Amino Acid Composition of Extracellular Protein from Six Mycobacteria.* (23703)

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The amino acid composition of defatted mycobacterial cells has been reported in an earlier communication from this laboratory (1). In continuing these investigations we have now determined the amino acid composition of the extracellular protein prepared by a standardized procedure from 6 strains of mycobacteria.

Methods. *Mycobacterium tuberculosis* var. *hominis* 599, *M. tuberculosis* var. *avium*, *M. tuberculosis* var. *bovis*, *Mycobacterium phlei* (336 289B), *Mycobacterium ranae*, and *Mycobacterium smegmatis*, the same strains as those employed previously(1), were maintained throughout this study on the egg medium of Steenken and Smith(2). Cultures for protein production were grown on a modification of Long's medium(3) prepared as follows. DL-Malic acid 100 g, 10% ammonium hydroxide 400 ml, potassium dihydrogen phosphate 30 g, sodium chloride 20 g, magnesium sulfate 10 g, and ferric ammonium citrate 0.4 g, were dissolved in 9 l of distilled water. The solution was filtered, and the filtrate adjusted to pH 7.2 with 3.N hydrochloric acid, was sterilized in 1 l Roux bottles, 180 ml per bottle, by autoclaving for 20 minutes at 121°. Prior to inoculation each bottle was supplemented with 20 ml of sterile 50% glucose solution. Inocula were prepared by seeding one or two such bottles with growth from the stock cultures. The remaining bottles, in each case, were inoculated from the fresh pellicles of the inoculum cultures. *M. smegmatis* was grown in a 15 l batch, *M. tuberculosis* var. *avium* in a 25 l batch and the other organism in 10 l batches. The inoculated media were incubated at 37° for the following lengths of time: *M. ranae* 14 days, *M. phlei* 38-43 days, *M. tuberculosis* var. *hominis* 30 days, *M. tuberculosis* var. *bovis* 115 days, *M. tuberculosis* var. *avium* 40 days (10 liters) and 142 days (15 liters), and *M. smegmatis* 31-43 days. The cultures were clarified by Seitz filtration, and the filtrate concentrated 5 to 10 fold by evaporation under reduced pressure, were acidified by adding 50% trichloroacetic acid to a final concentration of 5%. After holding the latter solutions at least 24 hours at 4° the precipitated proteins were removed by centrifugation and redissolved in distilled water (a few drops of 3.N sodium hydroxide were added to effect complete solution). The protein solutions were dialyzed against distilled water, treated with an equal volume of saturated ammonium sulfate solution, and allowed to stand 12 to 24 hours at 4°. The precipitate was collected by centrifugation and dissolved in distilled water. The procedure of precipitation in 50% ammonium sulfate solution was repeated, and the final precipitates, dissolved in distilled water, were dialyzed against distilled water for 4 days at 4°. The protein solutions were filtered and lyophilized. This procedure yielded the following amounts of lyophilized extracellular mycobacterial protein: *M. ranae* 685 mg, *M. tuberculosis* var. *hominis* 1.50 g, *M. tuberculosis* var. *bovis* 650 mg, *M. smegmatis* 700 mg, *M. phlei* 2.10 g, and *M. tuberculosis* var. *avium* 175 mg. The preparations were analyzed for moisture by drying them to constant weight in a vacuum oven at 56°, for ash by igniting them to constant weight in a closed crucible with a Fisher burner,† for nitrogen by the semi-micro Kjeldahl procedure, and for amino acids by microbiological assay methods. The samples for amino acid analyses were hydrolyzed by refluxing them

* Paper 121. The material presented here has been taken in part from a thesis by Sarah L. Lovett, submitted in partial fulfillment of the requirements for degree Master of Science, Jan., 1956. This work was supported by grants from Amer. Trudeau Soc., the Los Angeles County Tuberculosis and Health Assn. and the University of California. The authors are indebted to Evelyn Brown, Audree Fowler and Ben Ginsberg for technical assistance.

† Moisture and ash analyses were carried out by Heather King.

TABLE I. Percentages of Moisture, Ash, Total Nitrogen* and Amino Acid Nitrogen† in Mycobacterial Proteins.

Constituent	In extracellular protein (%)									In dried, defatted cells (%)
	M. phlei	*M. ranae*	*M. smegmatis*	*M. tuberculosis* var. *avium*	*M. tuberculosis* var. *bovis*	‡	*M. tuberculosis* var. *hominis*	§	‖	¶
Moisture	6.7	5.7	6.4	3.7	5.6		6.0			4.9 (1.9– 7.0)
Ash	2.2	12.9	5.2	17.3	1.0		1.6			3.9 (1.9– 7.8)
Nitrogen	15.3	14.7	14.3	13.0	12.6		15.3			10.2 (7.0–12.0)
Glutamic acid	13.3	12.5	12.5	10.6	13.5	*8.0*	12.1	*8.7*		6.2 (5.7– 9.6)
Alanine	10.3	8.1	11.8	8.9	10.0	*13.2*	10.1	*9.2*		9.6 (8.5–11.5)
Aspartic acid	9.9	8.3	10.7	9.0	9.3	*7.9*	10.9	*8.0*	*8.1 (6.7–13.1)*	4.4 (4.1– 4.8)
Leucine	10.1	7.8	8.6	8.9	9.4	*6.3*	9.0	*6.3*		5.8 (5.3– 6.3)
Valine	8.1	6.6	8.2	6.5	8.4	*2.7*	7.9	*5.4*		4.9 (4.4– 5.3)
Arginine	7.5	6.0	5.9	5.7	7.3	*15.9*	6.4	*15.6*	*4.8 (3.1– 5.8)*	15.4 (12.8–17.2)
Isoleucine	5.3	3.9	4.7	4.5	5.1	*4.6*	4.8	*4.3*		2.9 (2.7– 3.0)
Lysine	5.0	3.6	5.2	4.6	4.6	*6.0*	4.5	*4.1*	*4.1 (3.0–10.4)*	4.5 (3.5– 5.5)
Phenylalanine	4.6	3.8	4.4	3.8	3.7	*2.1*	4.7	*2.2*	*1.9 (.8– 3.8)*	1.6 (1.5– 1.7)
Serine	3.0	3.6	4.1	3.7	4.1	*4.3*	5.0	*3.1*	*3.8 (3.4– 5.7)*	2.8 (2.4– 3.1)
Tyrosine	4.2	3.4	4.0	4.2	3.1	*1.8*	3.5	*1.6*		1.1 (1.0– 1.2)
Histidine	2.5	1.7	2.0	2.2	2.0	*3.5*	2.2	*4.5*	*2.9 (2.0– 4.3)*	3.2 (2.8– 3.5)
Methionine	2.0	1.7	1.6	1.3	1.6	*1.2*	1.8	*1.3*		.9 (.8– 1.0)
Amino acid total	85.8	70.9	83.6	73.2	82.3	*78.5*	83.7	*74.3*		64.0 (59.6–65.9)

