Journal of Bioenergetics and Biomembranes, Vol. 24, No. 6, 1992

Minireview

Structural Features of Archaebacterial Cell Envelopes

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Received May 30, 1992; accepted June 15, 1992

Regularly arrayed surface (glyco)proteins—often referred to as S layers—are a common feature of the cell envelopes of almost all archaebacteria. We have selected some examples (*Halobacterium*, *Sulfolobus*, *Thermoproteus*, *Pyrobaculum*, *Staphylothermus*), and we describe the structure of their surface layers as revealed primarily by electron crystallography. In spite of a considerable diversity in shapes and dimensions, some common structural features emerge from the comparison. The glycoprotein arrays are composed of oligomeric units which are anchored in the plasma membrane; extended spacer or linker domains maintain the bulk of the more or less porous surface layers at a constant distance above the membrane surface, thus creating a quasi-periplasmic compartment. Functions ascribed to surface layers, such as compartmentalization, shape maintenance and determination, and adhesion are discussed.

KEY WORDS: S layers; surface glycoproteins; plasma membrane; electron crystallography.

INTRODUCTION

The cell envelopes of archaebacteria exhibit considerable structural diversity. However, nearly all incorporate a regular crystalline surface layer (S layer) of protein or glycoprotein subunits (König, 1988). Only *Thermoplasma*, *Halococcus*, *Methanobrevibacter*, and *Methanosphaera* appear to be devoid of an S layer. The most common type of an archaebacterial cell envelope consists solely of an S layer, which is intimately associated with the cytoplasmic membrane and covers the entire cell surface: Thus the S layer is the predominant macromolecular component of the cell envelope. This is observed in members of the *Halobacteriales*, the *Methanococcales* and *Methanomicrobiales*, the *Sulfolobales*, *Thermoproteales*, and *Thermococcales*. In some of the "Gram-positive" archaebacteria (Kandler and König, 1985), the cell envelope consists of an S layer in combination with a polysaccharide or pseudomurein layer. *Methanosarcina* possesses a layer of methanochondroitin, an acidic polysaccharide reminiscent of the chondrochitin of animal connective tissue, external to an S layer (Kreisel and Kandler, 1986). The envelope of *Methanothermus* consists of a sacculus of pseudomurein adjacent to the cell membrane, and an externally located glycoprotein S layer (Nußer *et al.*, 1988). *Methanospirillum* and *Methanotrix* possess a particularly complex type of envelope: Each cell is surrounded by a matrix layer of carbohydrate and protein components, and linearly associated cells are enclosed by a protein sheath; individual cells are separated by spacer plugs made up of two different regular glycoprotein arrays (Zeikus and Bowen, 1975; Shaw *et al.*, 1985; Beveridge *et al.*, 1986).

A few well-established cases apart, where two distinct layers composed of different proteins form a composite, those layers investigated in greater detail appear to be made up of a single protein species. Primary structures are available only for *Halobacterium halobium* (Lechner and Sumper, 1987) and *Haloferax volcanii* (formerly *Halobacterium volcanii*) (Sumper *et al.*, 1990) and the thermophilic methanogens *Methanothermus fervidus* and *Methanothermus sociabilis* (Bröckl *et al.*, 1991). While the two *Methanothermus* species have highly conserved primary structures, with only three amino acid exchanges

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0145-479X/92/1200-0567$06.50/0

in 593 amino acid residues, they exhibit no similarity to the primary structures of *Halobacterium halobium* and *Halobacterium volcanii* nor to any other known protein sequences. This is similar to the situation in eubacteria where the few primary structures of S layer proteins available to date show only weak indications of relatedness, if any. S layers are merely defined by their location at the cell surface and, as it appears, they do not belong to one family of proteins. One perhaps significant common feature on the primary structure level is the relatively high content of the hydroxyaminoacids serine and threonine which often occur clustered in the sequences; such motives also occur in many eukaryotic adhesion proteins (see, e.g., Noegel *et al.*, 1986; Cunningham *et al.*, 1987). Most archaebacterial surface proteins appear to be glycoproteins, although detailed information about the structure of the glycan chains and their linkage to the protein is rather scarce. Notable exceptions in this respect are the S layers of *Halobacterium halobium* (Sumper, 1987) and of *Haloferax volcanii* (Sumper *et al.*, 1990).

While the speculations are many, few functions have been rigorously assigned to S layers. The porous nature of S layers is intuitively regarded as an indication for a role in controlling the passage of small and medium sized molecules across the cell envelope. In addition to this molecular sieving function, phenomena as diverse as shape determination and maintenance (Mescher and Strominger, 1976b; Messner *et al.*, 1986; Wildhaber and Baumeister, 1987) and cell–cell or cell–substrate interactions have been ascribed to surface layers (Baumeister and Hegerl, 1986; Phipps *et al.*, 1991).

