycoproteins present a diversity of sugar subunits not seen in either Eukarya or Bacteria [24]. With these points in mind, attempts at developing an archaeal glyco-engineering system capable of generating designer glycoproteins tailored for enhanced activity, stability or longevity in the face of extreme conditions are warranted. Specifically, with a relatively well-defined N-glycosylation pathway and the availability of appropriate tools for genetic manipulation, *Hfx. volcanii* is a promising candidate upon which to establish a glyco-engineering platform. However, before such efforts can proceed, two criteria must be met. First, it is necessary to develop a series of *Hfx. volcanii* strains capable of performing differential N-glycosylation. Secondly, it is necessary to show that *Hfx. volcanii* is capable of N-glycosylating non-native proteins. Of late, progress has been made on both of these fronts. By replacing components of the Agl pathway with genes encoding homologous proteins from *Halobacterium salinarum*, *Haloquadratum walsbyi* or *Haloarcula marismortui*, *Hfx. volcanii* strains capable of decorating the S-layer glycoprotein with N-linked glycans distinct from the pentasaccharide normally decorating this protein were created [25,26]. At the same time, it was shown that VP4 (viral protein 4), the major structural protein of HRPV-1 (*Halorubrum* pleomorphic virus 1), is N-glycosylated at the same sites when expressed in either the native host, *Halorubrum* sp. strain PV6, or in *Hfx. volcanii*. The composition of the N-linked glycan in each case was, however, species-specific, with *Hfx. volcanii* adding the same pentasaccharide as N-linked to the S-layer glycoprotein [27]. These proof-of-concept results justify the continuation of efforts to develop *Hfx. volcanii* into a versatile glyco-engineering platform.
Different haloarchaea, different N-glycosylation strategies

The insight into archaeal N-glycosylation gained by studying *Hfx. volcanii* has served to elucidate aspects of this post-translational modification in other Haloarchaea. Recent efforts revealed that the S-layer glycoprotein of both *Hfx. volcanii* and *Har. marismortui* are decorated with the same N-linked pentasaccharide [28]. Nonetheless, differences in the N-glycosylation pathways of these two Haloarchaea exist. Whereas the N-linked pentasaccharide decorating the *Hfx. volcanii* S-layer glycoprotein is derived from a tetrasaccharide sequentially assembled on a common DolP carrier and a final mannose residue derived from a distinct DolP carrier [6], the same pentasaccharide N-linked to the *Har. marismortui* S-layer glycoprotein is fully assembled on a single DolP, from where it is transferred to the protein [28]. As such, haloarchaeal N-glycosylation relies on pathways reminiscent of either the parallel eukaryal or bacterial processes.

The *Hfx. volcanii* N-glycosylation pathway, involving multiple glycan-charged DolP carriers, recalls its counterpart in higher Eukarya. Here, the first seven subunits of the 14-meric oligosaccharide assembled in the ER (endoplasmic reticulum) are sequentially added to a common dolichol pyrophosphate carrier; the second set of seven sugar subunits are derived from single mannose- or glucose-charged DolP [20,29]. The finding that the final mannose of the N-linked pentasaccharide decorating the *Hfx. volcanii* S-layer glycoprotein is added to the tetrasaccharide already attached to the protein [28] shows further the eukaryal-like nature of N-glycosylation in this organism. In Eukarya, N-glycosylation begins with the delivery of a lipid-linked oligosaccharide to the target protein in the ER. Modification continues in the Golgi, where additional sugar subunits are attached to the oligosaccharide already N-linked to the target protein. By contrast, by relying on a single glycan-charged DolP carrier, N-glycosylation in *Har. marismortui* is similar to the parallel bacterial process, as exemplified by *Campylobacter jejuni*. Here, a heptasaccharide is assembled by the sequential addition of seven soluble nucleotide-activated sugars on to a common undecaprenol phosphate carrier. The complete heptasaccharide is then delivered to the target protein and does not undergo further processing [21,22,30].

N-glycosylation as an adaptive response to environmental salinity

Although considerable advances in our understanding of archaeal N-glycosylation have been realized using *Hfx. volcanii* as a model system, numerous questions remain unanswered. One of the more pressing open questions concerns the identity of the flippase(s) responsible for translocating glycan-charged DolP across the plasma membrane during N-glycosylation. Indeed, little is known of lipid-linked oligosaccharide flippases in any glycosylation system. The agents responsible for several other predicted steps of the *Hfx. volcanii* Agl pathway also have yet to be reported, such as the enzyme responsible for delivering mannose from its DolP carrier to the S-layer glycoprotein-bound tetrasaccharide. Furthermore, little is known of DolP biosynthesis in *Hfx. volcanii*.

The fact that the *Hfx. volcanii* S-layer glycoprotein undergoes differential N-glycosylation as a function of environmental salinity raises a distinct set of questions. What advantage does this differential N-glycosylation offer the cell? What enzymes contribute to the biogenesis of the ‘low-salt’ tetrasaccharide? Is AglB responsible for delivering the ‘low-salt’ tetrasaccharide from its DolP carrier to the S-layer glycoprotein-bound tetrasaccharide? If this is indeed the case, then *Hfx. volcanii* AglB would have to be more versatile that any known oligosaccharidetransferase. Finally, one can ask whether additional variations in S-layer N-glycosylation occur in response to other environmental conditions.

Clearly, further study of *Hfx. volcanii* N-glycosylation will continue to reveal new twists on this universal post-translational modification. The future indeed looks sweet.

What next?

Although considerable advances in our understanding of the ability of *Hfx. volcanii* to grow in high salt, S-layer stability and architecture, and S-layer resistance to added protease [4,17–19], cells lacking AglB, and hence unable to perform N-glycosylation, are viable [4]. Thus, although not essential for *Hfx. volcanii* survival, N-glycosylation is, nonetheless, advantageous to *Hfx. volcanii* in certain scenarios. As such, one can propose that *Hfx. volcanii* modifies aspects of N-glycosylation in response to changing growth conditions. Recently, this hypothesis was tested and shown to be true when N-glycosylation of the S-layer glycoprotein was compared in cells grown in 3.4 or 1.75 M NaCl-containing medium [31]. At the higher level of salinity, S-layer glycoprotein Asn13 and Asn83 were shown to be modified by the pentasaccharide described above, whereas DolP was shown to be modified by the tetrasaccharide comprising the first four pentasaccharide residues, again as discussed above. In contrast, cells grown at the lower level of salinity were shown to contain DolP modified by a distinct tetrasaccharide comprising a sulfated hexose, two hexoses and a rhamnose. No such glycan was detected linked to DolP in cells grown at the higher level of salinity. The same tetrasaccharide-modified S-layer glycoprotein Asn98 in cells grown in low salt, whereas no glycan decorated this residue in cells grown in the high salt medium. At the same time, Asn13 and Asn83 were modified by substantially less pentasaccharide under the low salt conditions. Hence, in response to the degree of environmental salinity, *Hfx. volcanii* modulates not only the composition of the N-linked glycans decorating the S-layer glycoprotein, but which residues undergo this post-translational modification.

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References


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