A NEW ADENINE NUCLEOTIDE ISOLATED FROM PIG BLOOD

Takashi Hashimoto and Haruhisa Yoshikawa

Department of Physiological Chemistry and Nutrition,

Faculty of Medicine, University of Tokyo, Tokyo.

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In the course of work in this laboratory on the phosphorus metabolism of red blood cells, a hitherto unknown adenine nucleotide has been isolated from ATP fraction obtained from pig blood by Dowex 1-formate chromatography. The presence of a similar adenine nucleotide in human red blood cells has been noticed by previous workers (Bishop et al., 1959; Mills and Summers, 1959; Bartlett, 1959; Gabrio et al., 1959). Further studies revealed that this compound corresponds in composition to adenylyl diphosphoglyceric acid.

Oxalated pig blood was extracted with ice-cold perchloric acid, and acid-soluble phosphates were precipitated as barium salts. Nucleotides were separated from other phosphates after removal of barium with Amberlite IR 120-H^+ and by treating with charcoal, chromatographed on a column of Dowex 1-formate with gradient elution according to Hurlbert et al. (1954). The minor peak which was eluted just after ATP (X in Fig. 1) gave a color reaction similar to that described by Bartlett (1959) for glyceric acid when heated with chromotropic acid in sulfuric acid. This peak was separated from a peak containing non-nucleotide phosphorus (Y in Fig. 1) and 2,3-diphosphoglyceric acid which had not been completely removed from the nucleotide fraction with the charcoal treatment. The new nucleotide was adsorbed on charcoal, eluted with ammonia alcohol and chromatographed on a column of Dowex 1-chloride with 0.1 N HCl.

To remove the contaminating ATP, the nucleotide was rechromatographed on a column of Dowex 1-formate using gradient elution from water to 4 M formic acid + 0.4 M ammonium formate. The treatment with charcoal and Dowex 1-

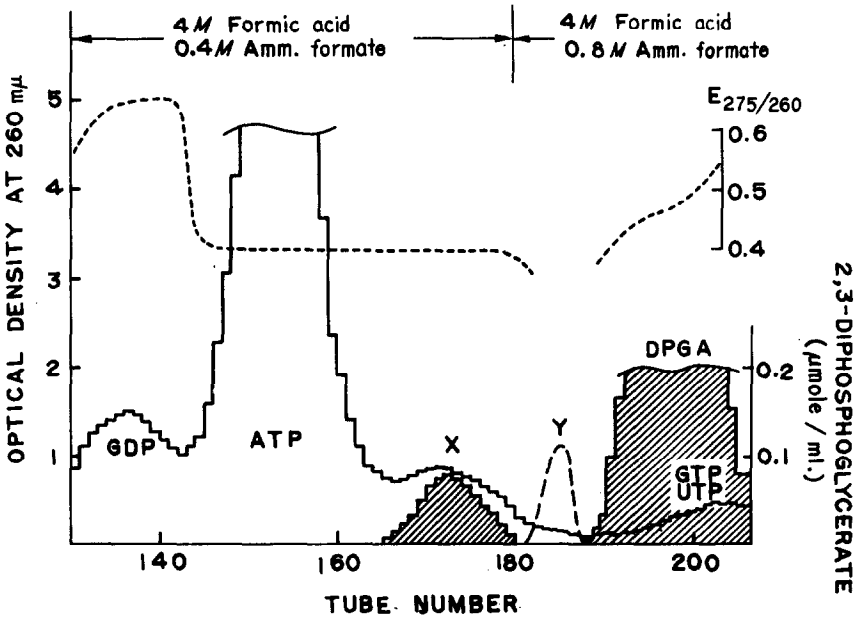

Fig. 1. Separation of nucleotides obtained from pig blood by Dowex 1-formate chromatography with gradient elution. The open column represents optical density at 260 mμ; the hatched column, glyceric acid in μmoles/ml. of eluant solution. The dotted line shows the position of unknown non-nucleotide phosphate.

formate was repeated and the purified compound was precipitated as the barium salt.

This material had an ultraviolet spectrum similar to that of adenosine phosphate but it behaved differently upon paper chromatography using the procedure for differentiating nucleotides described by Wade and Morgan (1955), and it gave the characteristic spectrum of 2,3-diphosphoglyceric acid in the chromotropic acid reaction. Analytical results from one such preparation of the new nucleotide are compared with simultaneous analyses on the ATP from the same run (Table I).

A portion of this material was hydrolysed in 1 N HCl at 100°C for 10 minutes and after removal of HCl in a desiccator under reduced pressure it was submitted to Dowex 1-chloride chromatography using stepwise elution. Approximately 1 equivalent each of adenine, ribose 5-phosphate and 2,3-

Table I

Analyses of the New Nucleotide Compared with ATP

	Adenine	Ribose	Labile P	Total P	Glyceric acid
ATP	1.00	1.01	1.88	2.94	-
New nucleotide	1.00	0.98	0.15	3.04	0.99

Analytical values are expressed as moles per mole of adenine. The molar extinction coefficient of adenine nucleotides at 260 mμ in acid was taken as 14,300. Ribose was determined by the orcinol reaction (Mejbaum, 1939) using AMP as a standard, phosphorus by the method of Berenblum and Chain (1938), and glyceric acid by the chromotropic acid reaction (Bartlett, 1959) using 2,3-diphosphoglyceric acid as a standard.

diphosphoglyceric acid were recovered in the expected position. Adenine was identified by the ultraviolet spectra at different pHs and paper chromatography. Ribose 5-phosphate was identified by its spectrum in the orcinol reaction and by the finding of a ratio of 1 ribose to 1 phosphorus. 2,3-Diphosphoglyceric acid was identified by paper chromatography, paper electrophoresis, its spectrum in the chromotropic acid reaction, and the yield of 1 glyceric acid to 2 total phosphorus after total hydrolysis. The liberated glyceric acid was determined by periodate oxidation.

By the action of nucleotide pyrophophatase obtained from snake venom of Agkistrodon Blomhoffii (Suzuki and Iwanaga, 1958), this nucleotide was split into 2,3-diphosphoglyceric acid and 5'-AMP. The latter was identified by paper electrophoresis, periodate oxidation and the reactivity to muscle 5'-adenylate deaminase.

The formulation of the new nucleotide as adenylyl diphosphoglyceric acid with a pyrophosphate bond is consistent with the above observations.

It is of interest that the new nucleotide possesses 2,3-diphosphoglyceric acid as a component which is the most abundant organic phosphate

in mammalian red blood cells. Further studies to clarify the molecular configuration of this compound are now in progress.

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