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Elution sequence and characterisation of nucleotides from *Mycobacterium smegmatis*

Using anion-exchange chromatography with a formic acid–ammonium formate eluent developed by INGLE[1], ethanol–acid extracts of *Mycobacterium smegmatis*[2,3] were resolved, and the nucleotides in eluate fraction identified by spectrophotometry[4,5], paper chromatography[6,7], and hydrolysis to the base by perchloric acid[8], followed by paper chromatography[7]. These characterisations[4,5], together with further reference to the literature for UV absorption data[9–11], confirm the tentative elution sequence obtained previously for the common 5′-ribonucleotides to be: CMP, NAD, AMP, GMP, NADP, UMP, ADP, FAD, GDP, ATP.

However, guanosine and cytosine were found to be not well resolved, and UTP was not separated from GTP. Furthermore some nucleotides resolved by this technique were unidentified[1]. These materials were characterised by chromatography, spectrophotometry and hydrolysis (Table I).

TABLE I

SPECTROPHOTOMETRIC CHARACTERISATION OF UNKNOWN NUCLEOTIDES IN ETHANOL–ACID EXTRACTS OF *Mycobacterium smegmatis*

The extracts were fractionated at 15° on Dowex columns eluted by formic acid–ammonium formate[1].

	Absorbance ratios[a]			*pH*	*Purine or pyrimidine by hydrolysis*[b]
	250/260	*280/260*	*290/260*		
Peak fractions eluted between AMP and GMP					
1	0.914	0.40	0.24	2	—
	0.85	0.46	0.28	12	
2	0.785	0.272	0.011	2	—
3	0.92	0.48	0.30	2	—
	1.02	0.59	0.43	12	
4	1.01	0.665	0.45	2	Guanine
Peak fractions eluted between GMP and NADP					
5	0.93	0.54	0.34	2	—
	1.01	0.52	0.36	12	
6	1.68	0.99	0.441	2	—
	1.87	0.99	0.68	12	
7	1.05	0.605	0.37	2	Guanine
	1.08	0.607	0.43	12	
Peak fractions eluted between FAD and GDP					
8	0.77	0.29	0.051	1.5	Adenine
9	0.64	0.41	0.035	1.5	Uracil
10	0.67	0.41	0.05	2	Uracil

[a] Fractions from both sides of a peak were spectrophotometrically characterised as a test for homogeneity of the peak. Absorbances were measured against an appropriate eluate blank. In some cases formic acid and ammonium formate were removed from the nucleotides by sublimation *in vacuo* (2 mm Hg, 36 h, 40°) and the resultant crystals dissolved in phosphate buffers or aqueous sodium hydroxide.

[b] Separate samples of eluate fractions, evaporated under vacuum, from both sides of a peak were hydrolysed by perchloric acid[8] and the resultant base identified by paper chromatography[7].

The nucleotides of eluate peak fractions (1–7, Table I) represented less than 1 % (as measured by absorbancy at 260 mμ, pH 2) of the total nucleotides extracted. Adenine and uracil nucleotides of peaks 8–10 (Table I) constituted about 5 % of the total pool.

Ethanol–acid extracts of *Mycobacterium smegmatis* (500 mg dry weight) were evaporated under vacuum to dryness, and the resultant crystals hydrolysed with perchloric acid (0.5 ml) to give a solution of purines and pyrimidines. Using the paper chromatographic technique of BENDICH[7], adenine and guanine were estimated in these hydrolysates. These values were compared with totals obtained by summing the adenine and guanine contents of the eluate fractions.

Four-day-old *Mycobacterium smegmatis* was found to contain 11.25 ± 0.75 μmoles adenine/g dry weight of organism extracted, by paper chromatography; and 10.45 ± 0.15 μmoles adenine/g dry weight of organism extracted, by column chromatography. Corresponding results for guanine were 1.1 ± 0.1 and 1.15 ± 0.35 μmoles/g dry weight extracted, by paper chromatography and column chromatography respectively.

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Chromatography of *im*-benzyl-L-histidine

The use of derivatives of *im*-benzyl-L-histidine[1] in the chemical synthesis of peptides containing histidine is well known[2]. As *im*-benzyl-L-histidine is strongly bound to the resins normally used in amino acid analyzers, the determination of this amino acid derivative in hydrolysates of synthetic peptides can pose problems. The use of a