Protein and Carbohydrate Composition of the Cell Envelope of *Halobacterium salinarum*¹

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The isolated cell envelope of *Halobacterium salinarum* strain 1 contained 15 to 20 proteins that were resolved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. All but one of these proteins had molecular weights of 130,000 or less and together accounted for 50 to 60% of the total envelope protein. The remaining 40 to 50% of the envelope protein was accounted for by a single protein with an apparent molecular weight of approximately 194,000 that stained for carbohydrate with periodate-Schiff reagent. The proteolytic enzymes trypsin and Pronase were used to show that the carbohydrate is covalently bound to the protein. Separation of amino sugar- and hexose-containing tryptic peptides by gel filtration indicated that all of the nonlipid carbohydrate of the cell envelope is covalently bound to protein. The results of partial purification by phenol extraction indicated that both the amino sugar and hexose are bound to the 194,000-molecular-weight protein. Exposure of isolated cell envelopes to low salt concentration resulted in solubilization of a majority of the envelope proteins. A relatively small number of proteins, including the high-molecular-weight, carbohydrate-containing protein, remained bound to the sedimentable cell membrane fraction.

The obligately halophilic bacteria of the genus *Halobacterium* lack the peptidoglycan layer characteristic of most bacteria. Although the cell envelopes of the *Halobacteria* possess an amino sugar-containing component, neither muramic acid, diaminopimelic acid (8), nor D-alanine (unpublished observation) has been found. Despite this lack of a rigid peptidoglycan layer, the organisms are able to maintain a rod-shaped morphology when grown under optimal conditions. Early studies of the envelopes of the *Halobacteria* failed to demonstrate the presence of a cell wall external to the plasma membrane (8). Improved methods of specimen preparation have shown, however, that the organisms do possess an external cell wall layer (2, 20, 21), and studies of *H. salinarum* by Steensland and Larsen (20), *H. halobium* by Stoetzenius and Rowen (21) and Marshall et al. (10), and *H. cutirubrum* by Kushner et al. (7) have shown that this layer is composed largely of protein.

A number of studies have been done on the effects of ion concentration on the structural stability of the cell envelopes of these orga-

nisms. The morphological changes and ultimate lysis of the cells that occur upon lowering the salt concentration of their suspending medium are not simply a result of osmotic imbalance, since similar morphological changes are seen in intact cells and in isolated cell walls. In the three halophiles that have been most extensively studied, *H. salinarum* (20), *H. cutirubrum* (7), and *H. halobium* (2, 21), a gradual lowering of the salt concentration results in a conversion from rods to spherical forms. This morphological change is accompanied by a breakdown in the structure of the outer envelope layer, resulting in disappearance of the patterned surface layer and a frayed appearance of the cell surface when seen in thin section. Similar structural changes are seen in cell envelopes prepared at high salt concentrations and resuspended in solutions of lower salt concentration. These changes in isolated cell envelopes are accompanied by the release of protein into solution. Further lowering of the salt concentration results in complete dissolution of the envelope, leaving only membrane fragments that have lost much of their protein.

Although the overall analysis of the envelopes of these organisms has been determined and the

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effects of salt concentration on their morphology and composition have been studied, their protein composition has not been examined nor has the nature of the amino sugar- and hexose-containing components been determined. During studies of cell envelopes of *H. salinarium*, it was found that the amino sugar behaved as if it were protein bound. This finding, together with the unusual nature of *Halobacterium* cell envelopes, prompted a study of the protein composition of the cell envelopes of *H. salinarium* and examination in more detail of the nature of the carbohydrate-containing component(s).

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MATERIALS AND METHODS

Growth and harvesting. *H. salinarium* strain 1 (ATCC 19700) was grown in liquid culture in the medium described by Steensland and Larsen (20) with the exceptions that yeast extract (Difco) was substituted for yeast autolysate and the sodium chloride used was reagent grade (Fisher). The bacteria were grown at 37°C in 1-liter shake cultures inoculated with 50 ml of a 2-day shake culture and harvested at the end of log phase (38 to 42 h) in a refrigerated centrifuge. Uniformly labeled cell envelope proteins were obtained by growing cells in the medium described above, to which 150 μ Ci of mixed 14 C-labeled amino acids (New England Nuclear Corp.) per liter had been added.

