A NEW ADENINE NUCLEOTIDE

by

D. H. MARRIAN

Department of Radiotherapeutics, The University, Cambridge (England)

During the course of work in progress in these laboratories on the metabolism of adenine by the rat[1,2,3], it became necessary to develop a separation of adenine nucleotides by modifying the procedures described by COHN AND CARTER[4]. This latter method, excellent as a fast separation of impurities known to be present in commercial adenosine-5'-triphosphate (ATP), was, of course, not designed to separate unexpected contaminants. By using the methods outlined, a hitherto unknown nucleotide has been consistently obtained.

METHODS AND RESULTS

Ion exchange separations

150–200 mg of barium ATP (Boot's Pure Drug Co.) was almost completely dissolved in 0.1 *N* HCl, brought to pH about 4, and loaded on a column of Dowex 2 anion exchange resin (8–10 cm × 1 sq. cm). After elution of adenosine monophosphate (AMP) by 0.002 *N* HCl, of adenosine diphosphate (ADP) by 0.01 *N* HCl, and of ATP by 0.01 *N* HCl/0.1 *M* NaCl, the new nucleotide was eluted as an apparently homogeneous band by 0.01 *N* HCl/0.2 *M* NaCl. Concentration to almost salt free solutions was achieved by addition of barium chloride solution to the neutralised eluate evaporated to about 1/10th bulk and precipitation with 1 volume of alcohol. The barium salt, contaminated with a little barium phosphate, was spun off, washed with 50% alcohol, suspended in a little water, cleared by addition of a trace of 0.01 *N* HCl, and the solution passed through a small column of Dowex 50 cation exchanger (Na form). The investigations listed in Table I were carried out on this solution.

Larger scale preparations were carried out using up to 2 g barium ATP on 14 cm × 10 sq.cm columns. Separations on these large columns were faster achieved using 0.01 *N* HCl/0.025 *M* NaCl to elute AMP and ADP, 0.01 *N* HCl/0.2 *M* NaCl to elute ATP, and finally 0.01 *N* HCl/0.3 *M* NaCl to remove the new compound. On working up as before, a precipitate formed immediately on adding barium chloride to the evaporated neutral solution. This precipitate was spun off, and 1 volume of alcohol added to the supernatant as usual to precipitate the barium salt of the new nucleotide. In 5 separations of 2 different batches of barium ATP, the new compound was present in amounts of about 1/3 of the ATP.

References p. 281.

Phosphorus analyses: by the method of ALLEN[5].

Easily hydrolysable phosphorus: defined as organic phosphorus liberated as inorganic phosphate by 1 *N* HCl at 100° for 10 minutes.

Pentose estimation: by the modified[6] method of MEJBAUM[7].

Phosphate liberated by washed myofibrils: method of PERRY[8].

Enzymic assay of energy rich phosphate bonds and of ATP: method of SLATER[9].

The presence of an intact α-glycol link was clearly shown when solutions of the nucleotide were dried off on filter paper and treated with a 1% solution of lead tetra-acetate[10].

Ultraviolet spectroscopy: by means of a Unicam SP 500 ultraviolet spectrophotometer. The molar extinction coefficient of adenosine phosphates at 260 mμ (pH 2) was taken as 14,200[4]. The ultraviolet spectrum of the new nucleotide was identical with that of adenylic acid at pH 2, 7, and 12, (maxima at 257–8, 259, and 259–260 mμ respectively) with the extinction at the higher pH values some 6% higher than in acid.

Hydrolysis products

Solutions of the new nucleotide were refluxed for 1 hour in 1 *N* HCl. Addition of aqueous picric acid gave a yellow solid which, when recrystallised from 25% acetic acid, was shown to be identical with an authentic specimen of adenine picrate by infrared spectroscopy. Portions of the hydrolysate, when submitted to paper chromatography in *n*-butanol/water/acetic acid (4/5/1 v/v/v), and in 72% *iso*propanol/ammonia, gave, after spraying with aniline phthalate[11], a spot corresponding to ribose-5-phosphate (prepared from ADP by the same procedures). More prolonged acid hydrolysis (0.1 *N* H_2SO_4 refluxed for 4 hours) gave spots indistinguishable from ribose and ribose-5-phosphate in the same solvents. Analytical results from one such preparation of the new nucleotide are compared with simultaneous analyses on the ATP from the same run in Table I.

