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SHORT COMMUNICATIONS

Identification of Collagen-Like Sequences in Proteins from the Cell Envelope of *Halobacterium salinarium*

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One of the cell-envelope proteins of gram-negative obligately halophilic archaebacteria of the genus *Halobacterium* is a glycoprotein, which is very similar to the glycoproteins of eukaryotic cells with respect to the type of glycosylation and the composition of oligosaccharides [1]. This glycoprotein contains the cluster of threonine residues glycosylated by disaccharides in a way typical of the collagen of animal cells [2]. The possibility could not be excluded that the cell-envelope proteins of *H. salinarium* are homologous to the animal-type collagen not only in the type of glycosylation but also in amino acid sequence. If so, this would provide evidence for an evolutionary relatedness of archaea, particularly *H. salinarium*, and animal cells. The aim of the present work was to attempt to detect collagen-like sequences in the cell-envelope proteins of the archaebacterium *H. salinarium.*

The strain *H. salinarium* ET-1001 used in this work was obtained from the culture collection of the Max Planck Institute for Biochemistry, Germany. The strain was cultivated aerobically at 37°C for 3 days in a medium containing (g/l) NaCl, 250.0; KCl, 2.0; sodium citrate, 3.0; $MgSO_4 \cdot 7H_2O$, 20.0; and peptone, 7.0 (pH 6.8–7.0). The cell envelopes of *H. salinarium* were prepared by the method previously described [3]. Proteins were extracted from the cell envelope with Tris–HCl buffer (pH 6.7) containing 3% SDS and 5% β-mercaptoethanol [3]. Proteins were analyzed by electrophoresis in PAAG under denaturing conditions [4].

To detect proteins containing collagen-like sequences, we used the highly specific enzyme clostridiopeptidase (collagenase), which was purified to an apparent homogeneity [5]. The enzyme actively hydrolyzed collagen and was inactive with respect to globular proteins, such as casein and albumin. It is recognized that the treatment of cell envelopes with clostridiopeptidase is an efficient test for collagen-like sequences in microbial proteins [6, 7].

Preliminary electron-microscopic studies showed that collagenase caused some alterations in the structures of the cell envelopes of *H. salinarium.* The electrophoresis of the cell-envelope proteins before and after treatment with clostridiopeptidase showed that the treatment led to the disappearance of proteins with a molecular mass of 40 and 130 kDa and certain minor proteins and to a decrease in the content of protein with molecular masses of 69 kDa, whereas the amounts of proteins with molecular masses of 194 and 23 kDa did not change (see Fig. 1).

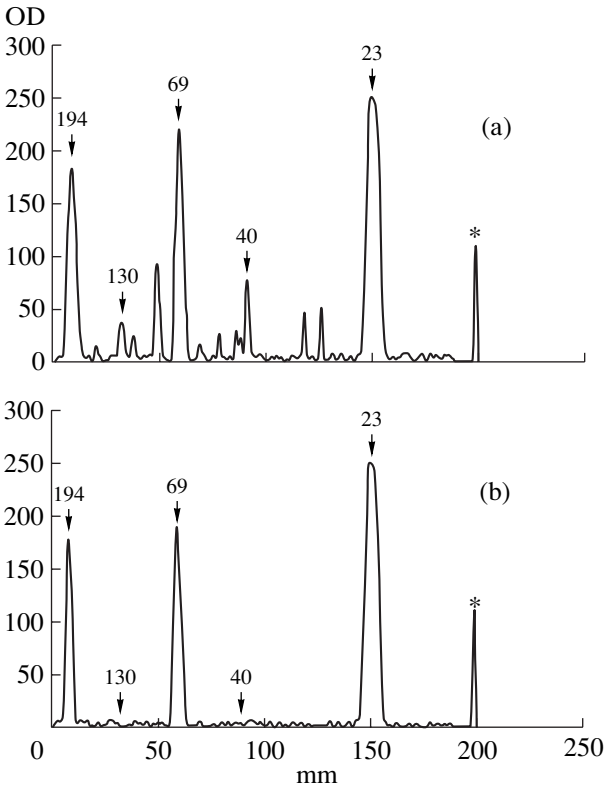

Fig. 1. Electrophoretic densitograms of proteins extracted from the cell envelope of *H. salinarium* with 3% SDS and 5% β-mercaptoethanol: (a) before treatment with clostridiopeptidase and (b) after such treatment. The treatment with clostridiopeptidase was performed at 37°C for 20 min and then at 45°C for 30 min. The ordinate shows the optical density (OD) of the gel at 600 nm in arbitrary units and the abscissa shows the gel length in mm. The numerals above peaks indicate the molecular masses of proteins in kDa. (*) Gel boundary.

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Our search in the GeneBee database showed that the proteins with molecular masses of 194 and 23 kDa contain no collagen-like sequences. This finding agrees with the electrophoretic data, according to which these proteins are resistant to the action of clostridiopeptidase. The amino acid sequences of the proteins with molecular masses of 130, 69, and 40 kDa were not found in the GeneBee database.

The disappearance of some cell-envelope proteins after the clostridiopeptidase treatment suggests that either these proteins contain collagen-like sequences, they are fixed in the cell envelope by proteins or polypeptides containing such sequences, or their disappearance is the result of the action of a cell-envelope proteinase activated by clostridiopeptidase.

The data presented are indicative of the presence of collagen-like sequences in some proteins of the cell envelope of *H. salinarium*. This finding is of interest from an evolutionary point of view.

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