Cell envelope preparation. After harvesting, cells were washed twice in basal salts solution having the same composition as the growth medium: 25% (wt/vol) NaCl, 1.0% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02% (wt/vol) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.5% (wt/vol) KCl. Washing and all subsequent operations were done at 0°C. The washed wet-cell pellet was suspended in 3 volumes of basal salts and 2 volumes of acid-washed, 120- μ m glass beads (3-M Co.) and ground for 5 min in a Mini-mill (Gifford-Wood Co.). The broken-cell suspension was then centrifuged at $4,500 \times g$ for 10 min to remove glass beads, debris, and whole cells. The supernatant solution was centrifuged for 40 min at $100,000 \times g$. The resulting envelope pellet was then washed twice by resuspending in 50 volumes of basal salts and again centrifuged at $100,000 \times g$ for 40 min. The washed cell envelope pellet was stored at -70°C if not used immediately.

Quantitative determinations. Salt-free dry weights were determined by drying samples to constant weight at 105°C and subtracting the ash remaining after heating at 1,800°C for 2 h. Lipids were extracted by suspending envelopes in 10 ml of 2:1 chloroform-methanol per g of wet cell envelopes and mixing with a Vortex mixer. The suspension was allowed to stand for 15 min at room temperature with occasional mixing and then centrifuged. The pellet was reextracted twice in the same manner and then

dried by lyophilizing. The supernatant solutions were combined and evaporated to dryness for determination of cell envelope lipid content.

Protein was determined by the method of Lowry et al. (9), with bovine serum albumin as standard. Neutral hexoses were determined by the anthrone assay, using the procedure described by Spiro (19) with glucose as the standard. Hexosamine was determined by a modification of the Morgan-Elson assay (4), with glucosamine-hydrochloride as the standard.

Two-dimensional thin-layer chromatography of amino acids and amino sugars was done on Silica Gel G plates by using chloroform-methanol-17% ammonium hydroxide (2:2:1, vol/vol) in the first direction and phenol-water (75:25, wt/wt) in the second direction (14). Amino acids were detected by spraying with ninhydrin and amino sugars by the method of Partridge (13), using *p*-dimethylaminobenzaldehyde.

Protease treatment. Proteolytic digestion of envelope protein was done by using trypsin (Calbiochem) in 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 8.2, at an enzyme to envelope protein ratio of 1:10 and Pronase (Calbiochem) in 0.01 M Tris-hydrochloride buffer, pH 7.8, at an enzyme to envelope protein ratio of 1:10. Mixtures were incubated at 37°C for 24 to 48 h with 0.02% sodium azide added to prevent bacterial growth.

Gel filtration. Gel filtration of trypsin-digested cell envelope protein was done on a column (2.5 by 70 cm) of Sephadex G-100 (Pharmacia), using 10 mM ammonium bicarbonate, pH 7.0 (with 0.02% sodium azide added), for elution. Fractions of 10 ml were collected and assayed for absorbance at 280 nm, amino sugar, and neutral hexose.

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Sodium dodecyl sulfate (SDS)-gel electrophoresis was done by the procedure of Weber and Osborn (22). Protein was visualized with Coomassie brilliant blue and carbohydrate by the periodate-arsenite-Schiff method (PAS stain) of Fairbanks et al. (3). Samples were dissolved in 0.01 M sodium phosphate buffer, pH 7.2, containing 1% SDS, 0.14 M 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue, placed in a boiling water bath for 10 min, cooled to room temperature, and applied to the gel. For determination of the radioactivity profile of labeled gels, they were cut into 1-mm slices and the slices were shaken overnight at 30°C in 10 ml of toluene scintillation fluid containing 3% Protosol (New England Nuclear Corp.) before counting.

RESULTS

Preparation of *H. salinarium* cell envelopes by mechanical disruption in a salt solution having the same ionic composition as the growth medium results in the isolation of envelopes in the form of closed vesicles. Cell envelope fragments prepared in this manner retain their outer wall layer as evidenced by the presence of the hexagonal surface patterns in these preparations (20). The cell envelope ac-

counts for approximately 20% of the salt-free dry weight of the whole cell. Analysis of the envelopes (Table 1) gave values for protein, lipid, and carbohydrate similar to those found by Steensland and Larsen (20). As in their preparations, spectrophotometric data indicated that the material unaccounted for is nucleic acid. Lipid extraction was found to remove approximately 25% of the neutral hexose present in the envelopes. Glycolipid has been reported in *H. cutirubrum* (6), and it appears that approximately one-quarter of the hexose in *H. salinarium* envelopes is in the form of glycolipid. All of the amino sugar remained in the lipid-insoluble fraction.