Our knowledge of the three-dimensional organization of bacterial surface layers has been obtained almost exclusively through the agency of electron crystallography. In this review we will describe the structure of some selected archaebacterial surface layers; on the one hand, this will reflect the structural diversity of S layers in archaebacteria; on the other hand, some common principles of organization will emerge.

THE CELL ENVELOPE OF HALOBACTERIA

As early as 1956 Houwink described a hexagonal pattern of globular particles about 13 nm wide on the surface of *Halobacterium halobium*. He reasoned: "If the globular particles in the bacterial cell wall are macromolecules—and this does not seem too wild a speculation—this layer of the cell wall may be a two-dimensional crystal lattice. Since a crystal grows by apposition only, and not by intussusception, this cell wall will have definite growth zones, coincident with the borders of the crystalline areas. Such borders must occur at the poles of the cell, where the shape of the cell wall passes from the tube into the dome. Additional growth zones may exist in other parts of the cell wall, wherever a dislocation occurs in the regular pattern of the globules." This observation and the conclusions Houwink drew from them are quite accurate; this is particularly remarkable in view of the quality of the electron micrographs that could be produced at the time.

Mescher and Strominger (1976a) purified the *Halobacterium halobium* surface layer protein and established its glycoprotein nature; this was actually the first report of a prokaryotic glycoprotein. In the meantime the saccharide structure and biosynthesis of this glycoprotein have been elucidated in great detail (e.g., Sumper, 1987 and Lechner and Wieland, 1989). Mescher and Strominger (1976b) also described experiments strongly indicating that the surface protein is involved in maintaining the shape of *Halobacterium halobium*. Removal of an N-terminal peptide by limited proteolysis or the inhibition of complete glycosylation by bacitracin converted the rod-shaped cells into spheres. Lechner and Sumper (1987) determined the primary structure of the *Halobacterium halobium* surface protein. The 86.5-kDa polypeptide has a hydrophobic stretch of 21 amino acid residues at the C-terminus which is supposed to serve as a membrane anchor. Close to the C-terminus is a cluster of threonine residues; to most of them neutral disaccharides are O-glycosidically attached. Sulfated oligosaccharides are linked to a number of N-glycosylation sites distributed throughout the polypeptide chain and a glycan chain, composed of repeated motives of sulfated pentasaccharides N-linked to asp 2. The latter may actually be critically involved in maintaining the integrity of the lattice and hence in shape maintenance, since it is the glycosylation of this asp residue which is inhibited by bacitracin.

Electron microscopy of Halobacteria has always been hampered by the high salt concentration which is required to maintain the integrity of the regular surface arrays. In contrast to *Halobacterium halobium*, the *Haloferax volcanii* surface layer can be maintained intact at low concentrations of divalent cations. This

has allowed one to perform a low-resolution three-dimensional reconstruction of this S layer (Kessel *et al.*, 1988). It reveals a dome-shaped hexameric structure with a relatively narrow pore at the apex which is directed outward and a wide opening directed toward the cell membrane (Fig. 1).

On the primary structure level the surface glycoproteins of *Halobacterium halobium* and of *Haloferax volcanii* show a moderate degree of similarity (Sumper *et al.*, 1990). Actually, stretches of nearly complete homology are interspersed with stretches of unrelated sequences; the degree of similarity is higher in the C-terminal part and drops toward the N-terminus. Features such as the putative C-terminal membrane anchor and the cluster of O-glycosylated thre residues adjacent to it are conserved. Otherwise, the pattern of glycosylation, particularly the N-glycosydically linked saccharides, appears to be quite different in the two species (Mengele and Sumper, 1992).

In retrospect, the overall similarity seems to justify putting forward a model which combines the primary structure data from *Halobacterium halobium* with the electron microscopic data from *Haloferax volcanii* (Kessel *et al.*, 1988). The model (Fig. 1f), which also draws from X-ray data obtained with pellets of *Halobacterium halobium* cell envelopes, indicates that the bulk of the surface protein layer is separated from the cell membrane by a 6.5-nm-wide interspace of low electron density (Blaurock *et al.*, 1976). The existence of such an interspace is corroborated by electron microscopy of freeze-fractured *Halobacterium* cells.

