SUT

South East Asian Technical University Consortium

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"Empowering Communities through Innovative Engineering, Science and Technology"

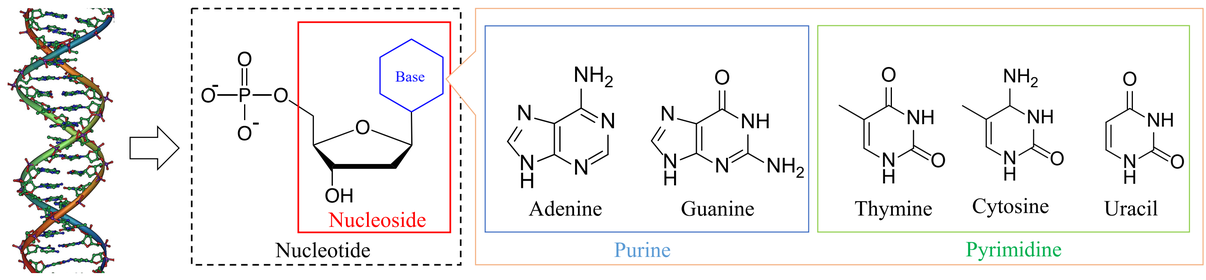
Effects of Functional Groups Recognition of Nucleoside substrate for Phosphorolysis with Nucleoside Metabolic Enzyme PyNP

Abstract

Unnatural nucleoside, which is chemically transformed nucleoside, is known to function as an antiviral agent and anticancer with the fact that it is incorporated into enzymes involved with nucleic acid biosynthesis and metabolism to show inhibitory activity. Our laboratory has revealed that it is possible to synthesize various unnatural nucleosides based on pyrimidine nucleoside phosphorylase (PyNP). PyNP could convert each of the pyrimidine nucleosides to 2-deoxy-D-ribose-1a-phosphate and free pyrimidine base in phosphate buffer, however, the detailed substrate specificity of ribosyl parts of this enzyme had been unknown. This study examined the substrate recognition ability of PyNP using modified nucleosides in which the hydroxyl group and hydrogen atom of the ribose moiety of the nucleoside were chemically converted to amino groups, thiol groups, and iodine. The study has revealed that compounds with 5'-hydroxyl groups of nucleosides transformed into hydrogen atoms show lower reaction rates than the hydrogen group. Compounds with 3'-hydroxyl groups transformed into hydrogen atoms exhibited further lower reaction rates than compounds without 5'-hydroxyl groups. This fact suggests that the substrate recognition based on 3'-hydroxyl groups may be involved with hydrogen bonding

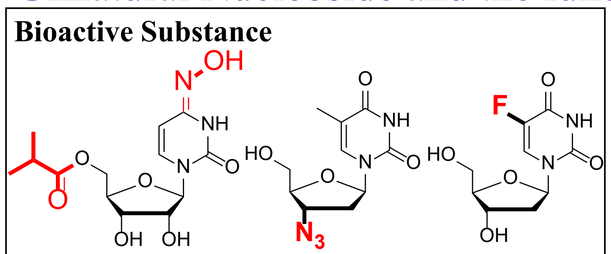
Introduction

Natural Nucleoside



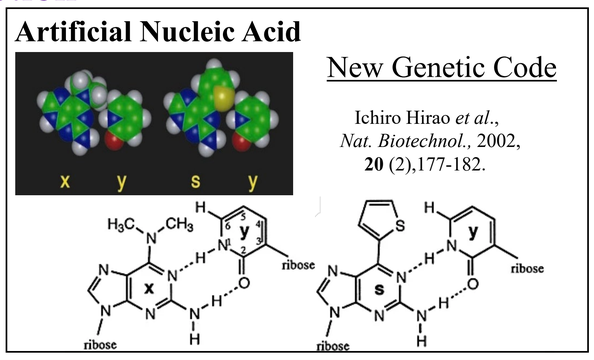
Unnatural Nucleoside and the function

Bioactive Substance



Jadd Shelton *et al*., *Chem. Rev.* , 2016, **116**, 14379-1455
Wendy P. Painter *et al*, *Antimicrob. Agents Chemother.*, 2021, **65** (5)