Lipid-extracted cell envelopes were solubilized by boiling in 1.0% SDS and 0.14 M 2-mercaptoethanol. The resulting material was electrophoresed on SDS gels and stained with Coomassie blue. Approximately 15 to 20 protein bands were observed (Fig. 1A). The majority of these proteins had molecular weights of 130,000 or less, similar to the findings of Schnaitman (16) for *Escherichia coli* envelopes, in which all of the proteins were found to have molecular weights of less than 120,000. Two of the bands, however, had lower mobilities than β -galactosidase (molecular weight, 130,000), the major one being a broad band having the same mobility as myosin (molecular weight, 194,000). When an identical gel was stained for carbohydrate with the PAS reagent, two bands were seen (Fig. 1B), a broad band with the same mobility as myosin and a more faintly staining, lower-molecular-weight band. The mobilities of these bands corresponded to those of the two high-molecular-weight, Coomassie blue-staining bands. If the envelopes were solubilized directly in SDS, without lipid extraction, a third PAS-positive band appeared having approximately the same mobility as the bromophenol blue dye marker. This material was apparently the glycolipid present in the envelope.

It was observed that the age and prior treatment of the envelope preparation caused a change in the relative intensity of the two high-molecular-weight bands as well as the occasional appearance of lower-molecular-weight, PAS-positive bands, suggesting that the envelope preparations contain a contaminating protease activity. To examine this possibility, envelopes were prepared in the usual manner and then suspended in either distilled water or basal salts and incubated for varying periods of time at 37 C. After the incubation period, SDS was added to a final concentration of 1% and 2-mercaptoethanol was added to 0.14 M. The

sample was placed in a boiling water bath for 10 min before being frozen and stored. When all of the samples had been prepared, portions containing equal amounts of protein were run in parallel on SDS gels and stained with the PAS reagent (Fig. 2). It is readily apparent that the high-molecular-weight component was being degraded with a resulting increase in all of the lower-molecular-weight PAS bands with increasing incubation time, including an increase in the amount of the minor PAS-positive band seen in Fig. 1B. Furthermore, the pattern of degradation products observed depended upon the salt composition of the incubation medium. At low salt concentration there was a more heterogeneous distribution of products, suggesting that more sites for proteolysis had been exposed either due to an unfolding of the protein or to the loss of the structural integrity of the envelope, or both. Coomassie blue-stained gels of identical samples showed no noticeable alteration from the initial pattern observed, except for a decrease in intensity of the highest-molecular-weight protein and an increase in the intensity of the next-highest-molecular-weight component (corresponding to the minor PAS-positive band). This result may be due to the greater probability of the high-molecular-weight protein being degraded by a protease because of its large size and high concentration relative to the other envelope proteins (see below). *Halobacteria* are known to produce extracellular proteases (11), and it appears likely that isolation of the envelopes results in sufficient disruption of their structural integrity to make them susceptible to these proteases. However, the possibility that the observed degradation may be due to glycosidase activity is not ruled out. In either case, the results suggest that the cell envelopes of intact *H. salinarium* cells possess a single high-molecular-weight, carbohydrate-containing protein; the minor PAS-positive protein is probably a degradation product.

TABLE 1. Cell envelope analysis^a

Substance	Amt in cell envelope	
	mg per 100 mg (dry weight)	% remaining after lipid extraction
Protein	63	98
Hexose	3.6	74
Amino sugar	0.6	95
Lipid	21	0

^a Values are averages obtained from two separate cell envelope preparations.

FIG. 1. SDS gels (5% acrylamide) of envelope proteins (600 μ g per gel). (A) Coomassie blue stained-gel; (B) PAS-stained gel (arrow on right indicates position of minor PAS-staining band). The positions of standard proteins run on parallel gels are indicated: (1) myosin (194,000); (2) β -galactosidase (130,000); (3) bovine serum albumin (69,000); and (4) aldolase (40,000).

