A 5'-AMINO ANALOG OF ADENOSINE DIPHOSPHATE

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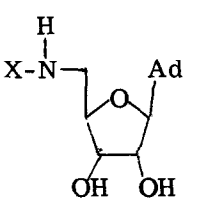
Summary. The synthesis and properties of the 5'-amino analog of adenosine diphosphate are described. The 5'-amino-5'-deoxyadenosine 5'-N-diphosphate ($_N$ADP) is prepared from the previously described aminonucleoside triphosphate by the hexokinase catalyzed transfer of the terminal phosphoryl to glucose. The $_N$ADP is stable in neutral or basic media, and is similar to natural ADP in chromatographic, electrophoretic, and spectrographic properties. Snake venom phosphodiesterase degrades $_N$ADP to the monophosphate $_N$AMP, and acid degrades both $_N$ADP and $_N$AMP to 5'-amino-5'-deoxyadenosine.

The synthesis of a phosphoramidate analog of dTTP and the utilization of this substance in the enzymatic synthesis of phosphoramidate analogs of polynucleotides was described in a previous communication (1). In extending these studies we wished to investigate the possibility of obtaining phosphoramidate analogs of polynucleotides by the action of polynucleotide phosphorylase on aminonucleoside diphosphates. The present communication reports a two step procedure for preparation of an appropriate diphosphate, 5'-amino-5'-deoxyadenosine 5'-N-diphosphate ($_N$ADP), from an aminodeoxynucleoside (equation 1)



$_N$A; X = H

$_N$AMP; X = $(HO)_2\overset{O}{\overset{\|}{P}}-$

$_N$ADP; X = $(HO)_2\overset{O}{\overset{\|}{P}}-O-\overset{O}{\overset{\|}{P}}(OH)-$

$_N$ATP; X = $(HO)_2\overset{O}{\overset{\|}{P}}-O-\underset{OH}{\overset{O}{\overset{\|}{P}}}-O-\underset{OH}{\overset{O}{\overset{\|}{P}}}-$

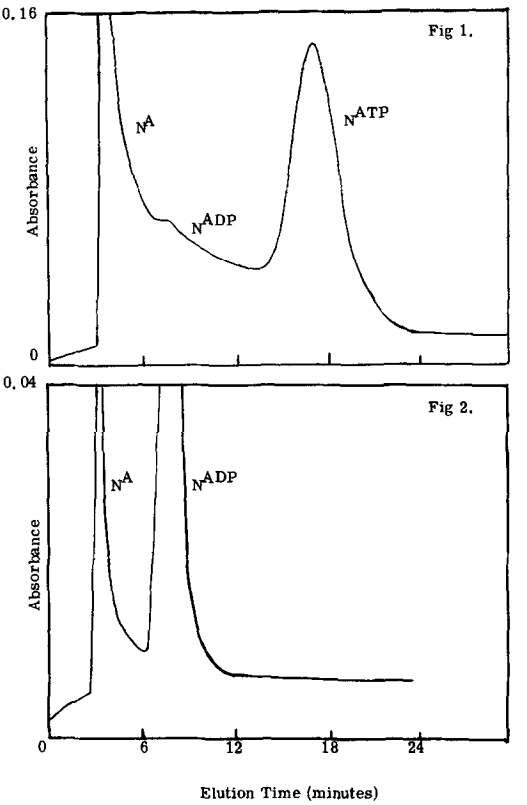
EXPERIMENTAL SECTION

5'-Amino-5'-deoxyadenosine ($_N$A)(58 mg) prepared from 5'-tosyladenosine and ammonia (2) and purified by elution from Dowex 50W-X8 (H^+ form) with

2M NH_4OH, was allowed to stand in 1 ml. of water with an equimolar amount of sodium trimetaphosphate hexahydrate for 48 hours. Paper chromatography revealed two spots, corresponding to the nucleoside ($_N$A) and the triphosphate ($_N$ATP). This result is analogous to that for the reaction of d_NT (1). Chromatography on a Varian LCS-1000 high pressure chromatograph equipped with an anion exchange column showed, however, that the mixture contained small amounts of substances corresponding to $_N$AMP and $_N$ADP as well as $_N$ATP and $_N$A. When the mixture was allowed to stand for a longer time (72 hours), greater amounts of $_N$AMP and $_N$ADP were observed, suggesting that these products arise by hydrolysis of $_N$ATP. Subsequent to this work, Trowbridge et al reported the synthesis of $_N$ATP from $_N$A by the same reaction(3).

For the enzymatic reaction the $_N$ATP was partially purified by precipitation with methanol (which removes much of the $_N$A) or by chromatography on DEAE Sephadex (3). It was then treated with glucose and hexokinase under conditions used for the reaction of ATP (4). The $_N$ATP was rapidly converted to $_N$ADP (Fig 1, 2). Analyses as a function of time showed that the rate of transfer of phosphoryl to glucose from $_N$ATP (0.57 μmole/min) is comparable to that for transfer from ATP (0.66 μmole/min) under the same conditions. Formation of a sugar phosphate was verified by tlc on DEAE-cellulose with aqueous 0.2M LiCl solution, which showed a spot at R_f 0.64, corresponding to a standard sample of glucose-6-phosphate (the spot was visualized by spraying with Hanes-Isherwood reagent (5)).