A structure basically very similar to the *Halobacterium halobium* surface layer is found in *Methanoplanus limicola* (Cheong *et al.*, 1991), a mesophilic methanogenic archaebacterium belonging to the *Methanomicrobiales*. The surface glycoprotein of *Methanoplanus limicola*, which has an apparent M_r of 135 kD (115 kD after deglycosylation), also forms a lattice composed of hexameric dome-shaped units; on micrographs of freeze-fracture replicas, the surface layer appears to be separated from the membrane by an interspace of about the same width as in Halobacterium. Also, *Archaeoglobus fulgidus*, a thermophilic archaebacterium which is regarded as a "missing link" between the methanogen/halophile branch and the sulfur-dependent extreme thermophiles (Achenbach-Richter *et al.*, 1987), has a surface layer structure reminiscent of *Halobacterium halobium* (Kessel *et al.*, 1990). Because of the somewhat higher resolution, the

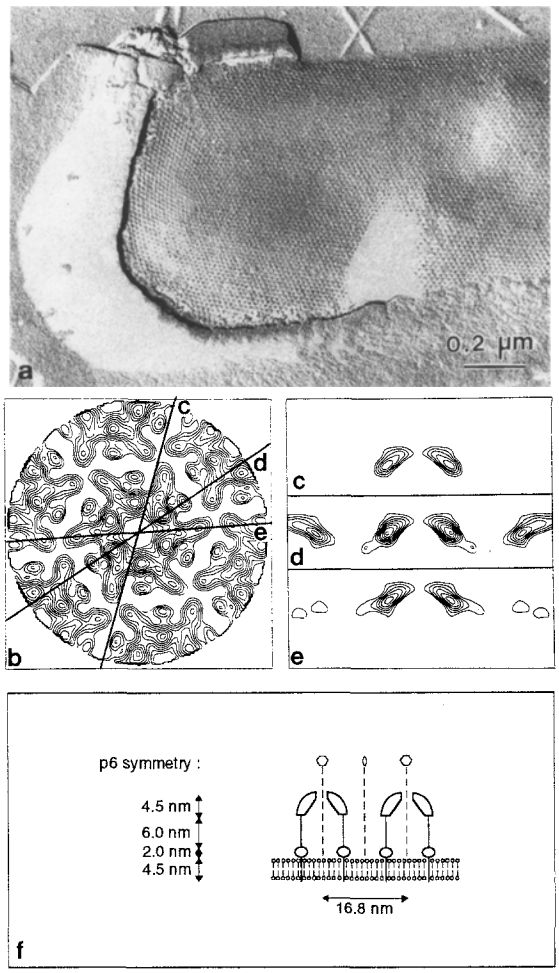

Fig. 1. (a) Metal replica showing the regular surface layer of *Halobacterium halobium*. Over the cylindrical part of the cell body the glycoprotein surface lattice is free of gross defects; at the cell poles disclinations and dislocations are visible. (b) Projected structure of the surface layer of *halobacterium volcanii* (now *Haloferax volcanii*) obtained by correlation averaging of micrographs from negatively stained cell envelopes. The average shows the hexameric organization of the glycoprotein subunits. (c,d,e) Vertical sections through the three-dimensional structure along the lines indicated in (b). The sections show the dome-shaped structure of the hexamers. (f) Schematic drawing combining the available structure information from X-ray studies of *Halobacterium halobium* envelopes (Blaurock *et al.*, 1976), the three-dimensional reconstruction of the *Haloferax volcanii* S layer obtained by electron crystallography (Kessel *et al.*, 1988), and the primary structure of the *Halobacterium halobium* surface protein (Lechner and Sumper, 1987). The three-dimensional structure determined by electron crystallography depicts only the upper dome-shaped structure which is separated from the cytoplasmic membrane by "spacer elements." It is proposed that the cluster of glycosylated threonine residues adjacent to the putative C-terminal membrane anchor forms the spacer domain. As indicated by the crystallographic symbols, the section runs from the 6-fold to another 6-fold axis via the 2-fold axis.

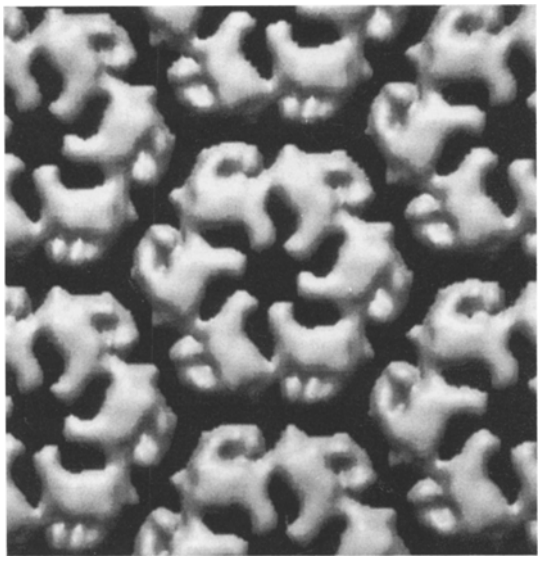

Fig. 2. View of the outer surface of the surface layer of *Archaeoglobus fulgidus*. The individual subunits in the hexameric complex appear only weakly interconnected. (For details, see Kessel *et al.*, 1990.)