To obtain an estimate of the contribution of this high-molecular-weight component to the total envelope protein, cells were grown in 1 liter of medium containing 150 μ Ci of 14 C-labeled amino acids. The 14 C-labeled amino acid mix-

ture was added to the normal growth medium before inoculation. Inoculation, growth, and harvesting were carried out as described in Materials and Methods. Approximately 30% of the added radioactivity was incorporated, and

FIG. 2. Effect of incubation at 37 C on carbohydrate-containing cell envelope proteins. Scans at 560 nm of PAS-stained SDS gels (7.5% acrylamide) are shown. The regions of the gels to the right of the dotted line were scanned at 10 times greater sensitivity than the high-molecular-weight band. Envelopes (A) immediately after preparation; (B) after incubation for 1.5 h in basal salts; (C) after incubation for 16 h in basal salts; (D) after incubation for 16 h in distilled water.

26% of the incorporated activity was present in the isolated cell envelopes. After lipid extraction, the envelopes were run on SDS gels, sliced, and counted (Fig. 3A). Parallel gels containing an equal amount of protein were stained with Coomassie blue (Fig. 3B) and PAS reagent (Fig. 3C). All of the radioactivity was recovered from the gel except for approximately 5% remaining at the top of the gel. Because of the relatively low specific activity (3.6×10^4 counts/min per mg) of proteins labeled in this manner, the resolution of the lower-molecular-weight components was poor. The high-molecular-weight PAS-positive band was well resolved, however, and accounted for 40 to 50% of the radioactivity on the gel. (The ^{14}C -labeled, carbohydrate-containing protein has subsequently been purified and found to have the same specific activity as the whole envelope protein; therefore, it is not preferentially labeled.) Comparison of the sliced gel and the Coomassie blue-stained gel indicates that the high-molecular-weight band stained very poorly with Coomassie blue in comparison with the lower-molecular-weight

proteins. A similar phenomenon is observed with the acidic glycoprotein of erythrocyte membranes (3).

To determine whether the carbohydrate portion of the high-molecular-weight component is, in fact, covalently bound to protein, lipid-extracted envelopes were treated with the proteases, trypsin, and Pronase. After extensive digestion with trypsin, a single, low-molecular-weight, PAS-positive band was seen on gels (Fig. 4). It also stained lightly with Coomassie blue and was the only Coomassie blue-positive band present. Treatment of the trypsin-digested material (or of lipid-extracted envelopes) with Pronase further degraded it so that no PAS-positive band was found on the gel (Fig. 4).

The trypsin degradation products were further examined by gel filtration. Before trypsin digestion, all of the nonlipid carbohydrate (both hexose and amino sugar) of cell envelopes solubilized in 10 mM ammonium bicarbonate ran as a single peak in the excluded volume on a Sephadex G-100 column. After trypsin degrada-

FIG. 3. SDS gels (5% acrylamide) of ^{14}C -labeled cell envelopes (lipid free). Gels were run in parallel with equal amounts of protein (120 μg) applied to each. Positions of standard proteins run in parallel are shown by arrows. (A) Radioactivity profile of sliced gel; (B) Coomassie blue-stained gel; (C) PAS-stained gel.

tion, the profile shown in Fig. 5 was obtained. The hexose remained in the excluded peak and the amino sugar was found in a single, included peak. SDS-gel electrophoresis of the material in the hexose peak resulted in a single PAS-positive band having the same mobility as the PAS-positive band seen in the tryptic digest of whole-cell envelope protein (Fig. 4A). No bands were seen on SDS gels of the material in the amino sugar peak, probably because of the low molecular weight of the peptide. Two-dimensional thin-layer chromatography (see Materials and Methods) of acid hydrolysates (6 N HCl for 16 h at 105 C, in vacuo) of pooled amino sugar- and pooled hexose-containing fractions indicated that most of the common amino acids were present in both. Two amino sugars were also detected in the amino sugar peak by both the ninhydrin and *p*-dimethylaminobenzal-

dehyde sprays, one having mobilities identical to those of glucosamine. The other ran in the region of galactosamine and mannosamine, which were not resolved in the system used. The susceptibility of the carbohydrate-containing material to trypsin suggests that all of the nonlipid carbohydrate of the cell envelope of *H. salinarium* was covalently linked to protein.