Artificial Nucleic Acid



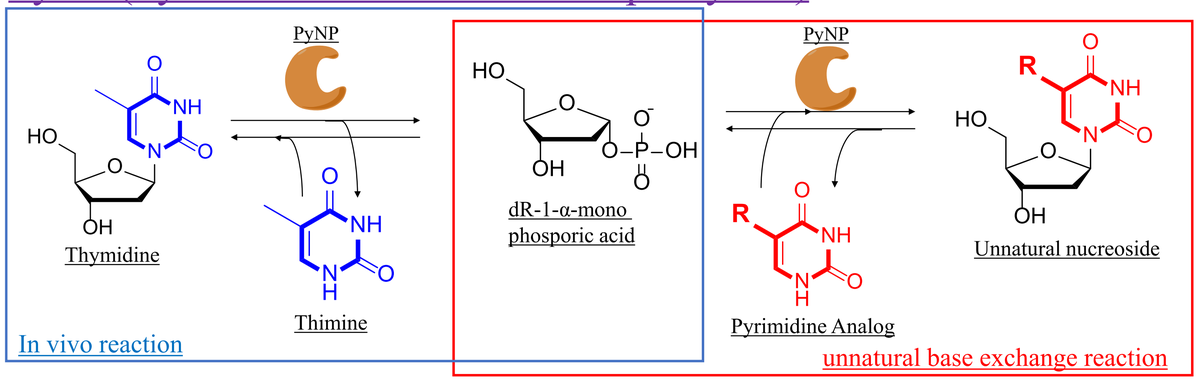
New Genetic Code

Ichiro Hirao *et al*., *Nat. Biotechnol.*, 2002, **20** (2),177-182.

In order to synthesize these unnatural nucleosides, there are problems such as a decrease in yield due to multi-step synthesis and an environmental impact due to a variety of organic sites.

Motivation

PyNP (Pyrimidine Nucleoside Phosphorylase)

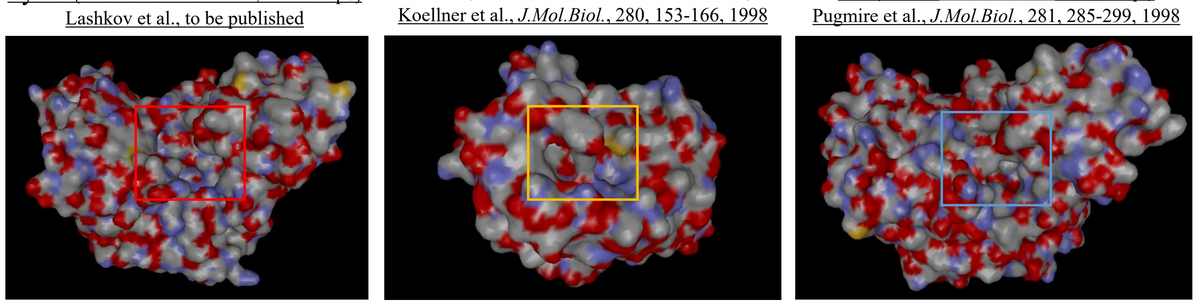


Research of ribose site recognition ability of PyNP

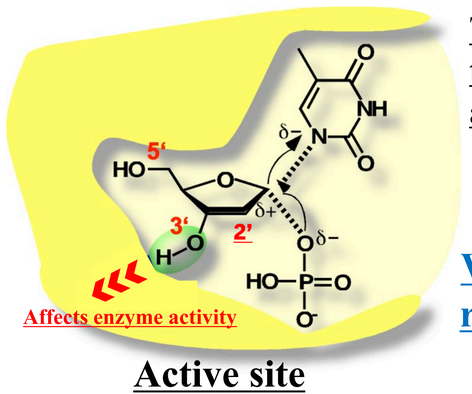
PyNP (*From Escherichia coli*, PDB : 5ep8)
Lashkov et al., to be published

PNP (*From Escherichia coli*, PDB : 1a69)
Koellner et al., *J.Mol.Biol.*, 280, 153-166, 1998

TP (*From Escherichia coli*, PDB : 2tpt)
Pugmire et al., *J.Mol.Biol.*, 281, 285-299, 1998



PyNP has a wider active site than other similar nucleic acid metabolic enzymes as TP and PNP.