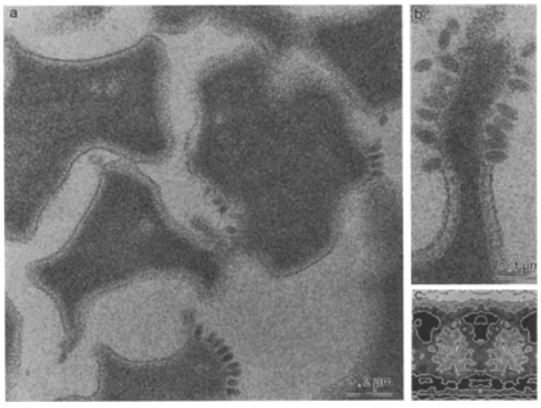

Fig. 3. (a) Micrograph of a thin section of *Sulfolobus shibatae* cells obtained by using the freeze-substitution method (courtesy of I. Wildhaber). The surface layer is represented by the electron-dense line which is separated from the underlying cytoplasmic membrane by an approximately 18-nm-wide electron-light interspace of constant width. (b) Where the virus SSV1 (Schleper *et al.*, 1992) is attached to the membrane, the surface layer is removed. (c) An average obtained by linear superposition along the cell boundary shows the regularly spaced protomers of the surface layer (top) and indications of spacer elements linking them to the underlying membrane (bottom).

three-dimensional reconstruction of this surface layer reveals more details about the shape of the subunits and their arrangement within the layer (Fig. 2).

THE CELL ENVELOPE OF SULFOLOBUS

Thin sections of *Sulfolobus shibatae* (formerly *Sulfolobus spec.* B12) cells (Fig. 3) reveal an S layer located at a uniformly large distance (approximately 18 nm) from the plasma membrane and enclosing a "quasi-periplasmic" interspace (Baumeister *et al.*, 1988, 1989). The layer appears to contact the plasma membrane via filiform linker elements which are more clearly seen in averages along the envelope cross section (Fig. 3c). As early as 1982 the *Sulfolobus acidocaldarius* S layer was investigated by electron crystallographic techniques and the three-dimensional reconstruction was performed, revealing a spongy structure with a network of channels and caves created by multidomain protomers apparently arranged on a *p*6-lattice (Taylor *et al.*, 1982). We recently reinvestigated this structure (Lembcke *et al.*, 1991), on the one hand as a part of a broader attempt to compare the surface proteins of several species of *Sulfolobus* in order to identify common and variable features in their design, and on the other hand with the goal in mind of attaining a significantly higher resolution. Surface layers, like many other two-dimensional crystals, natural as well as synthetic, suffer notoriously from lattice imperfections and distortions. In order to avoid detrimental effects on resolution, image averaging has to be adapted to this situation. Instead of relying on perfect crystallinity, unit cell displacements from ideal lattice positions must be determined by cross-correlation before the precisely aligned motifs are superimposed and thus averaged (Saxton and Baumeister, 1982). When analyzing micrographs of several S-layer fragments of *Sulfologus acidocaldarius* with this technique, the results turned out to be alarmingly inconsistent with regard to symmetry; some averages showed almost perfect *p*6 symmetry while others were clearly *p*3 and many were somewhere between these two extremes. Careful examination of cross-correlation functions of large arrays gave a first hint; they showed local variations with characteristic interpenetrating domains of intrinsically uniform peak height and shape. Congruent domain patterns were obtained by an entirely independent approach, namely multivariate statistical analysis of eigenvector–eigenvalue data. Selective intradomain averages yielded clear *p*3 structures, and it turned out that a 60° rotation of the trimeric motifs, indicative of the existence of twin boundaries, was causing the variations observed. Obviously nondiscriminative averaging over image

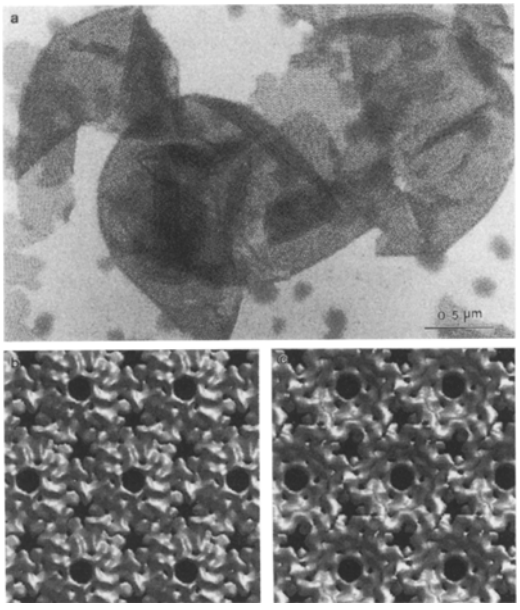

Fig. 4. (a) Cell "ghosts" of *Sulfolobus shibatae* obtained by detergent extraction and composed exclusively of the S layer (glyco)protein. (b) View of the outer surface as revealed by electron crystallography; the resolution is about 1.5 nm. (c) View of the inner surface, i.e., the surface directed toward the cytoplasmic membrane in the intact cell envelope. The narrow outlet of the dome-shaped units is directed outward, the wide opening inward. S layers of *Sulfolobus* are an example of a tightly interconnected surface protein. The unit cell is $a = b = 20.1$ nm.