The phenol extraction procedure originally used by Westphal et al. (23) for extraction of lipopolysaccharide from envelopes of gram-negative bacteria has been used by a number of investigators for isolation of cell surface glycoproteins from erythrocyte membranes (5). It was found to be effective for obtaining partial purification of the carbohydrate-protein complex of *H. salinarium* cell envelopes. The extraction was carried out by using an equal volume of 0.05 M Tris-hydrochloride buffer, pH

FIG. 4. SDS gels (5% acrylamide) of cell envelope protein after incubation with added proteases. Staining was done with the PAS reagent. (A) Trypsin-digested whole-cell envelopes; (B) Pronase-digested whole-cell envelopes.

7.2, containing 0.5% NaCl and buffer-saturated phenol and was equally effective when done for 30 min at either room temperature or at 85 C. All of the amino sugar was recovered in the

aqueous phase along with approximately 75% of the neutral hexose. SDS gels of the two phases (after dialysis to remove the phenol) show that the only major protein present in the aqueous phase was the high-molecular-weight, carbohydrate-containing protein (and its proteolytic degradation products) (Fig. 6C and C'). All of the remaining envelope proteins were recovered in the phenol phase (Fig. 6B). Trypsin digestion of the material recovered in the aqueous phase resulted in amino sugar- and hexose-containing peptides eluting at the same positions on a Sephadex G-100 column and having identical mobilities and staining properties on SDS gels as those observed for whole-envelope digests. Co-purification of the amino sugar and hexose moieties suggested that both are bound to the high-molecular-weight protein.

Lowering of the salt concentration of the suspending medium results in release of protein from isolated cell envelopes of *H. salinarium* (20). Steensland and Larsen (20) have shown that exposure of cell envelopes to low salt concentration followed by centrifugation results in the isolation of an envelope fraction that is missing the outer layer, and the only organized structure that appears to remain is the cell membrane. This cell membrane fraction is considerably enriched in lipid by comparison with the intact cell envelope. To determine whether all of the envelope proteins are solubilized to an equal extent under these conditions or whether a specific group of proteins remain bound to the sedimentable membrane fraction, cell envelopes were prepared in the usual manner and the wet pellet was suspended in dilute salt solution to give a final concentration of approximately 0.14 M NaCl, 5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 10 mM KCl. The resus-

FIG. 5. Elution profile on Sephadex G-100 (2.5 by 70 cm) of whole-envelope protein (600 mg) after trypsin digestion. Samples of fractions were assayed for hexose (absorbance at 620 nm), amino sugar (absorbance at 585 nm), and absorbance 280 nm.

FIG. 6. Phenol extraction of cell envelopes. SDS gels (5% acrylamide) of: (A) whole envelopes (800 μ g of protein) (Coomassie blue stained); (B) phenol phase after phenol extraction (400 μ g of protein) (Coomassie blue stained); (C) aqueous phase after phenol extraction (400 μ g of protein) (Coomassie blue stained); (C') aqueous phase after phenol extraction (400 μ g of protein) (PAS stained; arrow on right indicates position of minor PAS-staining band).

pended cell envelopes were stirred for 5 min and then centrifuged at $100,000 \times g$ for 1 h. Spectrophotometric scans of Coomassie blue-stained gels of whole-cell envelopes (Fig. 7A) and of the pellet obtained after exposure to low salt concentration (Fig. 7B) showed that the material remaining sedimentable under the above conditions was greatly enriched in several of the envelope proteins, among them the high-molecular-weight, carbohydrate-containing protein. The majority of the envelope proteins remained in very low amounts, if at all, in the sedimentable cell membrane fraction relative to their concentration in whole cell envelopes.