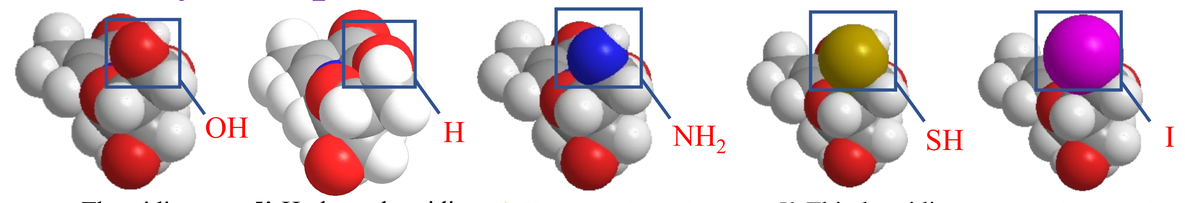
Active site

The hydroxyl groups at the 2′, 3′ and 5′ positions of thymidine are made into other functional groups and reacted with PyNP in a release reaction.

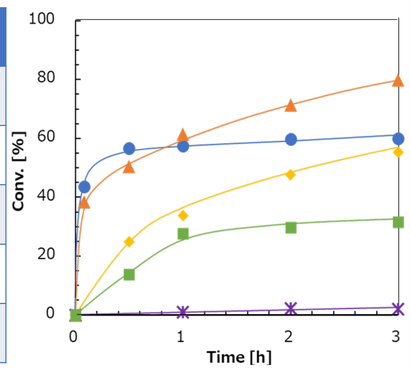
We might synthesize enzymes of a wide range of unnatural nucleosides, such as inhibitors and anticancer drugs.

Results and Discussion

Reactivity of 5' position modified ribose

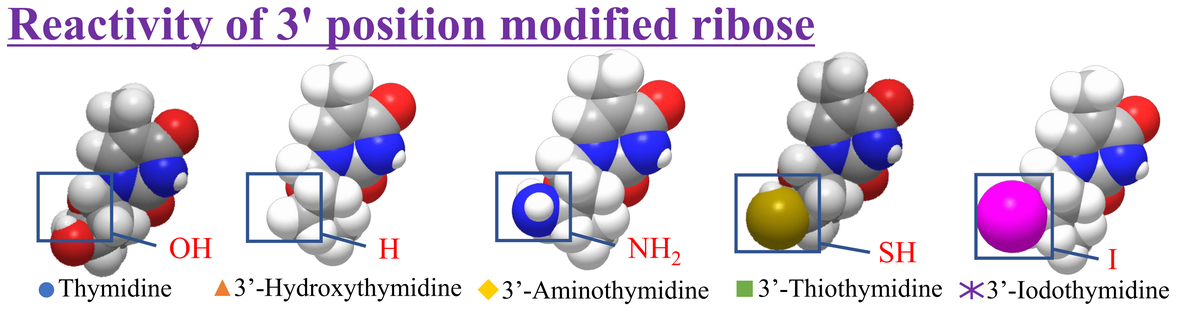


5'-modified thymidine	R^5	V_0[mM/h]	Max Conv.[%]
	-OH	53	99
	-H	46	99
	$-NH_2$	5.0	85
	-SH	2.8	33
	-I	0.1	16

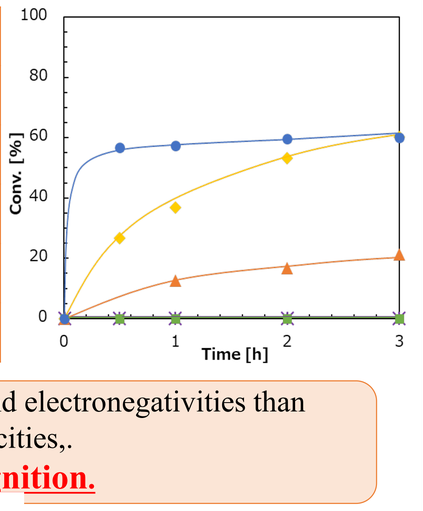


As the atomic radius of the modified functional group increased, the initial velocity decreased significantly, but the initial velocity was slightly higher for -OH than for -H.
→ **Hydrogen bonds are also thought to be slightly involved.**