* Calculated for moisture and ash-free material.

† Calculated as % of total nitrogen. Literature data from other than the authors' laboratory are given in italics.

‡ Data of Jones *et al.*(7) for bovine tuberculin prepared by precipitation of culture filtrate at pH 4.95.

§ Data of Jones *et al.*(7) for human tuberculin purified by a series of precipitations at pH 3.8 to 5.0 (material precipitating at pH 5.0 was excluded and final product was precipitated at pH 4.82).

‖ Median and range of values of Seibert(8) for 5 tuberculin fractions obtained by alcohol precipitation, ammonium sulfate precipitation and urea extraction. Similar values reported by Nishihara(9) for a single human tuberculin preparation are omitted because they could not be presented on a comparable basis.

¶ Median and range of values of Ginsberg *et al.*(1) for dried defatted cells of 11 strains of mycobacteria (4 species), including 6 strains employed in the present study.

in 3*N* hydrochloric acid for 24 hours. Alanine was determined with *Leuconostoc citrovorum*(4), serine with *Lactobacillus casei*,‡ methionine with *Leuconostoc mesenteroides* P-60(5), and all other amino acids with the organisms and methods employed previously (6).

Results. Moisture, ash, total nitrogen, and amino acid nitrogen content of the extracellular mycobacterial proteins are given in Table I. Analogous results from other laboratories (7,8) and the earlier data from this laboratory on dried, defatted mycobacterial cells(1) are included in the table for convenience of comparing them with the present values. It may be seen (Table I) that the authors' 6 extracellular protein preparations obtained by a uniform procedure from different mycobacteria are quite similar in amino acid composition to each other, albeit, markedly different in this respect from the preparations of Jones *et al.*(7) obtained from closely related strains by a somewhat different procedure. The principal differences between the authors' preparations and those of Jones *et al.* are that the latter contained a much higher percentage of arginine nitrogen and a much lower percentage of glutamic acid nitrogen than the former. Smaller, but still significant differences in content of aspartic acid, leucine, valine, phenylalanine, tyrosine and histidine are also apparent. It appears, therefore, that different protein fractions derived from a single culture are generally less similar in composition than uniform fractions from different species, and this conclusion is borne out by the data of Seibert(8) (Table I) for 5 tuberculin fractions, all from *M. tuberculosis* var. *hominis*.

‡ Unpublished method of Camien and Dunn.

It may be seen that the spread of amino acid values for these five homologous tuberculins is in every case considerably greater than the corresponding spread of values for the authors' uniform preparations from six different strains, representing four mycobacterial species.

The extracellular proteins prepared by the authors' procedure are apparently quite different in amino acid composition from the dried, defatted cells of the strains from which they were derived. The arginine content, in particular, is much higher in the cells than in the extracellular material. Histidine, likewise, is appreciably higher in the cells. Glutamic acid, aspartic acid, leucine, valine, isoleucine, phenylalanine, tyrosine and methionine, on the other hand, are of significantly greater abundance in the extracellular proteins than in the corresponding preparations of cellular material.

Summary. Extracellular protein has been obtained from 6 strains of mycobacteria (4 species) by a uniform procedure. The amino acid composition of the protein preparations is presented.

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Effect of Modification of Insulin on Its Degradation and Biological Activity.* (23704)

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Studies on the degradation of insulin labeled with I^{131} have shown that it is rapidly attacked by an enzyme or enzymes present in slices and homogenates of liver, kidney, pancreas, spleen, adipose tissue, and muscle(1-3). A large number of inorganic and organic compounds have been shown to inhibit insulin-I^{131} degradation in extracts of these tissues (4-7). The unusually high rate of insulin-I^{131} degradation observed after its injection into intact animals(8) poses many interesting questions for speculation, such as: Is the degradation linked in any way to the biological activity of insulin or are the two processes, degradation and biological action, independent of one another? In an attempt to gain information on this aspect of the problem, we have carried out a series of experiments in which the insulin-I^{131} molecule was modified by reagents which react with one or more groups of the insulin molecule. The modified insulins were injected into eviscerated-nephrectomized rabbits and their rate of degradation was measured. The biological activity of the injected materials was determined simultaneously.

Procedure. General treatment of animals and determination of presence of biological activity in modified insulins. Adult female rabbits were eviscerated-nephrectomized and maintained as previously described(9). The plasma sugar was kept at a normal concen-

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