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MINI REVIEW

Bacterial glycoproteins

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Glycoproteins are a diverse group of complex macromolecules that are present in virtually all forms of life. Their presence in prokaryotes, however, has been demonstrated, and accepted, only recently. Bacterial glycoproteins have been identified in many archaeobacteria and in eubacteria. They comprise a wide range of different cell envelope components such as membrane-associated glycoproteins, surface-associated glycoproteins and crystalline surface layers (S-layers), as well as secreted glycoproteins and exoenzymes. Even their occurrence in the cytoplasm cannot yet be ruled out. This minireview tries to cover the whole subject as completely as possible and refers to available information on presence, structure, biosynthesis, and molecular biology of bacterial glycoproteins.

***Keywords*: prokaryotic glycoprotein, glycoconjugate, eubacteria, archaeobacteria, surface layer (S-layer), flagellin, pilin, exoenzyme**

Introduction

Glycosylation is an important covalent modification of proteins [1–4]. While detailed characterizations of the glycan structures including genetic analyses and elucidation of their biosynthesis have been performed on a great number of eukaryotic glycoproteins [1–6], information concerning glycoproteins of prokaryotic organisms is relatively scarce [7–12]. Until the mid-1970s, glycosylation of proteins was believed to be restricted to eukaryotes. The first bacterial glycoprotein to be described in detail is the surface layer (S-layer) glycoprotein of the archaeobacterium *Halobacterium salinarium* [13, 14]. Since then, S-layer glycoproteins of archaeobacteria [for reviews see refs 8, 11] as well as eubacteria [for reviews see refs 9, 12] have been continuously investigated.

Although the presence of cell-associated or secreted (extracellular) prokaryotic glycoproteins has now been known for two decades, very little attention has been paid to these glycoconjugates. There is now an increasing number of reports in the literature on prokaryotic glycoproteins, unrelated to S-layer glycoproteins, from both the domains Archaea (archaeobacteria) and Bacteria (eubacteria). Among eubacterial glycoproteins, Sandercock *et al.* [15] have suggested to distinguish between non-S-layer glycoproteins and S-layer glycoproteins.

The presently known bacterial glycoproteins are listed in Table 1. Not all of the cited reports are discussed in the present paper. In previous investigations the glycoprotein nature was very often only inferred from a positive periodic acid-Schiff (PAS) staining reaction. Some of the data are still under dispute. Especially in cases of positive PAS staining reactions from Gram-negative eubacteria, contamination of the glycoprotein preparations by lipopolysaccharides needs to be excluded.

The classification into different sections of the particular glycoconjugates follows the topographical location of the respective glycoprotein within the bacterial cell or in the surrounding medium.

Surface layer glycoproteins

Crystalline surface layers (S-layer) composed either of identical protein or glycoprotein subunits represent the outermost cell envelope component of many archaeobacteria and eubacteria [12, 16]. A list of S-layer-carrying bacteria is presented in ref. 16. Most of the S-layers of archaeobacteria are glycosylated [17, 18]. Among eubacteria, glycosylated S-layer proteins have been demonstrated only for organisms belonging to the family of the *Bacillaceae* (e.g. *Bacillus* sp., *Paenibacillus* sp., *Clostridium* sp., *Thermoanaerobacter* sp., *Thermoanaerobacterium* sp., *Desulfotomaculum* sp.) [12] and for *Lactobacillus* sp. [58]. The glycan structures of all S-layer glycoproteins investigated so far, together with the available information on the composition of analysed carbohydrate-protein linkage regions, have been summarized recently [12]. In comparison to eukaryotic glycoproteins, the number of prokaryotic glycoproteins known is

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Table 1. Bacterial glycoproteins.

