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CHEMICAL PROPERTIES OF PROTEINACEUS CELL WALL FROM AN ACIDO-THERMOPHILE, Sulfolobus acidocaldarius

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Proteinaceus cell wall from an acido-thermophilic archaebacteria, Sulfolobus acidocaldarius, contains two glycoproteins of molecular weight 40 000 and 100 000 as major subunits. Glucose and mannose were major carbohydrate components. The cell wall proteins were rich in hydrophobic amino acid residues. In the electron microscopy, a hexagonal array was seen on the surface of the isolated cell wall as well as the intact cell.

Sulfolobus acidocaldarius is an acido-thermophile inhabiting in solfataric hot springs and characterized by the lobed shape and autotrophic growth in the presence of elementary sulfur in addition to the ability to grow under hot and acidic conditions.[1] This organism is classified into archaebacteria.[2] Like other archaebacteria, S. acidocaldarius lacks the peptide glycan cell wall, and has rigid cell wall consisting of proteins.[3,4] In this communication, some chemical properties of the cell wall will be described.

S. acidocaldarius strain 7 used in this study was isolated from a hot-acid spring in Japan. Isolation and microbial properties will be described elsewhere. The strain is capable of growing optimally at pH 3, 75°C. In the electron microscopy, the ultra-thin section of the cell showed the presence of cell wall membrane of about 50 nm thick covering the cytoplasmic membrane.

The organism was grown in a medium consisted of 0.1% yeast extract, 0.1% casamino acid, 0.1% glucose, 0.02% NaCl, 0.03% KH_2PO_4, 0.13% $(NH_4)_2SO_4$, 0.025% $MgSO_4 \cdot 7H_2O$ and 0.005% $CaCl_2 \cdot 2H_2O$, at 75°C. The medium was adjusted to pH 3.0 with 1N H_2SO_4.

The cell wall was isolated as follows; the cells were suspended in 0.5 M Tris-HCl buffer, pH 8, containing 2 mM EDTA and ground with alumina for 15 min at 4°C (all procedures were carried out at 4°C). Alumina and unbroken cells were removed by centrifugation at 1 500 × g for 10 min. The supernatant was further centrifuged at 8 000 g for 20 min. The resulting pellet was suspended in 0.1 M 3-[cyclohexyl-amino]-1-propane-sulfonic acid(CAPS)-NaOH buffer, pH 10.5, containing 2% Triton X-100. The Triton-insoluble pellet was resuspended in 0.1 M CAPS-NaOH buffer, pH 10.5, containing 2% sodium dodecylsulfate (SDS), and centrifuged at 60 000 g for 1 h. Upper white layer of the precipitate was saved. This procedure was repeated three times. The remaining SDS was removed by washing with distilled water from the final preparation. In the electron microscopy, it was found that the cytoplasmic membrane was completely removed from the cell wall preparation.

The cell wall fraction thus obtained was empty vesicles as shown in Fig. 1-A.

