Short Communication

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MOLECULAR WEIGHT ANALYSIS OF PROTEIN UNITS IN THE *VIBRIO CHOLERAE* CELL ENVELOPE

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The nature of the membrane protein units is one of the expressions of the genetic make up of the organism concerned. Molecular weights of different protein units in the envelopes of *Pseudomonas aeruginosa* (1) and *Vibrio parahaemolyticus* (2) have recently been reported. Similar analysis of the proteins in the envelope of *Vibrio cholerae* (classical) possessing some degree of similarity with the above organisms is reported in this communication.

Freshly harvested *V. cholerae* cells were broken by sonication at 10 kHz and the envelopes were isolated by differential centrifugation. The envelopes were purified by treatments with DNase and RNase in the presence of MgCl₂, and by repeated washing by centrifugation at 20,000×*g* at 4° till the supernatant was free from 260 and 280 nm absorbing materials. The purity of the isolated cell envelopes was assessed by electron microscopy, visible and ultraviolet spectrophotometry, and the absence of contaminating particulate materials, nucleic acids, and cellular proteins was established (3, 4). Studies on the chemical composition including infrared absorption characteristics and biological activity including phage inactivating and O-antigenic properties of the envelope fraction thus isolated, are under publication elsewhere (4). Polyacrylamide gel electrophoresis of the envelope materials was done by the phenol-acetic acid-water method of TAKAYAMA *et al.* (5) and also by the SDS-gel method of WEBER and OSBORN (6). In the acid method, proteins dissolved in a small volume of phenol-acetic acid-water (2: 1: 0.5, w/v/v) were subjected to electrophoresis with a current of about 3 mA/tube. The *R_f* values were determined as the ratios of the distances migrated by the bands and the length of the gel. For the SDS-gel method, the standard proteins obtained from Serva Feinbiochemica, Germany, and used for calibration purposes were serum albumin (68,000), ovalbumin (43,000), pepsin (35,000), β-lactoglobulin (18,400),

RNase (13,700), and cytochrome-*c* (12,000). The envelope materials were dissolved in 0.01 M sodium phosphate buffer (pH 7.0) containing 1% SDS and 1% β -mercaptoethanol and heated at 70° for 30 min. The supernatant obtained after centrifugation was dialysed for 24 hr against a buffer containing 0.01 M sodium phosphate (pH 7.0), 0.1% SDS, and 1% β -mercaptoethanol. In some experiments, the sample was dissociated in the 1% SDS dissociating mixture described above by heating for 1 min at 100° and then dialysed as described above. Sucrose to a final concentration of 20% (w/v) and 0.05% of Bromophenol Blue in water were mixed with the dialysed sample containing proteins. Electrophoresis was carried out for about 3.5 hr with a current of 8 mA/tube. Mobility was measured following the method of WEBER and OSBORN (6), and molecular weights determined from the calibration curve.

The electrophoretic pattern as obtained by the phenol-acetic acid-water method resolved the presence of at least 14 different protein bands. The measured *R_f* values, as averaged over a number of independent experiments, of these bands were 0.185, 0.215, 0.240, 0.255, 0.280, 0.310, 0.360, 0.410, 0.465, 0.515, 0.615, 0.665, 0.705, and 0.750. The presence of a large number of protein bands was also detected by identical method in the envelope fraction of *V. parahaemolyticus* (2). In the SDS-gel method, the presence of 13 bands could be resolved in the electrophoretic pattern (Fig. 1), 3 or 4 of which comprised the major fraction of the envelope proteins.

The protein units had the molecular weights of 167,000; 155,000; 128,000; 108,000; 83,000; 70,000; 57,000; 53,000; 45,000; 35,000; 25,000; 20,000; and 13,000. The 13,000 molecular weight polypeptide had the largest share in the total envelope protein. Experiments carried out with the envelope fractions of different batches revealed that the individual variations in the molecular weight values of different bands were within a maximum limit of $\pm 8\%$. No additional band could be detect-

Fig. 1. SDS-acrylamide gel electrophoretic pattern of proteins extracted from the isolated cell envelopes.

ed when the sample was heated for 1 min at 100°. However, when the sample was not heated at all in the 1% SDS mixture some bands were missing, indicating some degree of aggregation of the polypeptides.

The present observations substantiate the statement of SCHNAITMAN (7) that the presence of one or few major protein species could be a common feature of all gram-negative bacterial cell walls. A polypeptide unit of molecular weight in the neighbourhood of 45,000 units, as detected presently in *V. cholerae* envelope, was also detected in the envelopes of *P. aeruginosa* (1), *Escherichia coli* (7), *Klebsiella aerogenes* (8), *V. parahaemolyticus* (2), and some other gram-negative organisms. However, the 30,000 and 90,000 molecular weight proteins of the envelope of *V. parahaemolyticus* (2) do not fit well with the molecular weight spectrum of *V. cholerae* envelope proteins within the limits of experimental error.

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