Communications to the Editor

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Interactions between Synthetic Nucleotide Analogs and Snake Venom 5′-Nucleotidase

Among several enzymes contained in the snake venom, phosphodiesterase, 5′-nucleotidase, and phosphomonoesterase are known as the phosphatase related to nucleotides. Although detailed investigations on phosphodiesterase were reported by Suzuki,[1] Laskowski,[2] and Khorana,[3] little is known as yet on the action of 5′-nucleotidase. As to the systematic study of the substrate specificity of this enzyme a report of Heppel and Hilmoe[4] appeared in the literature. Cleavage of the phosphate group in other 5′-nucleotides, such as 8-azaguanosine 5′-phosphate,[5] 8-azaxanthosine 5′-phosphate,[5] 5-fluorouridine 5′-phosphate,[6] and 6-azathymidine 5′-phosphate,[7] was reported for elucidation of their structure. In addition, pseudo-uridine 5′-phosphate, which was isolated from soluble RNA*1 was also hydrolyzed by this enzyme.[8] Cohen[9] reported the hydrolytic activity of snake venom on the enzymatically synthesized spongo-uridine and spongo-cytidine 5′-phosphate.

A number of 5′-nucleotides were chemically synthesized in this laboratory and the action of whole venom of *Trimeresurus flavoviridis* HALLOWELL (Japanese Habu) on these nucleotide was examined, especially with 5′-nucleotidase.

In the present work following analogs were tested as the substrate : Pyrimidine analogs : 5-Hydroxyuridine 5′-phosphate (5-hydroxy-UMP)[10] (I), 5-bromouridine 5′-phosphate (5-bromo-UMP)[10] (II), 5-dimethylaminouridine 5′-phosphate (5-dimethylamino-UMP)[11] (III), 1-(β-D-ribofuranosyl)-2-oxo-2,3-dihydropyrimidine 5′-phosphate (4-deoxy-UMP)[12] (IV), 3-methyluridine 5′-phosphate (3-methyl-UMP)[11] (V), 1-β-D-ribofuranosyl-4-dimethylamino-2-oxo-2,3-dihydropyrimidine 5′-phosphate (4-dimethyl-CMP)[13] (VI).

Purine analogs : 6-Methylamino-9-β-D-ribofuranosylpurine 5′-phosphate (6-methyl-AMP)[14] (VII), 6-dimethylamino-9-(β-D-ribofuranosyl) 5′-phosphate (6-dimethyl-AMP)[14] (VIII), 1-β-D-ribofuranosylbenzimidazole 5′-phosphate (benzimid.-MP)[15] (IX), 1-β-D-ribofuranosyl-4-nitro-benzimidazole 5′-phosphate (nitrobenzimid.-MP)[15] (X).

Analogs changed in sugar moiety : 9-(2′-Hydroxyethyl)-6-aminopurine 2′-phosphate[16] (AR_1) (XII), 9-(3′-hydroxypropyl)-6-aminopurine 3′-phosphate[16] (AR_2) (XIII), 9-(4′-hydroxybutyl)-6-aminopurine 4′-phosphate[16] (AR_3) (XIV), 1-β-D-arabofuranosyluracil 5′-phosphate[17] (spongo-UMP) (XV).

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*1 Following abbreviations are used : RNA, ribonucleic acid; AMP, adenosine 5′-monophosphate; UMP, uridine 5′-monophosphate; GMP, guanosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; TMP, thymidine 5′-monophosphate.

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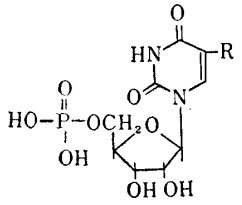
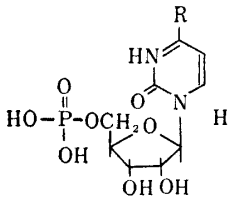
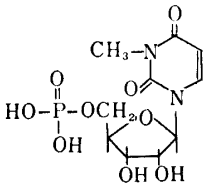


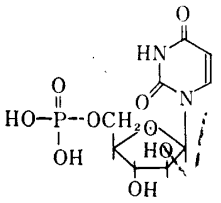
(I) R=OH
(II) R=Br
(III) R=N<CH3 CH3



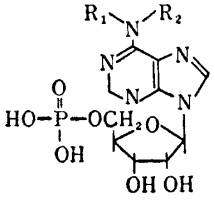
(IV) R=H
(VI) R=N<CH3 CH3



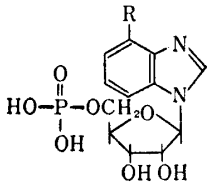
(V)



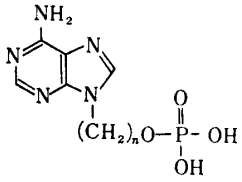
(XV)



(VII) R_1=H R_2=CH_3
(VIII) R_1, R_2=CH_3



(IX) R=H
(X) R=NO_2



(XII) n=2
(XIII) n=3
(XIV) n=4

Venom Solution——Lyophilized venom was dissolved in deionized H_2O, insoluble material removed by centrifugation, and the protein content was estimated spectrophotometrically by ultraviolet absorption at 280 mμ. A mixture of 0.5 cc. of enzyme solution,*2 50 μmoles of tris-HCl buffer (pH 8.5), 60 μmoles of $MgCl_2$, and ca. 3 μmoles of the substrate was incubated (total volume, 2.2 cc.) at 37° for 15 min. The reaction was stopped by the addition of 0.8 cc. of 60% $HClO_4$ solution, except in the case of *p*-nitrophenyl phosphate. The liberated phosphate was determined by the Allen method.[18] All runs

Table I.

