RIBONUCLEOSIDE 5′-AMINOMETHANEPHOSPHONATES: SYNTHESIS AND AFFINITY TOWARDS SOME PHOSPHOMONOESTERASES

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1. Introduction

In our previous papers [1–4] we have described the behaviour of snake venom 5′-nucleotidase towards some analogues of 5′-nucleotides modified at the phosphoric acid residues. The comparison of enzyme activity on ribonucleoside 5′-methanephosphonates and 5′-hydroxymethanephosphonates [3, 4] (the latter of which are substrates for the enzyme) suggests that a hydrophilic (or non-hydrophobic) character of the surroundings of the phosphorus atom to be bound is required for the substrate activity. In order to develop this line of investigation we attempted to synthesize and study the behaviour of nucleotide analogues bearing an amino group attached near to the phosphorus atom–the ribonucleoside 5′-aminomethanephosphonates.

2. Materials and methods

N-Benzyloxycarbonylaminomethanephosphonic acid (II) was prepared from aminomethanephosphonic acid [5] by the reaction with benzyl chloroformate in aqueous alkali under usual conditions [6]. 2′, 3′-*O*-Isopropylidene derivatives of uridine and N^6-dimethylaminomethyleneadenosine were prepared by the known procedures [7, 8].

Snake venom 5′-nucleotidase (*Crotalus adamanteus*) and bacterial alkaline phosphatase (*E. coli*) were purchased from Worthington Co. and were found to be free of any contaminating nucleolytic activities under the test conditions.

Paper chromatography was performed on Whatman No. 1 paper in systems: S1, 2-propanol–conc. aqueous ammonia–water (7:1:2); S2, 1-butanol–acetic acid–water (5:2:3); paper electrophoresis was performed on the same paper in S3, 0.1 M triethylammonium hydrogen carbonate pH 7.5 at 20 V/cm in an apparatus of Markham and Smith.

2.1. *Ribonucleoside 5′-aminomethanephosphonates–general procedure (scheme 1)*

The 2′, 3′-*O*-isopropylidene derivative (–uridine, –N^6–dimethylaminomethyleneadenosine) (1 mmole) and *N*-benzyloxycarbonylaminomethanephosphonic acid (1.4 mmole) were rendered anhydrous by repeated co-distillation with pyridine and treated with 2, 4, 6-triisopropylbenzenesulfonyl chloride (2.5 mmole) in pyridine (5 ml) for 16 hr at room temp. After treatment with water, the protected derivative III was separated by silica thin-layer chromatography (chloroform–methanol, 7:3), eluted with methanol and precipitated with ether. This material was quantitatively transformed to the 5′-aminomethanephosphonate IV on treatment with 36% hydrobromic acid in acetic acid (10 min at room temp) followed by precipitation with ether and paper chromatography in system S1 (6 sheets of Whatman No. 3 MM paper). Thus, the uridine derivative IVa was obtained in 17% yield; R_F 0.18 (S1), 0.15 (S2), E_{Up} 0.49 (S3). Adenosine derivative IVb was isolated in 22% yield; R_F 0.23 (S1), 0.16 (S2), E_{Up} 0.40 (S3).

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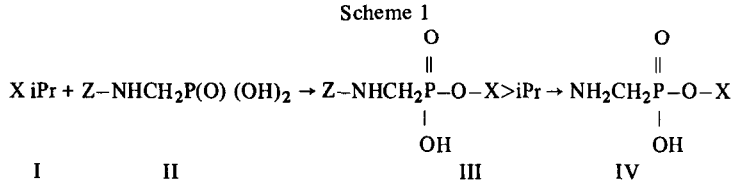
Scheme 1



In formulae I–IV, a X = uridine;
b X = adenosine; Z = benzyloxycarbonyl–; iPr = 2′, 3′-O-isopropylidene group.

2.2. *Enzymatic assays*

1 mg of compound IV in 50 μl 0.05 M Tris-HCl buffer pH 9.0 containing 0.005 M magnesium sulfate was treated with a) snake venom 5′-nucleotidase (100 γ protein) in 50 μl of the same buffer; b) bacterial alkaline phosphatase (10 γ protein in 10 μl ammonium sulfate suspension); incubation time, 4 hr at 37°. The mixtures were applied to Whatman No. 1 paper and analysed by chromatography in system S1. The spots were detected by ultraviolet absorption and ninhydrin reaction.

Kinetic measurements were made in 0.1 ml mixtures containing the substrate (2×10^{-2} M) and varied enzyme concentration (7 γ/ml–70 γ/ml) in 0.05 M Tris-HCl buffer pH 9.0 (0.005 M magnesium sulfate), aliquots were taken at time intervals, analysed as above and the percentage of splitting estimated by spectrophotometry (pH 2) of eluates.

3. Results and discussion

Both uridine and adenosine 5′-aminomethanephosphonates (IV) are quite resistant towards the action of bacterial alkaline phosphatase. This result is consistent with previous experiments showing that the enzyme in question requires a presence of two electronegative charges at the phosphorus acid moiety. This condition is not fulfilled with the present analogues.

On the other hand, the treatment of compounds IV with snake venom 5′-nucleotidase results in a substantial cleavage (36% with uridine derivative, 45% with adenosine derivative under assay conditions) of phosphonic acid ester linkage affording the ribonucleoside and aminomethanephosphonic acid.

The structural requirements of snake venom 5′-nucleotidase include the following main principles: a) the substrate molecule is bound to the enzyme at 3 different regions including the nucleoside moiety; one of them is responsible for the interaction with the heterocyclic base [9, 10]; b) the substrate molecule bears a phosphorus acid ester at the 5′-position and a free hydroxylic group at 3′-position; the latter has to be in the *ribo* configuration [11]; c) the phosphorus acid residues bear at least one electronegative charge: thus, nucleoside 5′-phosphites are good substrates for the enzyme [2]. As shown by comparison of the other electronegatively charged species which are not substrates for the enzyme (5′-nucleotide esters, nucleoside 5′-methanephosphonates) with 5′-hydroxymethanephosphonates [3, 4] which are split under the same conditions, the lack of activity in the former cases is evidently due to the hydrophobic character of the substituent at the phosphorus atom.

The present results with 5′-aminomethanephosphonates IV support the theory of the necessary hydrophilic character of the phosphorus atom surroundings mentioned above. However, the relative rate constants† of the hydrolytic reaction (table 1) show a lower affinity of amino derivatives IV when compared with that of nucleoside 5′-hydroxymethane phosphonates.

Table 1
Relative rate constants of snake venom 5′-nucleotidase degradation (referred to uridine 5′-phosphatase, see Materials and methods).

Compound	K_{rel}
Uridine 5′-phosphate	1.00
Uridine 5′-hydroxymethanephosphonate	0.08
Uridine 5′-aminomethanephosphonate	0.02
Adenosine 5′-phosphate	1.50 (1.00*)
Adenosine 5′-hydroxymethanephosphonate	0.105 (0.07)
Adenosine 5′-aminomethanephosphonate	0.03 (0.02)

* Values in parentheses refer to adenosine 5′-phosphate.

† The Michaelis parameters could not be obtained, due to the complexity of metal ions requiring enzyme kinetics.

The requirement for the presence of 2 different hydrophilic regions in the molecule (at 3′-position and at the phosphorus atom) orientated around the moiety to be cleaved is rather unique in the series of nucleolytic enzymes and suggests a typical character of the active site in snake venom 5′-nucleotidase.

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