$_N$ADP was purified by elution from DEAE-Sephadex with a linear gradient of triethylammonium bicarbonate at pH 8.7 (as described for $_N$ATP, 3), which separates $_N$ADP from $_N$A and $_N$AMP, followed by chromatography on Whatman 3MM paper with solvent F (1-propanol/ammonium hydroxide/water 55/10/35), which separates $_N$ADP from inorganic phosphates. Two purifications by paper



Elution pattern for nucleotides on LCS-1000 chromatograph before (Fig 1) and after (Fig 2) treatment with hexokinase and glucose; elution rate, 27.23 ml/hr.; buffer, 0.3 M potassium phosphate pH 8.0; temperature, 65°. The concentration of nucleotidic material for Fig 2 is 1/4th that for Fig 1 because of dilution for the enzymatic reaction.

chromatography were necessary to yield material of sufficient purity to give a satisfactory adenine:phosphorus ratio.

The uv spectrum of $_N$ADP (λ max 259 nm at pH 7) is indistinguishable from that of ADP. The phosphate/adenine ratio in $_N$ADP was determined to be 2.08/1.00 from the absorbance of a solution at 259 nm and analysis for phosphate by the method of Fiske and Subbarow (6). As shown in Table I, $_N$ADP behaves similarly to ADP on chromatography and electrophoresis. Presence of an acid sensitive phosphoramidate bond was demonstrated by treating $_N$ADP with 50% aqueous acetic acid at 90° for 20 minutes, followed by analysis by paper chromatography (Solvent

Table I. Chromatographic Properties

Compound	R_f - Paper Solvent A	Chromatogram Solvent F	R_f Cellulose TLC	Rm (electrophoresis)
ADP	.05	0.36	0.30	+ 1.0
$_N$ADP	.01	0.33	0.31	+ 1.1
AMP	.05	0.4	--	+ 0.82
ATP	.03	0.35	--	+ 0.74
$_N$ATP	0	0.27	0.17	+ 0.70
$_N$A	0.33	0.56	0.63	- 0.42

Paper chromatography was carried out on Whatman 3MM paper; Solvent A (i-PrOH/NH_4OH/H_2O, 7:1:2), solvent F (n-PrOH/NH_4OH/H_2O, 55:10:35) were used as eluents. TLC was carried out as described by Randerath and Randerath (7). Electrophoresis was carried on Whatman 3MM paper at 2000 volts for 1 hr with a Savant flat-bed electrophoretor. The buffer was 0.05 M sodium phosphate pH 7.2. R_m values are relative mobility with respect to ADP.

F) and electrophoresis. In both tests, the spot corresponding to $_N$ADP was found to be replaced by a spot corresponding to the aminonucleoside (see Table 1 for values).

Like ADP, $_N$ADP is hydrolyzed in an aqueous solution containing snake venom phosphodiesterase to a nucleoside monophosphate [conditions: 0.1 ml solution containing 3 µmole of magnesium chloride, 4 µmole sodium tetraborate (pH 9.0), 0.25 mg enzyme (Worthington Biochem.) and 80-90 nmoles nucleoside diphosphate; temp. 37°]. Analysis of aliquots on the LCS-1000 chromatograph as a function of time showed that the reaction of $_N$ADP proceeded somewhat faster than that for ADP. A small amount of $_N$A was also found as a by-product. It probably arises by hydrolysis of $_N$AMP. Treatment of $_N$AMP with 50% acetic acid at room temperature for 10 minutes results in complete hydrolysis of $_N$AMP to $_N$A.

DISCUSSION

$_N$ADP can be prepared conveniently from $_N$A by the reactions:

$$_N\text{A} \xrightarrow{P_3O_9^{-3}} {}_N\text{ATP} \xrightarrow[\text{hexokinase}]{\text{glucose}} {}_N\text{ADP} \qquad \text{(eq. 1)}$$

$_N$ATP is relatively unstable; it is converted to $_N$A very rapidly in acid solution and it hydrolyses slowly to give $_N$ADP and $_N$AMP as well as $_N$A in alkaline solution (pH 10). In the presence of hexokinase and glucose, $_N$ATP is converted cleanly to $_N$ADP. A susbtance that appears to be $_N$ADP has also been reported as a product of $_N$ATP subjected to the rabbit muscle creatine kinase reaction (3).

The phosphoramidate link in $_N$ADP is much more stable hydrolytically than that in either $_N$AMP or $_N$ATP; however, $_N$ADP can be hydrolyzed to $_N$A in acid solution or to $_N$AMP by snake venom phosphodiesterase. Preliminary results

$$_N\text{A} \xleftarrow[H_2O]{H^+} {}_N\text{ADP} \xrightarrow[H_2O]{\text{S.V.P.}} {}_N\text{AMP}$$

of experiments with polynucleotide phosphorylase indicate that $_N$ADP is not a suitable substrate for polynucleotide phosphorylase in the absence of ADP. In the presence of ADP some incorporation of the amino analog does occur, albeit at a somewhat slower rate. Futhermore, the polynucleotides appear to be shorter than those formed with ADP alone. We are currently investigating this reaction further.

ACKNOWLEDGEMENT

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