DISCUSSION

Cell envelopes of *Halobacterium salinarium* lack the peptidoglycan layer and lipopolysac-

charide-containing outer membrane component normally found in cell envelopes of gram-negative bacteria but have been shown to possess an external cell wall layer in addition to a plasma membrane with a typical bilayer appearance (20). When the proteins of the cell envelope are examined by SDS-gel electrophoresis, the pattern obtained resembles in some respects that seen for *E. coli* (16, 17) and other gram-negative bacteria (15). Approximately 50% of the total envelope protein is present in 15 to 20 bands having molecular weights of 130,000 or less. The strikingly different feature of the protein composition of these envelopes is the absence of a major band at 42,000 daltons, which in *E. coli* accounts for approximately 40% of the envelope protein and which Schnaitman (17) has shown to be a group of at least three polypeptides associated with the outer membrane. Instead,

the remaining 40 to 50% of the protein in *H. salinarium* envelopes is accounted for by a protein with a mobility on SDS gels corresponding to a molecular weight of approximately 194,000. This protein has both all of the amino sugar and all of the nonlipid hexose covalently bound to it as indicated by PAS staining, its susceptibility to proteases, and its behavior on phenol extraction. SDS gels are known to give anomalous molecular weight values for proteins containing substantial amounts of carbohydrate (18), and the value of 194,000 may therefore be

in error. However, this protein would contain only approximately 12% carbohydrate (calculated from the data of Table 1 and Fig. 3). This relatively low carbohydrate content would not be expected to result in a large error in the molecular weight as determined by SDS gels. The results obtained by SDS-gel electrophoresis do not rule out the possibility that the high-molecular-weight band is actually two or more proteins with similar molecular weights. Thus, although both the amino sugar and hexose moieties are covalently linked to protein, it is not clear whether they are bound to the same protein. The protein runs as a broad band on gels loaded with sufficient material to allow adequate visualization of it and of the lower-molecular-weight envelope proteins by Coomassie blue (Fig. 1A and 6) but is sharpened considerably when lesser amounts are electrophoresed (Fig. 3A). Furthermore, if there is more than one PAS-staining protein, they copurify upon phenol extraction. Further purification and study of the high-molecular-weight protein will be necessary to resolve this question.

Several bacterial envelope proteins having covalently bound carbohydrate have been reported, and two types of linkage between the protein and the carbohydrate-containing moiety have been found. Braun and Bosch (1) have demonstrated the occurrence of a lipoprotein bound through the 6-amino group of its C-terminal lysine to the diaminopimelic acid of the peptidoglycan in *E. coli*. Wober and Alau-povic (24), working with *Serratia marcescens*, and Wu and Heath (25), working with *E. coli*, have shown that most of the lipopolysaccharide of these organisms is present as a protein-lipopolysaccharide complex covalently linked by a phenol-sensitive bond. In light of the absence of peptidoglycan and lipopolysaccharide and its stability in hot phenol, the carbohydrate-containing protein of *H. salinarium* appears to resemble neither of these molecules. Okuda and Weinbaum (12) have reported the presence of phenol-extractable "glycoproteins" in the cell envelope of *E. coli*. They have shown that the carbohydrate-containing moiety is covalently bound to protein but have not demonstrated a direct linkage between a carbohydrate molecule and an amino acid as occurs in plant and animal glycoproteins; the question of whether *E. coli* or other bacteria possess glycoproteins of the type found in higher organisms thus remains open.

The high-molecular-weight, carbohydrate-containing protein of *H. salinarium* is being studied further in order to determine the nature

FIG. 7. Effect of low salt concentration on envelope protein content. Scans at 620 nm of Coomassie blue-stained SDS gels are shown. (A) Whole-cell envelopes; (B) sedimentable envelope fraction after suspension in medium of low salt concentration.

of the linkage between the carbohydrate moiety and the protein and to attempt to determine its function. The large proportion of the high-molecular-weight protein in the cell envelope of *H. salinarum* suggests that it plays an important role in the structure of the envelope, e.g., in its shape. Unlike the majority of envelope proteins, it remains bound to the lipid-enriched cell membrane at low salt concentration and may serve as a bridge between the membrane and the less firmly bound envelope proteins. In addition to further study of the *H. salinarum* carbohydrate-protein complex, *H. cutirubrum* and *H. halobium* cell envelopes are being examined to determine whether they have similar carbohydrate-containing proteins. The cell envelopes of these bacteria have amino sugar and hexose compositions similar to those of *H. salinarum*, and a preliminary report on the occurrence of protein-bound hexose in the cell envelope of *H. halobium* has been presented (M. A. Koncewicz, *Biochem. J.* **128**:124, 1972).

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