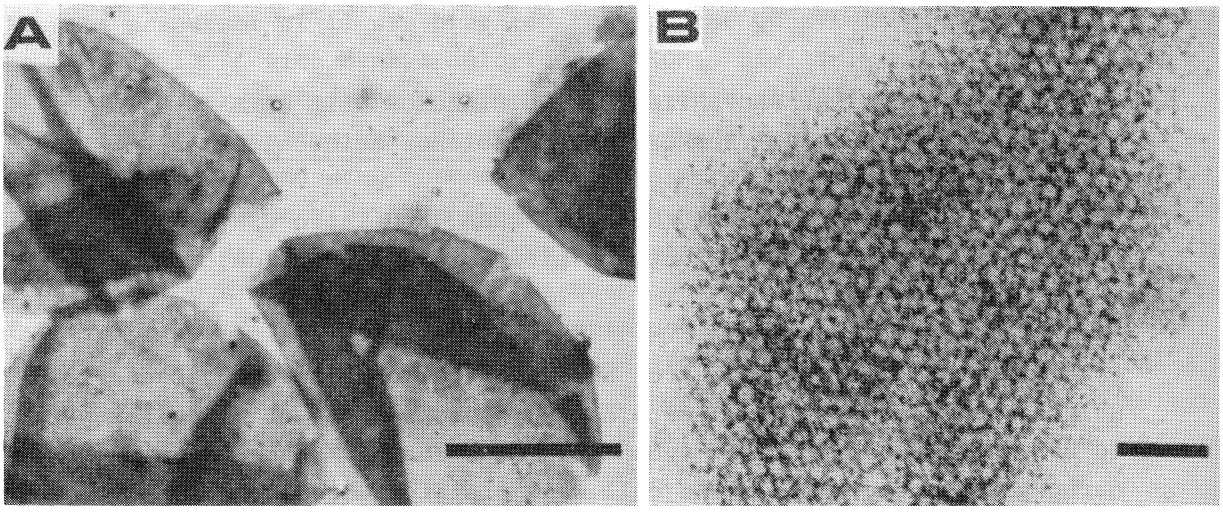

Fig. 1. Electron microscograph of the isolated cell wall from S. acidocaldarius. A, negatively stained. Bar=1.0 μm; B, thin section at high magnification. The hexagonal array is seen. Bar=100 nm.

The size of the vesicles was similar to that of the intact cells. A regular hexagonal arrangement[3,4] was seen on its surface as shown in Fig. 1-B. The center to center distance of this hexagon was about 20 nm. The results show that the hexagonal structure of the cell wall is stable to 2% Triton X-100 and 2% SDS treatments in the alkaline buffer.

The cell wall fraction contained no extractable lipids, since chloroform-methanol (2 : 1) extraction did not change the dry weight of the preparation. The chemical composition of the dried preparation was 34% carbohydrates and 60-70% proteins. The carbohydrates were determined by anthrone reaction using glucose as a standard,[5] and the protein by the method of Lowry[6] using bovine serum albumin as a standard. A small amount (about 3%) of hexosamine was detected by Elson-Morgan reaction[7] using glucosamine as a standard. The low hexosamine content can be interpreted as indicating the lack of peptideglycan in the preparation.

The cell wall preparation was dissolved in 4% SDS containing 5% 2-mercapto-ethanol and applied on an SDS-polyacrylamide gel plate for electrophoresis. Two protein bands of molecular weight 40 000 and 100 000 were detected with Coomassie blue. These bands were also stained with a periodate Schiff reagent[8] for the detection of carbohydrates (Fig. 2). This indicated that the main components are

Table 1. Amino acid composition of the cell wall proteins

Amino acid	μmol/100 mg	Amino acid	μmol/100 mg	Amino acid	μmol/100 mg
Lys	13.8	Glx	47.6	Ile	44.5
His	4.0	Pro	43.8	Leu	58.7
Arg	2.0	Gly	41.4	Tyr	44.9
Asx	56.9	Ala	51.8	Phe	36.4
Thr	74.8	Val	53.8	Cys	1.1
Ser	53.6	Met	8.3	Trp	0

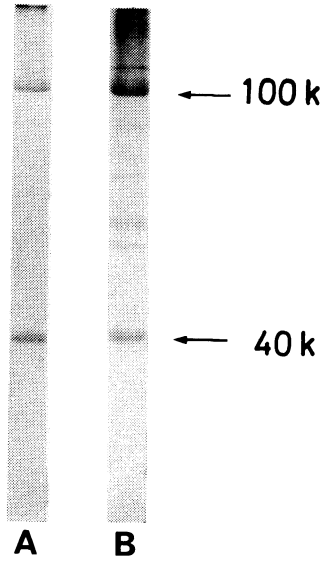

Fig. 2. SDS-gel electrophoresis of the isolated cell wall. A, stained with Coomassie blue; B, stained with the periodic acid - Schiff technique.