areas with domains differing in unit-cell orientation creates the higher *p*6 symmetry. Therefore, in producing a three-dimensional reconstruction, all the projection images have to be subjected to a pattern recognition procedure capable of classifying the unit cells according to their orientation in the lattice.

Figure 4 shows such a three-dimensional reconstruction of the *Sulfolobus shibatae* surface protein (Lembcke *et al.*, manuscript in preparation). The main feature is a large dome-shape structure centered on the three-fold axis and opening toward the membrane; the apex, which is perforated by a large pore (4 to 5 nm in diameter), lies at the outer surface of the layer which has a minimum thickness of 6.3 nm as indicated by the reconstruction. No protrusion that could serve as a linker and membrane anchor is visible at the inner face. However, this is not unexpected, since long and slender protein domains as required for linker elements tend to remain invisible in reconstructions by reason of their flexibility. Moreover, we cannot rule out that all or part of the linker is formed by a distinct protein species, anchored in the membrane and noncovalently bound to the S layer.

The three-dimensional structure of the *Sulfolobus shibatae* S layer is remarkably similar to the structure of the *Acidianus brierleyi* (formerly *Sulfolobus brierleyi*) S layer (Baumeister *et al.*, 1991). In spite of the great diversity in the molecular architecture of archaebacterial surface proteins (see, e.g., Baumeister *et al.*, 1990), closely related species (i.e., on the genus or family level) usually have very similar S layer structures; therefore S-layer structures are of (limited) usefulness as taxonomic features.

THE CELL ENVELOPE OF THERMOPROTEUS AND PYROBACULUM

Closely related to the *Sulfolobales* are the *Thermoproteales*, another group of extremely thermophilic archaebacteria, which include among others the genera *Thermoproteus*, *Pyrobaculum*, and *Staphylothermus*. *Thermoproteus* and *Pyrobaculum* are rod-shaped cells of variable length but strikingly constant in diameter (Zillig *et al.*, 1981, Huber *et al.*, 1987)—unlike *Sulfolobus* cells which appear as rather irregular cocci with edges and lobes (Weiss, 1974). When *Thermoproteus* cells are freeze-fractured (Fig. 5a), the surface protein array is revealed as a thin (3–4 nm) layer located at a uniform distance of 25 nm from the plasma membrane, and in contact with it by means of regularly spaced protrusions. When this S layer is isolated by detergent extraction, and examined in thin sections, it can be seen that the protrusions are an integral part of the surface array (Baumeister *et al.*, 1989). The protrusions are narrow pillarlike extensions of the surface layer which terminate in a globular mass; they extend across the entire interspace and appear to penetrate the plasma membrane (Wildhaber and Baumeister, 1987; Baumeister *et al.*, 1989). Detergent extraction of these layers produces "ghosts" that retain the shape of the cell. While these have so far resisted even the most vigorous attempts to dissociate them by chemical means, they can be partially ruptured by sonication to obtain single layered patches. Examination of the arrays by negative staining reveals a *p*6 lattice of unusually large center-to-center spacing (32.8 nm) and an elaborate network of delicate masses; on the inner surface pillarlike protrusions located at the six-fold axis are the most prominent feature in the three-dimensional reconstruction (Wildhaber and Baumeister, 1987).

