Adenosine pentaphosphate from commercial ATP

MARRIAN[1] has recently reported that commercial ATP from ox muscle contains up to one-third of the total adenine as adenosine tetraphosphate. Similar findings have been made in this laboratory with respect to ATP presumably obtained from yeast. In addition, evidence has been found for the presence of a compound in this material which corresponds in composition to adenosine pentaphosphate. In the preparation on which most of the experiments were carried out, obtained from Schwarz Laboratories, Inc., the tetraphosphate accounted for some 8% of the total adenine, and the pentaphosphate for less than 1%.

The method used for the separations was based on the ion exchange chromatography of COHN AND CARTER[2]. In one experiment, 2.75 g of "chromatographically pure" sodium ATP was dissolved in water, a small amount of dilute ammonia solution added, and the nucleotide materials adsorbed on a column of Dowex-1 anion exchange resin 17 cm high by 1.1 cm in diameter. AMP was eluted by 0.002 *N* HCl, ADP by 0.01 *N* HCl, and ATP by 0.01 *N* HCl plus 0.04 *M* NaCl. The progress of the elution was followed by measurements of the optical density of the eluate at 260 mμ in a Beckman Model DU spectrophotometer. A total of 533 mg of adenine as ATP was eluted with 4500 ml of this eluant. When the optical density of the eluate decreased below 0.05, the eluting solution was changed to 0.01 *N* HCl plus 0.2 *M* NaCl. Two distinct peaks of optical density were obtained, the first at 400 to 500 ml with optical density 12.7, and the second at 1200 to 1300 ml with optical density 0.705. The adenine equivalent in the first peak was 41.3 mg, and in the second, 4.6 mg. The middle portion of eluate containing each peak was neutralized with powdered barium hydroxide, and one volume of ethanol added. The solutions were let stand overnight in the refrigerator, and the precipitates collected by centrifugation. These were washed with ethanol, dissolved in 0.1 *N* HCl, and freed of barium by treatment with Dowex-50 cation exchange resin in the sodium form. Analyses were carried out on the resulting solutions. Determinations of adenine equivalent were made by ultraviolet spectroscopy, of pentose by the method of MEJBAUM[3], using AMP as a standard, to avoid the correction factor which LEUTHARDT AND EXER[4] found necessary on AMP with a ribose standard, and of total P and acid-labile P by the method of FISKE AND SUBBAROW[5]. Acid-labile P was determined by making the solution 1 *N* in sulfuric acid and heating for 15 minutes in a bath of boiling water. Some barium phosphate was formed by hydrolysis of the polyphosphates in the course of the elution. The analytical data are given in Table I, corrected for the amount of orthophosphate found.

TABLE I

ANALYSES ON NEW COMPOUNDS OBTAINED FROM COMMERCIAL ATP PREPARATIONS, AS MOLES PER MOLE ADENINE

	Adenine	*Pentose*	*Labile phosphate*	*Total phosphate*
Material in 1st elution peak	1.00	0.98	2.92	4.03
Material in 2nd elution peak	1.00	0.96	3.86	4.94

The absorption spectra were determined at pH 2, 7, and 12, and found to be indistinguishable from that of ATP.

Evidence for the presence of nucleoside polyphosphates with more than two acid-labile phosphate groups per mole of adenine equivalent in trichloroacetic extracts of liver has been published elsewhere[6]. These experiments were carried out on rabbit liver, in which a minimum of 60 g of tissue was available. The total amount of nucleotide material with a ratio of acid-labile to acid-stable P higher than 2 amounted to less than 1% of the total adenine equivalent of the extracts. It is therefore not surprising that MARRIAN[1] failed to find evidence for the adenosine tetraphosphate in experiments on rat viscera.

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