Organism, Component	*Linkage type*	*References*
S-Layer Glycoproteins		
Archaeobacteria		
Halobacterium halobium (*salinarium*)	Glc-Asn, GalNAc-Asn Gal-Thr	[8, 14, 19, 20]
Halogerax volcanni	Glc-Asn, Gal-Thr	[11, 21–23]
Haloarcula japonica	–	[24, 25]
Methanothermus fervidus	GalNAc-Asn	[26]
Methanoculleus (formerly *Methanogenium*) *liminatans*	–	[27]
Methanoculleus (formerly *Methanogenium*) *marisnigri*	–	[28]
Methanocorpusculum spp.	–	[29]
Methanolacinia paynteri	–	[30]
Methanosaeta (formerly *Methanothrix*) *soehngenii*	Rha-Asn	[31]
Thermococcus stetteri	–	[32]
Archaeoglobus fulgidus	–	[33]
Staphylothermus marinus	–	[34]
Pyrodictium abyssi	–	[35]
Sulfurococcus mirabilis	–	[36]
Gram-positive Eubacteria		
Bacillus stearothermophilus	Rha-Ash	[37–41]
Bacillus thermoaeroaerophilus	O-glycan	[42]
Bacillus thermoaeroaerophilus	O-glycan	[43]
Paenibacillus (formerly *Bacillus*) *alvei*	Gal-Tyr	[44, 45]
Thermoanaerobacter (formerly *Clostridium*) *thermohydrosulfuricus*	Gal-Tyr	[46–48]
Thermoanaerobacter (formerly *Clostridium*) *thermohydrosulfuricus*	Glc-Tyr	[49]
Thermoanaerobacter (formerly *Clostridium*) *thermohydrosulfuricus*	–	[50]
Thermoanaerobacter (formerly *Acetogenium*) *kivui*	Tyr-?	[51]
Thermoanaerobacterium (formerly *Clostridium*) *thermosaccharolyticum*	–	[52]
Thermoanaerobacterium (formerly *Clostridium*) *thermosaccharolyticum*	Tyr-?	[53, 54]
Clostridium symbiosum	–	[55]
Clostridium sp.	–	[56]
Desulfotomaculum nigrificans	–	[39]
Sulfobacillus thermosulfidooxidans	–	[57]
Lactobacillus buchneri	Glc-Ser	[58]
Corynebacterium glutamicum	–	[59]
Gram-negative Eubacteria		
Aquaspirillum sinuosum	–	[60]
Non-S-layer Glycoproteins		
(a) Membrance-associated glycoproteins		
Archaeobacteria		
Thermoplasma acidophilus, plasma membrane glycoprotein	GlcNAc-Asn	[68]
Eubacteria		
Micrococcus luteus (lysodeikticus), membrane glycoprotein	–	[69]
Bacteriodes nodosus, outer membrane protein	–	[70]
Myxococcus xanthus, peripheral membrane glycoprotein	–	[71]
Clostridium thermocellum, cellulosome complex	Gal-Thr	[72–75]
Bacteriodes cellulosolvens, cellulosome complex	Gal-Thr/(Ser)	[75, 76]
Borrelia burgdorferi, outer membrane protein	–	[77]
Fibrobacter succinogenes, cellulose-binding protein	–	[78]
(b) Surface-associated glycoproteins		
Archaeobacteria		
Halobacterium halobium, flagellin	Glc-Asn	[79]
Sulfolobus acidocaldarius, flagellin	–	[80]
Sulfolobus shibatae, flagellin	–	[81]
Thermoplasma volcanium, flagellin	–	[81]

Table 1. *(continued)*

Organism, Component	*Linkage type*	*References*
Methanospirillum hungatei, flagellin	–	[82, 83]
Methanococcus deltae, flagellin	–	[84, 85]
Methanothermus fervidus, flagellin	–	[84]
Eubacteria		
Streptococcus salivarius, cell wall-associated glycoprotein	–	[86]
Spirochaeta aurantia, flagellar filament protein	–	[87]
Azospirillum brasilense, flagellin	–	[88]
Campylobacter coli, flagellin	–	[89, 90]
Neisseria meningitidis, pilin	2,4-diacetamido-2,4,6-trideoxyhexose-Ser	[91, 92]
(c) Secreted glycoproteins, exoenzymes		
Eubacteria		
Cellulomonas sp., cellulase	–	[93]
Cellulomonas fimi, cellulase	Man/Gal-Thr	[15, 94, 95]
Streptomyces lividans, cellulase	Man/Gal-Thr	[15, 95]
Streptomyces lividans, cellulase	–	[96]
Thermoanaerobacterium (formerly *Clostridium*) *thermosaccharolyticum*, extracellular protein complex	–	[97]
Flavobacterium meningosepticum, endoglycosidase, protease	Man-Ser/Thr	[98, 99]
Corynebacterium sepedonicum, phytotoxin	Man-Thr	[100]
Mycobacterium tuberculosis, 55-kDa antigen	–	[101, 102]
Mycobacterium tuberculosis, 45-kDa glycoprotein	Man-Thr	[103]
Mycobacterium tuberculosis, 19-kDa antigen	–	[104]
Mycobacterium bovis, 55-kDa antigen	–	[102, 105]
(d) Cellular glycoproteins		
Eubacteria		
Bacillus megaterium, protein factor PG-1	–	[106]
Clostridium acetobutylicum, autolysin	–	[107]
Enterococcus hierae (formerly *Streptococcus faecium*), N-acetylmuramoylhydrolase	O-glycan?	[108]
Streptococcus pyogenes, acid glycoprotein	–	[109, 110]
Streptococcus snaguis, platelet aggregation-associated protein	GlcNAc-Asn	[111]
Bacillus thuringiensis subsp. *israelensis*, crystal toxin	GlcNAc?-Asn	[112, 113]