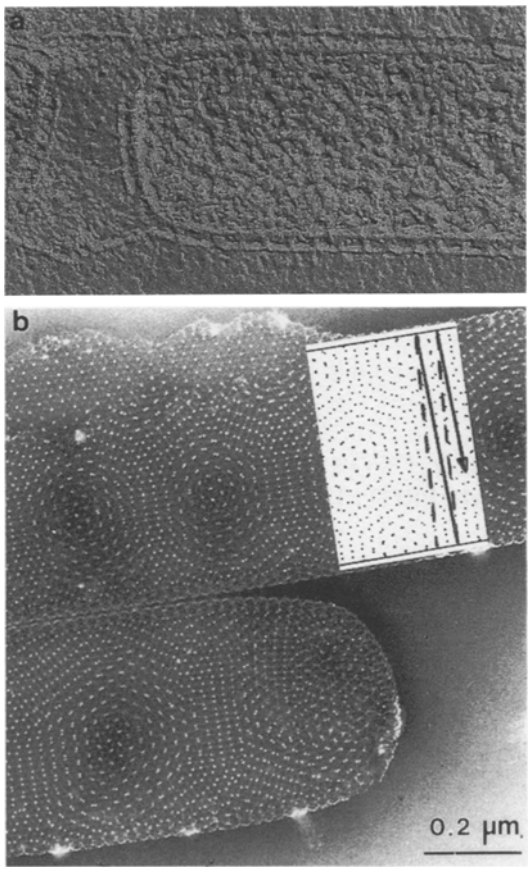

Fig. 5. (a) Freeze-fractured cells of *Thermoproteus tenax* show a distinct interspace between the S layer and the cytoplasmic membrane. Regularly spaced protrusions or "pillars" span the entire width of this space. Upon cell division, new S layer caps form at the two cell poles, while the old layer persists, eventually interconnecting two completely separated cells; at no stage is the "naked" plasma membrane exposed to the environment. (b) Negatively stained "ghosts" of *Thermoproteus tenax* obtained by detergent extraction. The superposition of two arrays, which results from the flattening of the cylinder-shaped ghosts, creates a hexagonal Moiré pattern. The array consists of bright spots corresponding to the pillars, interconnected by a filiform network. The inset shows how the intact S layer can be described as two parallel right-handed helical chains of morphological units. According to the helical template model, new units are added at the terminal ends of these helices near the cell poles. (For details, see Wildhaber and Baumeister, 1987.)

An analysis of the *Thermoproteus tenax* surface array revealed that one of the lattice base vectors is invariably offset from the perpendicular to the long axis of the cell by a small angle (3–4°). Consequently, the layer can be described in terms of two parallel helical strands, consisting of protein hexamers, which wrap around the girth of the cell at a shallow angle (Fig. 5b). The order of the array over the cylindrical portion of the cell is remarkably perfect; no lattice defects (edge dislocations, disclinations, etc.) of any kind are seen. Distortion analysis (Dürr, 1991) shows that the lattice is, in fact, capable of undergoing smoothly distributed long-range deformations, i.e., it behaves like an elastic rubber sheet. The ability to "flex" to a certain degree without abrogating intersubunit contacts is probably essential for the cell; it allows the cell to bend rather than rupture in response to strong bending moments exerted on it in its natural environment (Saxton *et al.*, manuscript in preparation). However, while the cylindrical part appears to be free of lattice defects, pentagonal wedge disclinations (Harris and Scriven, 1970) have been shown to exist at the poles of *Thermoproteus* cells (Messner *et al.*, 1986). Based on these data, a cogent hypothesis for the growth of the surface protein layer was put forward. This "helical template model" (Wildhaber and Baumeister, 1987) proposes that new morphological units are added at the ends of the helical strands where these meet the polar caps, generating edge dislocations which are absorbed by migration of the wedge disclinations. Thus, new subunits are added only at the end of the cells, and the cells grow by elongation while maintaining a strictly defined diameter, as observed.

All species of the genus *Pyrobaculum* possess a hexagonal protein array in their cell envelope which is almost congruent with the shape-maintaining layer of *Thermoproteus tenax*. However, in *Pyrobaculum* this layer is not the outermost component of the cell envelope. In *Pyrobaculum islandicum* (Phipps *et al.*, 1990) the outer surface is covered by a prominent fibrillar surface coat resembling an eubacterial capsule (Fig. 6). In *Pyrobaculum organotrophum* (Phipps *et al.*, 1991) an additional hexagonal protein layer covers the entire cell surface. This layer appears to be rather fragile and is only loosely associated with the outer surface of the inner (*Thermoproteus*-type) layer. From a three-dimensional reconstruction it emerges as a porous network of blocklike dimers with a lattice constant smaller than that of the inner layer (Fig. 7).

THE CELL ENVELOPE OF STAPHYLOTHERMUS

When the cell surface of *Staphylothermus marinus* is exposed by deep-etching a meshwork of rather filiform structural units, an unusually low degree or order becomes visible, which is reminiscent of the cell

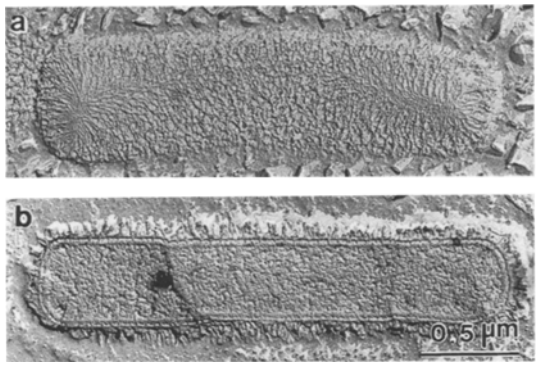

Fig. 6. *Pyrobaculum islandicum* (strain GEO3) has an S layer, which at a resolution of 2 nm is virtually identical to the S layer of *Thermoproteus tenax*. However, as deep-etching (a) and freeze-fracturing (b) reveal, an additional fibrous surface coat covers the entire outer surface. (For details, see Phipps *et al.*, 1990.)