TABLE I

ANALYSES (mole/mole OF ADENINE NUCLEOTIDE) OF THE NEW COMPOUND COMPARED WITH ATP

	Adenine nucleotide	*Pentose*	*Organic phosphorus*	*Easily hydrolysable phosphorus*	*Phosphate liberated by myofibril ATPase*
New nucleotide	1.00	1.15	4.0	2.95	0.4
ATP	1.00	1.05	2.95	2.0	0.9

TABLE II

ENZYMIC ASSAY OF THE NEW NUCLEOTIDE

	μmole/ml
Adenosine phosphate (by spectrum)	2.1
ATP (after 5 minutes)	0.47
Energy rich phosphate	2.8 (a slow reaction unlike ADP or ATP)

References p. 281.

Four other isolations have shown identical analyses. The consistently high figures for pentose (1.15–1.2 molar proportions) are in agreement with the observations of LEUTHARDT AND EXER[12] on AMP.

One batch of the new nucleotide was assayed for high energy phosphate bonds and for ATP content (hexokinase). The author is indebted to Dr. E. C. SLATER of the Molteno Institute, University of Cambridge, for these estimations which are shown in Table II.

DISCUSSION

The formulation of the new nucleotide as adenosine-5′-tetraphosphate is consistent with the above observations. A phosphate substituted amino group in the purine ring would not be expected to retain the adenine type of spectrum (compare 6-acetamino purine[13]), where the N-acetyl group, which reduces the possibilities of anionotropic shifts, causes the main peak to shift to 283–4 mμ and another peak to appear at about 210 mμ.

The presence of this tetraphosphate with its high proportion of acid labile phosphate doubtless explains SLATER's observations[9], that the acid labile phosphate in commercial ATP was more than could be accounted for by the ADP and ATP present.

The results shown in Table II appear to show only one energy rich phosphate bond in the molecule, but it should be noted that this value is minimal as the reaction was very much slower than was the case with ADP or ATP. Again, the slow apparent liberation of ATP by the nucleotide is interesting in that a similar slow activity was shown in the ATPase assay shown in Table I.

Although the new nucleotide has been isolated five times from three different batches of ATP, it has not so far been detected in trichloracetic acid extracts of rat viscera. This, as well as the phosphorus content, differentiates it from any of those nucleotides briefly reported by GOLDWASSER[14,15] or by SACKS AND LUTWAK[16].

It is difficult to envisage this type of structure arising as an artefact during the isolation of ATP, and the possibility must be considered that it is a naturally occurring nucleotide of unknown significance.

ACKNOWLEDGEMENTS

The author expresses his thanks to Miss S. M. WERBA for technical assistance, to Dr. E. C. SLATER for the enzymic assays, and to Professor J. S. MITCHELL, F.R.S. for his interest.

SUMMARY

A new nucleotide has been shown to be present in commercial samples of adenosine-5′-triphosphate (ATP). The analytical data suggest the structure adenosine-5′-tetraphosphate.

RÉSUMÉ

Un nouveau nucléotide a été observé dans des échantillons d'adénosine-5′-triphosphate (ATP). Des dates analytiques sont presentées qui lui assignent la constitution d'un adénosine-5′-tetraphosphate.

References p. 281.

ZUSAMMENFASSUNG

Ein unbekanntes Nucleotid ist in käuflichen Proben von Adenosin-5′-triphospbat (ATP) beobachtet worden. Die mitgeteilten Analysen entsprechen einem Adenosin-5′-tetraphosphat.

REFERENCES

1 D. H. MARRIAN, *Biochem. J.*, 51 (1952) i.
2 D. H. MARRIAN, *Biochim. Biophys. Acta*, 9 (1952) 469.
3 D. H. MARRIAN, *Biochim. Biophys. Acta*, 13 (1954) 282.
4 W. E. COHN AND C. E. CARTER, *J. Am. Chem. Soc.*, 72 (1950) 4273.
5 R. J. L. ALLEN, *Biochem. J.*, 34 (1940) 858.
6 H. G. ALBAUM AND W. W. UMBREIT, *J. Biol. Chem.*, 167 (1947) 369.
7 W. MEJBAUM, *Z. Physiol. Chem.*, 258 (1939) 117.
8 S. V. PERRY, *Biochem. J.*, 48 (1951) 257.
9 E. C. SLATER, *Biochem. J.*, 53 (1953) 157.
10 J. G. BUCHANAN, C. A. DEKKER AND A. G. LONG, *J. Chem. Soc.*, (1950) 3162.
11 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
12 F. LEUTHARDT AND B. EXER, *Helv. Chim. Acta*, 36 (1953) 507.
13 J. DAVOLL AND B. A. LOWY, *J. Am. Chem. Soc.*, 73 (1951) 1650.
14 E. GOLDWASSER, *Fed. Proc.*, 12 (1953) 210.
15 E. GOLDWASSER, *Nature*, 171 (1953) 126.
16 J. SACKS AND L. LUTWAK, *Fed. Proc.*, 12 (1953) 468.

Received September 24th, 1953