Compound	Salt	Concn. μmole/cc.	Relative rate of hydrolysis (%)
2′(3′)-AMP	Li	3.3	0
2′(3′)-GMP	Li	3.7	0
2′(3′)-CMP	Na	3.6	0
p-nitrophenylphosphate	Na	10.0	0
AMP	Na	3.2	100
GMP	Na	3.2	32.0
UMP	Na	2.9	38.9
CMP	Na	2.7	54.8
TMP	NH_4	3.1	41.2
5-Bromo-UMP	Ba	3.3	16.7
5-Hydroxy-UMP	Ba	2.7	70.9[a]
5-Dimethylamino-UMP	Ba	3.2	0
3-Methyl-UMP	Ba	3.1	38.4
4-Deoxy-UMP	Ba	3.0	0
4-Dimethyl-CMP	Ba	3.0	88.0
6-Methyl-AMP	Ba	3.8	16.5[a]
6-Dimethyl-AMP	Ba	1.2	24.1[a]
Benzimid.-MP	Ca	3.4	0
4-Nitrobenzimid.-MP	free	3.3	0
AR_1	Ba	3.1	0
AR_2	Ba	3.1	0
AR_3	Ba	3.1	0
Spongo-UMP	NH_4	2.1	7.0

[a] Data taken from pre-examination.

*2 E_{280}=0.486 and 0.506 were used.

18) R. J. L. Allen : Biochem. J., **34**, 858 (1940).

were made with authentic AMP or UMP, and percentage of hydrolysis was calculated from simultaneous experiment. When *p*-nitrophenyl phosphate was used as the substrate, the reaction was stopped by the addition of 0.25*N* NaOH solution (1 cc.), the reaction mixture was deproteonized by centrifugation, and estimated by the absorption at 400 mμ. In order to exclude additional phosphate liberation caused by non-specific phosphomonoesterase, the hydrolysis of *p*-nitrophenyl phosphate,[20] 2′(3′)-AMP,*3 2′(3′)-GMP,*3 and 2′(3′)-CMP*3 was tested.[19] None of these were hydrolyzed.

From these experimental results, 5′-nucleotidase in whole Habu venom seems to have a fairly strict requirement of substrate structure. Among the natural nucleotides, AMP was cleaved almost completely and CMP, fairly fast. UMP, GMP, and TMP have comparable activity. In synthetic pyrimidine 5′-phosphates, 5-substitution has some effect on the hydrolysis. Whereas 5-hydroxy- and 5-bromo-UMP were cleaved up to a certain extent, 5-dimethylamino-UMP was not hydrolyzed. Substitution of the 4-position in the pyrimidine ring also caused some effect; while 4-deoxy-UMP was not hydrolyzed, 4-dimethyl-CMP was cleaved 1.6 times faster than CMP. 3-Methyl-UMP also has activity comparable to natural UMP.

Among the synthetic purine analogs, benzimidazole derivatives (IX and X) and their analogs, which have no ribose moiety (XII to XIV), were not hydrolyzed at all. On the other hand, analogs (VII) and (VIII), which have purine and ribose moieties intact, were cleaved to a certain extent. As shown in the case of spongo-UMP (XV), together with Cohen's investigation,[9] arabinose configuration exerted an unfavorable effect on the enzyme action. It is also noted that pseudo-UMP was cleaved by this enzyme,[8] although it has a C-C linkage between the base and sugar moiety.

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Faculty of Pharmaceutical Sciences,
School of Medicine,
Hokkaido University,
Sapporo, Hokkaido.

Yoshihisa Mizuno (水野義久)
Morio Ikehara (池原森男)
Tohru Ueda (上田 亨)
Akihiko Nomura (野村哲士)
Eiko Ohtsuka (大塚栄子)
Fumiyoshi Ishikawa (石川文義)
Yoshio Kanai (金井良雄)

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*3 Obtained from RNA hydrolysate.

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Chemistry of Zygomycin-A, a New Antibiotic.*1 Isolation of Pseudoneamine, D-Ribose, and Two Diaminohexoses from Zygomycin-A Complex

In the previous reports,[1] it was shown that antibiotic No. 45449A, isolated from the culture filtrate of *Streptomyces pulveraceus*, closely resembled paromomycin[2] reported in the midst of the present work. Further studies revealed that antibiotic No. 45449A was different from paromomycin in the diaminohexose moiety and, therefore, the name zygomycin-A was assigned to the antibiotic.

In the present paper will be described the isolation of pseudoneamine,[3] D-ribose, and two diaminohexoses which were separated from the degradation products of zygomycin-A.

*1 Some part of this paper was presented at the 116th and 118th Meetings of the Japan Antibiotic Research Association, September 18, 1959, and January, 22, 1960.

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