surface of *Desulfurococcus mobilis* (Wildhaber *et al.*, 1987). Freeze-fracture replicas show that the surface glycoprotein protrudes more than 60 nm from the plasma membrane (Fig. 8a,b). The S-layer meshwork can be easily isolated by detergent extraction. The resulting "ghosts" (Fig. 8c) emphasize the filiform nature of this surface glycoprotein; at the edges of these ghosts, parabolic elements with long stalks may be discerned. While this surface network is resistant

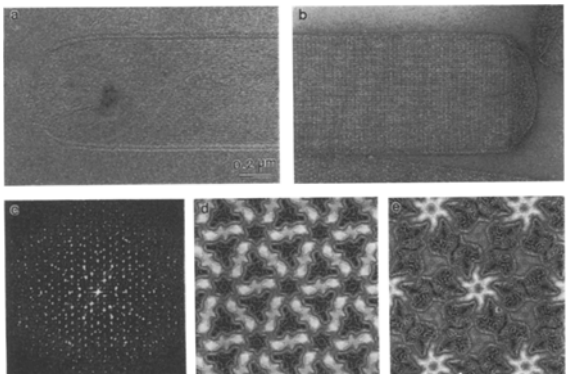

Fig. 7. *Pyrobaculum organotrophum* (strain H10) cell envelopes are composed of two distinct protein arrays. (a) Platinum/carbon shadowed "ghost" obtained by detergent extraction. At the polar cap the relatively labile outer layer is locally removed. (b) The negatively stained "ghost" gives a composite image of the two layers as clearly reflected by the corresponding power spectrum (c). The outer layer (d) is a porous network of blocklike dimers arranged on a hexagonal lattice with a spacing of 20.6 nm. The inner layer (e), which is again almost congruent with the *Thermoproteus tenax* S layer, has a lattice spacing of 27.9 nm. (For details, see Phipps *et al.*, 1991.)

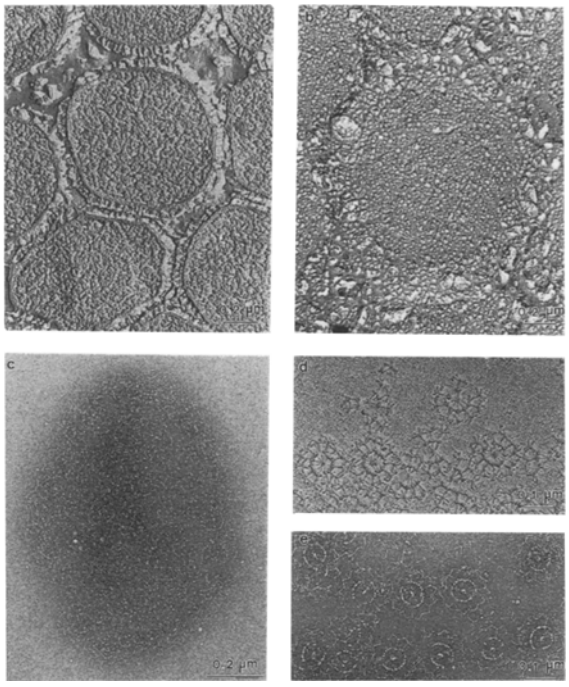

Fig. 8. Freeze-fracturing (a) and deep-etching (b) of *Staphylothermus marinus* cells reveal a poorly ordered surface network composed of filiform structural units. (c) The negatively stained "ghost" obtained by detergent extraction is a plain protein network. This network can be dissociated into the structural units using, for example, glycerol as a "chaotropic" agent. The structural units (probably tetramers) released from the surface layer spontaneously form protein "micelles" with the hydrophobic membrane anchor at the center (d,e), especially the negatively stained micelles emphasize the filigree structure of the protein. The linker or spacer domains radiate outward in the micelles. They are approximately 65 nm long and are "decorated" by a globular protein species near their middle. Further outward the spacer domain branches into four arms which interconnect the structural units within the S-layer meshwork.

to harsh detergent treatments, it dissociates upon exposure to glycerol, and the released (probably tetrameric) protomers spontaneously form "micelles" in order to shield their hydrophobic membrane anchors (Fig. 8d,e). The observation that glycerol and related compounds are capable of dissociating the meshwork into its structural units is indicative of carbohydrate residues being directly involved in maintaining the interactions within the meshwork. Adding detergent or propanol at this stage leads to a dissociation of the micelles, a process which is reversible upon dialysis (Peters *et al.*, manuscript in preparation). Thus, by virtue of its unusual shape and dimensions, the *Staphylothermus marinus* surface protein allows the direct visualization of the basic structural elements of