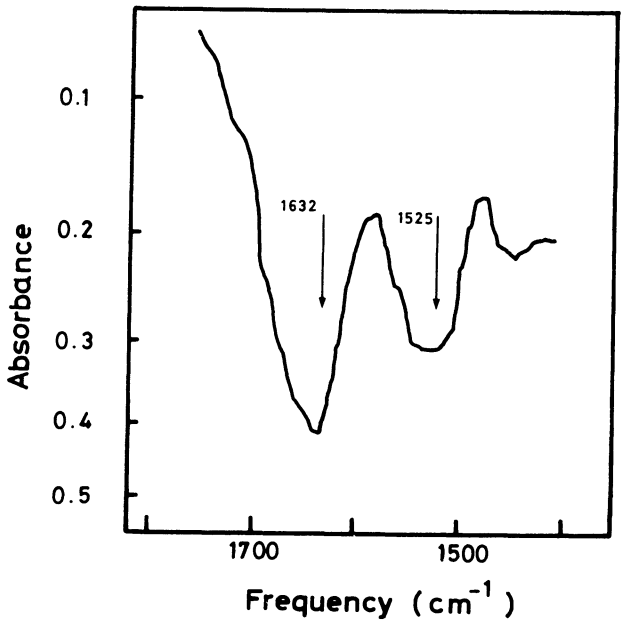

Fig. 3. Infrared spectrum of the isolated cell wall (solid film).

two glycoprotein subunits of M.W. 40 000 and 100 000.

Amino acid composition of the cell wall preparation is shown in Table 1. Tryptophan was determined by the method of Penke et al.,[9] and cysteine was analyzed after converted to cysteic acid by performic acid oxidation at 25°C for 4 h. As shown in the table, amino acid residues of chargeable side chains were poor. Whereas hydrophobic residues were abundant. No tryptophan residue was present.

Carbohydrate composition was analyzed by the method of Chambers and Clamp[10] using a Shimazu model GC-4CM gas chromatograph after methanolysis and trimethylsilylation. A glass column (3 mm × 20 cm) packed with 5% SE30 on 60/80 Celite 545 (Nihon Chromato Co.) was used with a temperature program from 140°C to 200°C at a rate of 0.5°C/min. Glucose and mannose were detected in a molar ratio of 2.6 : 1 as major components.

The preparation was treated in 0.1 M NaOH containing 0.3 M $NaBH_4$ according to Tanaka and Pigman.[11] It was reported that O-glycosylated serine and threonine degraded under the conditions. After hydrogenation in the presence of $PdCl_2$, the amino acid composition was analyzed. About 15% of threonine and 30% of serine detected by the acid hydrolysis were converted to 2-aminobutyric acid and alanine, respectively. The results suggest that linkages between proteins and carbohydrates of the cell wall are, at least in part, O-glycosidic bonds of serine and threonine. Methylation analysis of the cell wall preparation was carried out according to Hakomori.[12] No dimethylated carbohydrate was detected, suggesting the absence of branch in the carbohydrate chains. From the ratio of trimethylated to tetramethylated carbohydrates it was suggested that, as an average, short chains of four carbohydrate residues are attached to the proteins, although incomplete methylation of the insoluble material may cause errors in this estimation.

Infrared spectrum (amido region) of the preparation is shown in Fig. 3. The

cell wall suspended in distilled water was spread on a CaF_2 plate and dried at 50°C to prepare a solid film. The spectrum was recorded on a Hitachi model 260-50 infrared spectrophotometer. As shown in Fig. 3, bands at 1632 and 1525 cm^{-1} were characteristic, suggesting the presence of β-sheet structure in the proteins.[13]

The cell wall structure were resistant to 2% SDS, 2% Triton X-100, 4 M urea and proteases. No attack was detected when trypsin was added to the suspension. The proteins were only slowly digested with pronase, and several, discrete bands were observed on the SDS-acrylamide gel electrophoresis. The results show that the cell wall structure is extremely rigid, having a few pronase sensitive sites in it.

The cell wall proteins of S. acidocaldarius have been studied by Weiss,[3] Michel et al.,[4] and Taylor et al.[14] The hexagonal array with 20 nm intervals on the surface was observed by these investigators. The amino acid composition described here is similar to that reported by Michel et al.,[4] but different from the data reported by Weiss. In contrast to our results, the cell wall studied by Michel et al. consisted of homologous subunits of M.W. 140 000 to 170 000 and the protein was soluble in a buffer, pH 9, at 60°C. The discrepancy should be solved in future studies. The bacterial strain of the present study differs from the strain used in these previous reports, and the cell wall in the present study was washed with 2% SDS at alkaline pH before the chemical analyses were carried out. Thus the discrepancy observed in the present study with the previous reports may be due to the differences of the bacterial strain and/or of the procedure used for the cell wall preparation.

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