Abbreviations: Asn, asparagine; Thr, threonine,; Ser, serine; Tyr, tryosine; Glc, glucose; Gal, galactose; Man, mannose; Rha, rhamnose.

small (approximately 20 different S-layer glycans). Despite this limitation in numbers, some generalizations concerning the architecture of S-layer glycan chains are possible. With one exception, all known archaeobacterial S-layer glycans consist of short linear chains of only a few (approximately up to ten) sugar residues [11, 26]. The only long glycan, a heavily sulfated heteropolysaccharide, was found in *Halobacterium halobium* [8, 11]. Many of the glycan chains from archaeobacteria are linked, without a core region, directly to the S-layer polypeptide by N-glycosidic linkages such as Glc-Asn [19], GalNAc-Asn [20, 26] and Rha-Asn [31]. On the other hand, O-glycosidic linkages via Thr are also common [8, 14, 22]. Eubacterial S-layer glycoprotein glycans usually consist of long homo- or heteropolysaccharide chains, composed of identical repeating units [12]. They often possess core structures between S-layer polypeptide and repeating units [45]. Presently only two examples of short oligosaccharides have been described [49, 58]. In eubacterial S-layer glycoproteins, however, O-glycosidic linkages to the apoproteins are more frequently observed than N-glycosidic linkages (Fig. 1). In fact, only one N-glycan between Rha-Asn has been described [41].

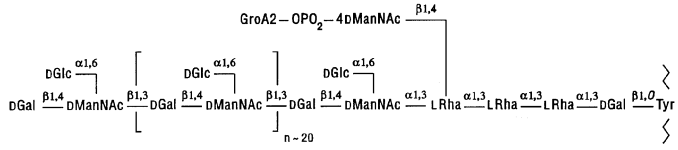

Figure 1. Typical structure of an O-linked eubacterial S-layer glycoprotein. The glycan chains of the S-layer glycoprotein of the Gram-positive bacterium *Paenibacillus* (formerly *Bacillus*) *alvei* CCM 2051 consist of the components D-Gal, D-Glc, D-ManNAc, L-Rha and GroA2 (2-phosphoglyceric acid) and are attached to the S-layer polypeptide via the linkage β-D-Gal-tyrosine (modified from [45]).

A similar linkage type has also been reported in the archaeobacterium *Methanosaeta* [31]. Beside the well-known O-glycosidic linkages via Ser (e.g. Glc-Ser [58] or Thr. T. Wugeditsch, P. Messner, unpublished), completely new types of linkages between O-glycans and polypeptide chains have been found in some eubacterial S-layer glycoproteins, such as tyrosine-glucose [49] or tyrosine-galactose [45, 48].

