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A Study of the Amino Acids and Proteins of Some Human T-Mycoplasma Membranes (39145)

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(Introduced by M. F. Shaffer)

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While the membranes of seven human T-mycoplasmas have been analyzed for their carbohydrate content (1), the proteins of only whole T-mycoplasmas have been studied (2). Therefore, membranes were prepared from the same seven human T-mycoplasmas, their proteins examined by isoelectric focusing, and their amino acid composition determined. The results are reported below.

Materials and Methods. The technique for preparing T-mycoplasma membranes from the four laboratory strains *T-McA*, *T-960*, *T-27*, and *T-58* and the three isolates from patients with nonspecific urethritis strains *T-210*, *T-220*, and *T-213* has been described (1). Exponential phase T-broth cultures were centrifuged at 14,500*g* for 30 min and the supernatants decanted. The pellets of T-mycoplasmas were resuspended in phosphate-buffered saline (PBS), pooled, sonicated for 1 min, washed twice in PBS, and resuspended in 50 ml of distilled water. The aqueous suspension was passed four times through a Sorvall cell fractionator at 40,000 psi and centrifuged twice at 14,500*g* to sediment whole T-mycoplasmas. Then the supernatant was centrifuged at 37,500*g* for 60 min to deposit the membranes, which were resuspended in a minimal volume of distilled water and checked for purity by examining by EM an aliquot after negative staining. Thereafter the suspension was lyophilized for chemical analysis (1). At least two membrane preparations were prepared independently from each strain and examined in duplicate.

Amino acid analysis. The amino acids were released from between 0.75 and 7.75 mg dried membrane by hydrolysis *in vacuo* in a sealed vial by 6 *N* HCl at 100° for 18 hr. Thereafter, the hydrolysate was dried *in vacuo* at room temperature over NaOH pellets, resuspended in citrate buffer (pH 2.2) and the amino acid content was determined using a Beckman 120 C Amino Acid Analyzer (3).

Isoelectric focusing. The dried membranes were extracted with 10% Tergitol 15 S-9 (Sigman Chemical Company, St. Louis, Mo.). A mixture of 4 mg of dried membrane/ml of Tergitol was kept at 2–4° for 48 hr. Thereafter, the suspension was clarified by centrifugation at 43,000*g* for 1 hr and the protein content of the supernatant was determined (4). Gels, 0.5 × 10 cm, were prepared containing 5% (w/v) polyacrylamide, 8 *M* urea, and 2% Ampholine carrier ampholytes (LKB Produkter AB) to form a gradient within the range pH 2.5 to 9.5. They were polymerized by means of ammonium persulfate (5). Each gel also contained 35–50 μg membrane protein. Focusing of the gels was continued for 5 hr at room temperature in a Canalco Disc Electrophoresis apparatus (Canalco, Rockville, Md.) having a constant voltage of 150 V and anode and cathode solutions, respectively, of 5% orthophosphoric acid and 5% 1, 2-ethylenediamine. Then duplicate gels were fixed and washed extensively with 12% trichloroacetic acid. One gel was stained for a minimum of 8 hr with 0.1% Coomassie Brilliant Blue (Bio-Rad Laboratories, Richmond, Calif.) in a methanol–glacial acetic acid–water mixture (45:10:45 v/v). Thereafter, the excess color was removed by several washings with a methanol–glacial acetic acid–water mixture (30:10:60 v/v). The other gel was treated with 0.5% periodic acid for 2 hr, followed by 0.5% sodium arsenite in 5% acetic acid for 1 hr.

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Then it was washed in 5% acetic acid for 10–20 min, stained overnight in Schiff's reagent, and decolorized in N/100 HCl. (6). Densitometer tracings of the stained gels were made in a Beckman spectrophotometer Acta C111 equipped with gel scanner 2.

The pH gradient of the gels was obtained by cutting a gel into 2-mm slices which were placed in 2 ml of distilled water, eluted overnight, and the pH determined.