Reactivity of 3' position modified ribose

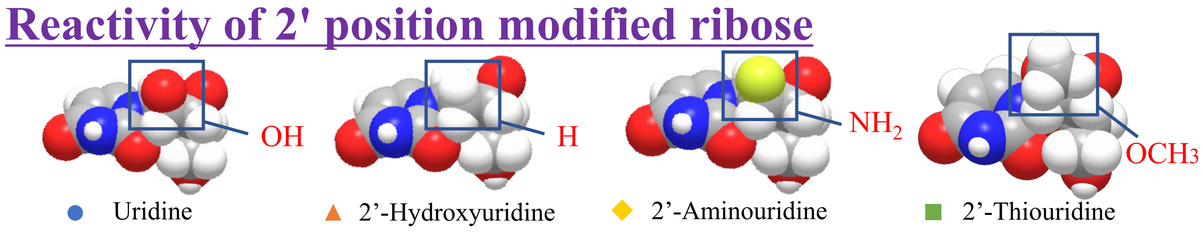


3'-modified thymidine	R^3	V_0[mM/h]	Max Conv.[%]
	-OH	53	99
	-H	1.3	79
	$-NH_2$	5.9	93
	-SH	0	0
	-I	0	0

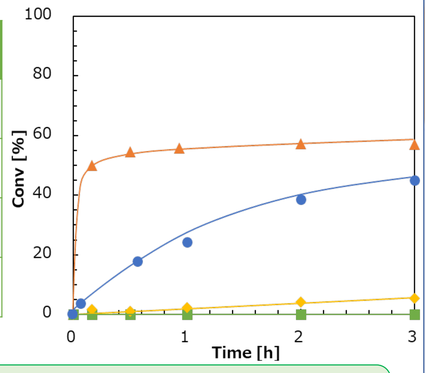


Hydroxy and amino groups, which have larger atomic radii and electronegativities than hydrogen atoms, have higher conversion rates and initial velocities,.
→ **Hydrogen bonding is involved in substrate recognition.**

Reactivity of 2' position modified ribose



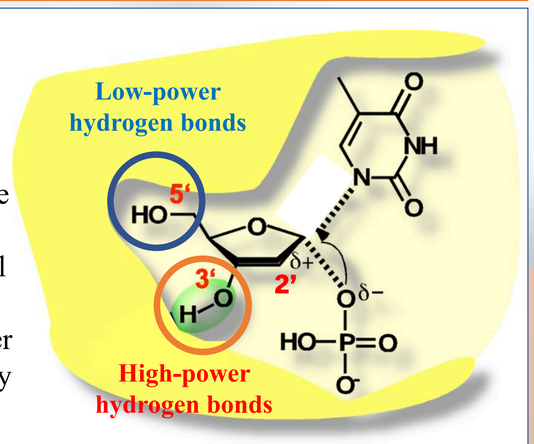
2'-modified thymidine	R^2	V_0[mM/h]	Max Conv.[%]
	-OH	3.2	99
	-H	30	99
	$-NH_2$	1.0	20
	$-OCH_3$	0	0



The initial velocity increases only when the sugar 2' position is modified with a hydrogen.
→ **Strictly identified by the size of the functional group**

Conclusions

At the 3' position, hydroxy and amino groups, which have larger atomic radii and electronegativity than hydrogen atoms, showed higher conversion rates and initial velocities, suggesting that hydrogen bonding is involved in substrate recognition. Furthermore, since the initial velocity increased only for the hydrogen atom at the 2' position, we thought that the size of the functional group was more strictly discriminated than at the 5'-position and the 3'-position. In the future, we will further clarify the recognition ability of 2′, 3′ and 5′ positions by enzymatic reaction of various modified nucleosides.



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