Biosynthesis of S-layer glycans and characterization of S-layer glycoprotein genes were predominantly investigated in archaeobacteria. A detailed description of the biosynthesis of the glycans from the archaeobacterium *H. halobium* was published recently in an excellent review by Sumper and Wieland [11]. The biosynthesis of the glycan chains of the archaeobacterium *Haloferax volcanii* was investigated using fluorescene-labelled substrates [23]. Using this approach, a single transferase was identified which is involved in glycosyl transfer. König *et al.* have investigated the S-layer glycoprotein of the methanogen *Methanothermus fervidus* [18, 61]. After isolating nucleotide-activated intermediates for the biosynthesis of the heterooligosaccharide of *M. fervidus* they found not only nucleotide-activated monosaccharides but also nucleotide-activated oligosaccharides [61]. Beside undecaprenol as the common prokaryotic carrier lipid, dolichols made of eleven [61] or twelve [62] isoprene units were found. König *et al.* suggested that the dolichols are responsible for the glycan biosynthesis of the S-layer glycoproteins, whereas undecaprenol is involved in the biosynthesis of pseudomurein [18]. Nucleotide-activated oligosaccharides and dolichol-C_{55} were also detected as activated intermediates of the biosynthetic pathway of the S-layer glycoprotein of *Paenibacillus* (formerly *Bacillus*) *alvei* CCM 2051 [63].

Up to now the genes for the S-layer glycoproteins of the archaeobacteria *H. halobium* [64], *H. volcanii* [21] and *M. fervidus* [65] have been cloned and sequenced. However, no eubacterial S-layer glycoprotein has been analysed so far. Detailed summaries of the genetic analyses are given in recently published reviews [8, 11].

The biological function of the glycan portion of S-layer glycoproteins is only poorly understood. There is only one function reported from archaeobacteria, concerning the shape-determination by the saccharide moiety of S-layer glycoproteins of *Halobacteria* [66, 67]. Upon removal of the Glc-Asn-linked glycan, the rod-shaped halobacteria change their structure to round spheres. Attempts to reverse this reaction have been unsuccessful. In eubacteria, due to a different cell wall architecture in comparison to archaeobacteria, S-layer glycoproteins are not essential for the survival of the organisms. For those strains which possess no lipopolysaccharides or other carbohydrate-containing components (e.g. capsules, teichoic acids etc.), glycosylated S-layer proteins may provide a specific selection advantage. However, detailed investigations of this particular problem have not been performed so far.

Non-S-layer glycoproteins

According to the localization of the glycoprotein molecules within the bacterial cell, available information about non-S-layer glycoproteins has been subdivided into several sections. Most reports concern eubacterial strains. However, a few examples of archaeobacterial glycoproteins include, for example, a membrane glycoprotein from *Thermoplasma acidophilum* [68] and glycosylated flagellins of halophilic and methanogenic archaeobacteria [79–85].

Membrane-associated glycoproteins

As with eukaryotic glycoconjugates, glycosylation is considered to be a a membrane-associated reaction [4, 5]. Therefore it is not surprising that a great number of prokaryotic glycoproteins have been isolated from membrane preparations.

One of the few non S-layer glycoproteins from archaeobacteria is the heavily glycosylated protein from the plasma membrane of *T. acidophilum* [68]. It was purified following phenol-water extraction and accounted for 32% (w/w) of the total membrane proteins with a carbohydrate content of less than 10% (w/w). The glycan is highly branched and consists mainly of mannose residues with $\alpha 1 \rightarrow 2$ linked mannoses at the non-reducing end. The presence of N-glycosidic linkage between Asn and GlcNAc was demonstrated by several techniques, including treatment with endoglycosidase H [68].

Glycoproteins have also been assumed to be present in membrane preparations of the Gram-positive eubacterium *Micrococcus luteus* [69]. Following incubation with GDP-[^{14}C]-mannose, several radiolabelled components containing [^{14}C]-mannosyl residues were obtained.

A peripheral membrane glycoprotein (VGP) has been isolated from vegetative cells of *Myxococcus xanthus* [71]. The glycoprotein has a molecular mass of approximately 74 000 Da and a sugar content of approximately 13.5%; its glycan portion is composed of mainly neutral sugars, but also smaller amounts of hexosamines and uronic acids. Binding of VGP to concanavalin A-agarose columns was not observed. However, the protein was bound to *Dolichos* lectin-affinity columns, indicating terminal GalNAc residues [71].