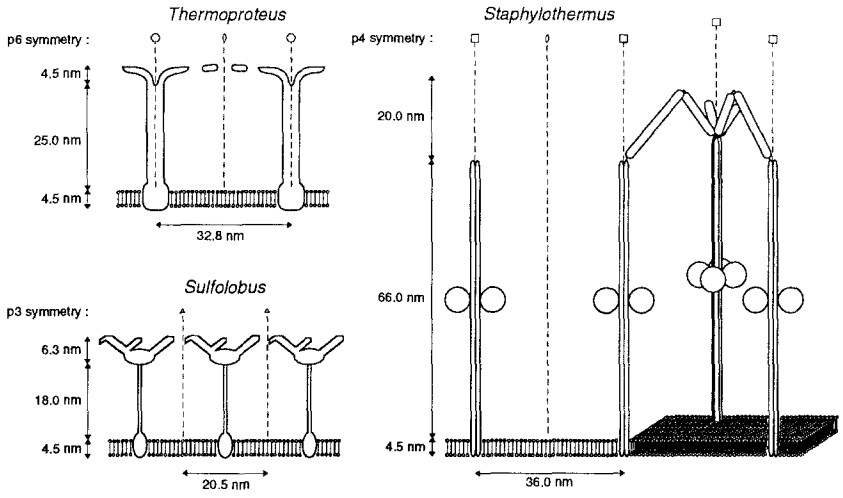

Fig. 9. Schematic representation of the structural organization of the surface proteins of *Thermoproteus, Sulfolobus,* and *Staphylothermus.* Common to them is an interspace between the membrane and porous outer canopy of the S layer which is maintained by spacer elements; it may serve as a kind of "periplasmic space" harboring assorted protein species.

an archaebacterial surface protein, i.e., the membrane anchor, a long filiform linker or spacer element, and more or less compact domains which form the S layer proper. In the case of the *Staphylothermus marinus* S layers, four filiform arms emanate from the spacer domains which interact with the ends of the arms from neighboring tetrameric units to form the meshwork.

SOME GENERAL FEATURES AND FUNCTIONAL IMPLICATIONS

Figure 9 shows schematically the structural organization of the surface proteins of *Thermoproteus, Sulfolobus,* and *Staphylothermus.* It is obvious from these examples that a distinct interspace of constant width is created by the S-layer proteins which is maintained by thin spacer elements which penetrate into the plasma membrane. At least in the case of *Thermoproteus* and *Staphylothermus* these elements are integral parts of the S-layer protein. The outer part of these S layers constitutes a highly interconnected, porous canopy supported by the pillar-shaped linker or spacer domains. If a periplasmic space is defined as a compartment outside the cytoplasm delineated by the plasma membrane and an outer layer of the cell envelope, then these archaebacteria can be said to possess a periplasmic space. It is different from the periplasmic space of Gram-negative eubacteria, primarily in the greater permeability of the outer boundary (Baumeister *et al.,* 1989). Nevertheless, it may serve as a holding compartment for proteins secreted by the cell, such as hydrolytic enzymes involved in breaking down macromolecular nutrients into transportable units, for binding proteins involved in nutrient transport across the plasma membrane, or enzymes of the glycosylation machinery. It has in fact been shown for *Halobacterium* that the transfer of carbohydrate moieties to the polypeptide chain of the S-layer protein occurs at the cell surface (Sumper, 1987).

In organisms such as *Thermoproteus* and *Pyrobaculum* it is very likely that the S layer plays a crucial role in maintaining and perhaps in determining the cell shape. On the other hand, this cannot apply to *Sulfolobus* cells which are pleomorphic and not at all rigid. Searcy (1987) presented some evidence that *Sulfologus* has a primitive cytoskeleton; one could imagine that the membrane-spanning domain of the S-layer protein interacts with such cytoskeletal elements and thus confers amoeboid properties on the cells.

One can also envisage that a highly organized protein array inserted into the plasma membrane exerts a strong influence on membrane lipids and proteins. This may be important for organisms living in extreme environments as a means of maintaining lamellar lipid structures and the lateral organization of the membrane proteins. Adhesion to animate and inanimate surfaces can clearly be mediated by surface proteins. However, as shown for *Pyrobaculum* (see above), the cell envelope components which are commonly referred to as S layer must not necessarily

represent the outermost component of the cell envelope. Other functions like, for example, transmembrane signalling, i.e., transducing environmental information to targets inside the cell, are so far pure speculation.

ACKNOWLEDGMENT

Parts of the work reviewed in this paper have been supported by the Deutsche Forschungsgemeinschaft (SFB 266/C2).

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