Results. Table I contains the amino acid composition of the membranes. The value for each amino acid is the mole% of the total amino acids in the acid hydrolysate. Most material eluted at times corresponding to amino acid standards. However, every sample contained four ninhydrin-positive components. One consisted of about 1% of the amino acids and was identical to the glucosamine standard while the others which comprised about 0.7% of the amino acids were not identified. No components corresponded to the galactosamine, mannosamine, and acid hydroxylysine standards. The total quantity of amino acids in the hydrolyzed samples comprised between 10 and 30% of the dry weight of the membrane.

TABLE I. AMINO ACID COMPOSITION OF MEMBRANE PREPARATIONS OF SEVEN HUMAN T-MYCOPLASMAS

Amino acid (mole %)	T-mycoplasma strains						
	T-McA	*T-960*	*T-58*	*T-27*	*T-213*	*T-210*	*T-220*
Asx	8.4	8.9	8.4	8.3	8.0	8.4	8.2
Thr	6.0	7.2	7.5	7.6	7.0	8.3	7.8
Ser	11.3	10.3	11.2	10.6	10.8	11.6	11.9
Glx	11.5	12.5	10.1	11.0	9.9	11.1	10.5
Pro	8.4	6.6	9.0	4.4	7.1	6.1	7.3
Gly	8.8	9.7	8.0	7.2	7.1	7.6	7.7
Ala	6.6	7.8	6.7	6.6	6.3	6.6	6.4
Cys	1.4	1.5	1.4	0.9	1.3	0.7	1.3
Val	6.1	8.3	7.8	7.7	7.2	9.1	8.7
Met	<0.1	<0.1	0.2	0.3	0.3	0.3	0.2
Ile	3.1	4.1	4.0	3.7	3.5	4.3	3.9
Leu	7.3	7.3	8.1	9.7	8.0	9.3	8.4
Tyr	1.7	0.7	2.7	3.2	3.1	3.2	3.2
Phe	3.4	3.1	3.0	3.3	3.1	3.2	3.2
"X"[a]	1.0	0.5	0.8	0.9	0.9	0.7	0.8
Lys	7.0	5.9	6.3	7.5	9.6	5.1	6.0
His	3.4	2.0	1.5	3.0	2.6	1.2	1.4
Arg	4.0	3.3	2.4	3.5	4.0	2.3	2.7
"Other"	<0.3	<0.3	0.6	0.6	0.3	0.7	0.5

[a] "X," ninhydrin-positive material eluting just before lysine. Its elution time coincides with that of glucosamine, but not with that of galactosamine, mannosamine, or hydroxylysine. "Other," composite of three minor unidentified peaks present in all samples. Tryptophan and amide content were not determined.

The isoelectric focusing patterns of the proteins of the seven T-mycoplasmas seen after staining with Coomassie Blue were reproducible and contained 7–10 protein bands, most of which were in range pH 4 to 7. The difference between the strains were mainly quantitative. They enabled the T-mycoplasmas to be differentiated. The protein bands of *T-960* (Fig. 1a) and *T-210* were similar being in the range pH 4 to pH 5.5 while those of *T-27* and *T-58* were found in two pH zones namely pH 4.5 to 6 and pH 6.5 to 7.5. Therefore, representative scans of *T-960* (Fig. 1a) and *T-27* (Fig. 2a) only are given. The protein bands *T-220* (Fig. 1b), *T-213* (Fig. 1c), and *T-McA* (Fig. 3) were concentrated mainly between pH 5 and 7. However, *T-213* (Fig. 1c) could be distinguished by a prominent basic band at pH 9 and *T-McA* (Fig. 3) by a distinctive acidic band at pH 3. The proteins of all seven strains were denatured during storage at −25°. The acidic proteins were affected most. The effect of storage for 7 days at −25° on the protein pattern of *T-27* is given in Fig. 2. Gels of *T-960, T-McA* and *T-220* stained with Periodic Acid–Schiff (PAS) had 5–7

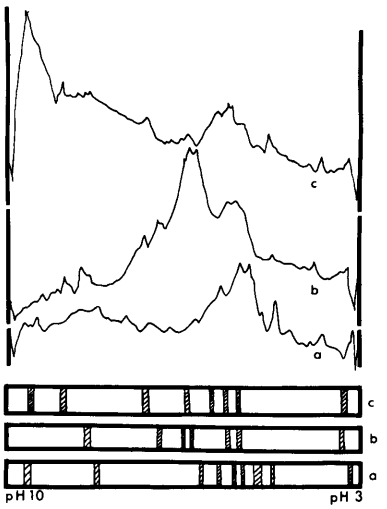

FIG. 1. Isoelectric focusing-densitometer tracings and schematic representations of Coomassie Blue stained patterns of Tergitol extracts of T-mycoplasma membranes of strains: (a) *T-960*, (b) *T-220*, (c) *T-213*.