Among the prokaryotic glycoproteins most thoroughly investigated are specific subunits of the cellulose-degrading multiprotein complexes (cellulosomes) of the cellulolytic bacteria *Clostridium thermocellum* and *Bacteroides cellulosolvens* [72]. Following proteolytic cleavage, glycopeptide fractions were analysed by monosaccharide analysis, amino acid analysis, methylation analysis and ^{1}H NMR spectroscopy [73–76]. The total carbohydrate content of cell-free forms of the cellulosomes from different *C. thermocellum* strains is 5–7% (w/w), present in the form of O-linked glycan chains. This result was substantiated by the observation that glycoprotein-containing material is resistant to the

action of peptide-N^4-(*N*-acetyl-β-glycosaminyl)asparagine amidase F (endoglycosidase F). Galactose residues, both as galactopyranose and galactofuranose, 3-O-Me-GlcNAc, and GlcNAc are present in the glycopeptides [74, 75]. The predominant amino acid is Thr, followed by Pro and small amounts of Ser [75]. While in *C. thermocellum* most of the glycans are linked via the Thr residues, Ser is also involved in the linkage regions in *B. cellulosolvens* [75, 76]. Not all cellulosomal subunits are glycosylated to the same extent. Although several biological functions have been proposed for the oligosaccharide moieties of the cellulosomal subunits, direct evidence for a functional role is still lacking.

The presence of glycoproteins in *Borrelia burgdorferi* was concluded from results of digoxygenin labelling together with PAS staining and an endoglycosidase F assay [77]. Metabolic labelling with [^{14}C]-*N*-acetylglucosamine resulted in incorporation of the amino sugar into the glycans of these outer membrane proteins. After treatment of borrelia cells with endoglycosidase F, the carbohydrate reaction was negative. This would happen if a chitobiose moiety linked to asparagine is present, suggesting the occurrence of N-linked glycans [77].

Fibrobacter succinogenes possesses several cellulose-binding proteins (CBP) in the other membrane [78]. Treatment of an 180 kDa CBP with periodate resulted in an almost complete loss of antibody binding. This observation suggested that the respective epitope is carbohydrate in nature and that the CBP is a glycoprotein [78].

Surface-associated glycoproteins

Flagellins are a group a surface-associated glycoproteins described very early; the first example is the flagellin of *H. halobium* [79]. These glycoproteins have the following features: all individual flagellar proteins contain identical sulfated saccharide moieties linked via glucose to asparagine residues of the apoprotein [19]. In all instances the sequon structure Asn-X-Ser/Thr, common in all N-linked glycopeptides determined so far, is present.

Other examples of glycosylated archaeobacterial flagella include those of the thermo-acidophilic species *Sulfolobus acidocaldarius* [80], *Sulfolobus shibatae* [81] and *Thermoplasma volcanium* [81] and the methanogens *Methanospirillium hungatei* [82, 83], *Methanococcus deltae* [84, 85] and *M. fervidus* [84]. In all strains the glycoprotein nature of the flagellins has been demonstrated either by PAS or thymol-sulfuric acid staining of samples following SDS-PAGE. Treatment of growing cells of *M. deltae* with different concentrations of bacitracin has indicated that at least some degree of glycosylation is necessary for the proper assembly of *M. deltae* flagella [85].

Among eubacteria, glycosylated falgellins have been described in great detail for the Gram-negative species *Azospirillum brasilense* [88] and *Campylobacter coli* [89, 90] only recently.

The glycoprotein nature of the flagellin from the polar flagellum of *A. brasilense* has been demonstrated on the basis of several findings. These include (i) a decrease of the apparent molecular mass after chemical deglycosylation using anhydrous trifluoromethanesulfonic acid, (ii) PAS staining or digoxygenin labelling upon periodate oxidation of blotted glycoproteins, and (iii) labelling with a carbohydrate-specific monoclonal antibody and observation with the transmission electron microscope. Two mutant flagellins which differ in the glycan composition in comparison to wild-type flagellin were also examined [88].

At about the same time Trust *et al.* [90] analysed a post-translational modification of *C. coli* flagellin. They have also identified and characterized the genes which are required for this post-translational modification [89]. Following periodate xidation and reaction with biotin hydrazide it was demonstrated that the flagellins of other *Campylobacter* sp. are also glycosylated. By labelling with *Limax flavus* lectin the presence of sialic acid has been demonstrated [90]. One of the genes required for the post-translational modifications of flagellins has shown significant homology to CMP-*N*-acetylneuraminic acid synthetase [89].

Until recently it has been assumed that the pilin (PilE) of *Neisseria meningitidis* pili is glycosylated [91]. Following reinvestigation of the meningococcal pilin, the presence of covalently linked carbohydrate chains on the major structural subunit of PilE has now been unambiguously demonstrated [92]. As constituents of the glycan, a terminal 1-4-linked digalactose unit linked to a 2,4-diacetamido-2,4,6-trideoxyhexose have been identified. This trisaccharide can be released by reductive elimination and, therefore, it is likely that it is attached to Ser or Thr. The 2,4-diacetamido-2,4,6-trideoxyhexose sugar described is particularly interesting as a novel linkage sugar because of its rarity in glycoconjugates [92].

Secreted glycoproteins, exoenzymes

There are no examples yet of secreted archaeobacterial glycoproteins. Among eubacteria most of the isolated glycoproteins show enzymatic activity and are therefore of considerable biotechnological importance.

About 20 years ago Béguin and Eisen [93] characterized extracellular cellulases from the aerobic, Gram-positive organizm *Cellulomonas* sp. Two of the cellulose-bound enzymes (cellulase CA and CB) have been identified as glycoproteins by PAS staining and by retention on a concanavalin A-sepharose column [93]. Warren *et al.* [95] have examined several cellulases of *Cellulomonas fimi.* Two of these, an endo-β-1,4-glucanase A (CenA) and a β-1,4-xylanase/exo-β-1,4-glucanase (Cex), are glycoproteins. They react with Schiff's reagent and bind concanavalin A. The glycans are mannose-rich and are assumed to be O-linked to Thr residues of proline-threonine-rich linker regions by mannose and galactose residues. A similar type of glycosidation has been observed when Cex was produced by

Streptomyces lividans 66 [15, 95]. Another *S. lividans* 66 strain also produces a glycosylated extracellular xylanase (xylanase B) [96].

From *Thermoanaerobacterium* (formerly *Clostridium thermosaccharolyticum*, an extracellular glycosylated protein complex with pectin methylesterase and polygalacturonate hydrolase activity has been isolated [97]. The protein consists of two subunits of which the large subunit (230 kDa) contains 10% (w/w) sugars (N-acetylgalactosamine and galactose). β-Elimination and endoglycosidase F treatment were not effective [97].

A new type of O-linked glycan has been identified on several proteins secreted by the Gram-negative bacterium *Flavobacterium meningosepticum* including endo-β-*N*-acetylglycosaminidases F_2 and F_3 and a P40 protease [98, 99]. In a detailed structural analysis the composition of the glycan portion has been determined to be (2-OMe)Man1-4GlcNAcU1-4GlcU1-4Glc1-4(2-OMe)GlcU1-4[(2-OMe)Rhal-2]Man. The terminal Man residues are O-linked to Ser or Thr residues at concensus sites corresponding to Asp-Ser* or Asp-Thr*-Thr [98]. The function of this unusual acidic heptasaccharide is as yet unknown.

Another glycoconjugate without enzymatic activity which has been isolated from culture supernatants is a phytotoxic glycopeptide of the Gram-positive eubacterium *Corynebacterium sepedonicum* [100]. This glycopeptide has been isolated from a pronase-treated, acid- hydrolysed preparation of the purified toxin. The carbohydrate portion, consisting of mannose and glucose residues, is O-linked to Thr residues of the apoprotein by mannoses [100].

The occurrence of glycosylated proteins in subcellular fractions of *Mycobacterium tuberculosis* has been widely reported [101–105]. Brennan *et al.* [103] have characterized a 45-kDa glycoprotein which has been isolated from the culture filtrate of *M. tuberculosis*. This glycoprotein fraction has been subjected to proteolytic digestion with different proteases. Glycopeptides were obtained following preparative SDS-PAGE and identification by reaction with concanavalin A. By mass spectrometry it was demonstrated that the glycopeptide comprising the N-terminus of the 45-kDa glycoprotein contained as the tenth amino acid a Thr residue which is substituted by an O-linked disaccharide of mannose residues [103].