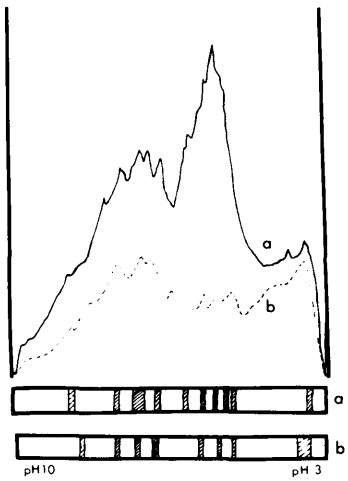

FIG. 2. Isoelectric focusing-densitometer tracings and schematic representations of Coomassie Blue stained patterns of Tergitol extracts of the membrane of *T-mycoplasma T-27* (a) before and (b) after storage at −25° for 7 days.

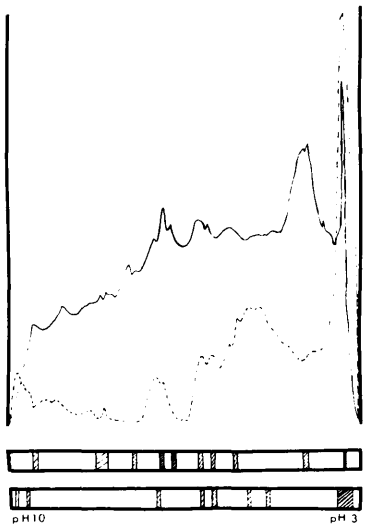

FIG. 3. Isoelectric focusing-densitometer tracings and schematic representatins of Coomassie Blue and PAS stained patterns of Tergitol extracts of the membrane of *T-mycoplasma T-McA*. The solid line shows the pattern obtained with Coomassie blue and the broken line that with PAS.

bands, of which at least two did not correspond to Coomassie Blue bands. *T-220* had several PAS-positive bands below pH 5, *T-960* had one at pH 6, and *T-McA* (Fig. 3) had a distinctive band at pH 3 corresponding to the Coomassie Blue band (Fig. 3).

Discussion. The amino acid content of the T-mycoplasma membranes were similar to those of other biological membranes (7). However, *T-213* contained a greater quantity of basic amino acids which correlated with the larger amount of basic protein detected by isoelectric focusing.

Isoelectric focusing of the T-mycoplasma membranes indicated that most proteins fell in the slightly acid pH range. The number of bands within a given pH range was basically similar and only *T-McA* and *T-213* could be distinguished, respectively, by a distinctive acid band and a strong basic band. It was not determined whether the PAS positive bands were glycolipids or glycoproteins. The membranes of the classical mycoplasmas contain both glycoprotein (8) and glycolipid (9). Glucose, mannose, and galactose are present in these strains (1), as were glucosamine and asparagine which can form a linkage between proteins and sugars (10). Their lipid content has not been determined. Therefore, the glycoproteins and glycolipids of the T-mycoplasma membranes require investigation. Thereafter, it may be possible to explain the resistance of the T-mycoplasmas (1) and the susceptibility of the classical mycoplasmas to lysis by osmotic shock (11).

Summary. Purified membranes were prepared from seven human T-mycoplasmas. Their amino acid composition was determined and was similar to that of other biological membranes. Their proteins were examined by isoelectric focusing and 7 to 10 protein bands were detected mostly in the pH 4 to 7 range of the gel. *T-mycoplasma T-McA* also had one distinctive band at pH 3 while *T-mycoplasma T-213* had a prominent basic band at pH 9. The proteins were denatured by storage at −25°. Five to seven Periodic Schiff-positive bands also were observed but were not identified.

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