Cellular glycoproteins

The glycoconjugates listed in this section have been isolated from intact or disrupted cells by various extraction methods. Therefore a topographic localization of the glycoproteins in the intact cells is not possible. Some of the glycoproteins may be localized in the cytoplasm. However, from a biosynthetic point of view, this is not very likely because bacteria do not possess compartments such as the Golgi apparatus, where the transfer of glycosyl residues would be effected.

Examples among eubacteria include a protein factor called PG-I which was extracted by lithium chloride extraction from toluene-treated *Bacillus megaterium* cells [106]. Another glycoprotein is an autolysin which has been isolated from, *Clostridium acetobutylicum* cells by acetone precipitation [107]. It lyses SDS-treated cells and cell wall preparations but has no effect on protein, DNA, or RNA synthesis. The gene appears to be chromosomal, since no plasmids were detected in this *C. acetobutylicum* strain [107]. In a detailed analysis, Kawamura and Shockman [108] showed that the autolysin of *Enterococcus hirae* (formerly *Streptococcus faecium*) is a glycoprotein. Following hydrolysis of the enzyme with cyanogen bromide or staphylococcal V8 proteinase, Concanavalin A-sepharose-purified autolysin produced at least three bands which showed a positive PAS staining reaction. β-Elimination of the glycans suggested that the enzyme contained O-linked sugars, such as glucose monomers and glucose oligosaccharides [108].

Other glycoproteins from *Streptococcus* strains include a streptococcal acid glycoprotein (SAGP) with antitumour activity from *Streptococcus pyogenes* Su strain [109] or the platelet aggregation-associated protein (PAAP) from protoplasts of *Streptococcus sanguis* [111]. Its glycoprotein nature was demonstrated using protease inhibitors. Bacitracin as an inhibitor of N-glycosylation was inhibitory but monensin or arylglycosides were not. Glycopeptides were obtained from PAAP by pronase digestion. NMR spectroscopy of an isolated glycopeptide fraction showed the presence of an N-glycosidic linkage between GlcNAc and Asn. Additionally, N-glycosidic linkages between Asn and GalNAc are considered likely [111].

In the case of *Bacillus thuringiensis* crystal proteins, the presence of glycoproteins was reported for several subspecies [112, 113]. However, the glycoprotein nature of the crystal protein from *B. thuringiensis* subsp. *kurstaki* [112] is still questioned. By lectin binding, the presence of an N-glycosidic linkage to GlcNAc was suggested for the *B.thuringiensis* subsp. *israelensis* crystal toxin although conclusive proof for a glycoprotein structure has not been presented [113].

Conclusions

The past two decades have seen the perception change that glycosylation of proteins is restricted to eukaryotic organisms. Today we can assume from different observations that prokaryotic glycoconjugates may well be as common as glycoproteins in higher organisms or plants. For example, almost all archaeobacterial S-layers consist of glycoslated proteins [16, 17]. Another example is the great number of glycosylated exoenzymes of flagellar proteins (see Table 1). A major difference to eukaryotic glycoconjugates, however, is that the glycan structures of prokaryotic glycoproteins differ considerably. For example, there is no common

structure such as the chitobiose core of eukaryotic N-glycans. Eubacterial S-layer glycans, for example, very often possess long linear or branched carbohydrate chains which can be linked via common N or O-glycosidic linkages. On the other hand, a few of them are O-linked via recently-discovered linkages to tyrosine [12]. In many prokaryotic glycoconjugates, O-glycosidic linkages from mannose or threonine have been found (Table 1). In contrast, many of the archaeobacterial glycans are N-linked via asparagine (Table 1). The main problem at the moment is the lack of sufficient coherent structural information to draw a picture of the general architecture of prokaryotic glycoproteins.

Presently there is not much information available on the biological function of the glycan portion of bacterial proteins. In general, it is assumed that the glycans fulfill similar protective functions as have been suggested for eukaryotic glycoconjugates [114]. One specific function, for example, is the determination of the cell shape by the glycan portion of halobacterial S-layer glycoproteins [66, 67].

Since only a few well-characterized prokaryotic glycoproteins are presently known (Table 1) many important questions about structure, biosynthesis, molecular biology and function of these glycoconjugates are still unanswered. The aim of this review was to shed some light on these fascinating molecules and to attract new investigators of this fast developing field.

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