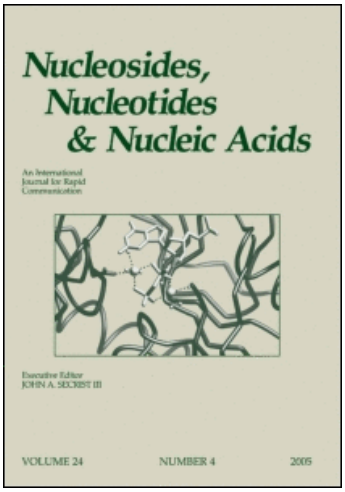

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Substrate Specificity of Thymidine Phosphorylase of *E. Coli*: Role of Hydroxyl Groups

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To cite this Article Panova, Natalya G. , Alexeev, Cyril S. , Polyakov, Konstantin M. , Gavryushov, Sergei A. , Kritzyn, Anatoliy M. and Mikhailov, Sergey N.(2008) 'Substrate Specificity of Thymidine Phosphorylase of *E. Coli*: Role of Hydroxyl Groups', Nucleosides, Nucleotides and Nucleic Acids, 27: 12, 1211 — 1214
To link to this Article: DOI: 10.1080/15257770802257895
URL: http://dx.doi.org/10.1080/15257770802257895

Nucleosides, Nucleotides and Nucleic Acids, 27:1211–1214, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802257895

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SUBSTRATE SPECIFICITY OF THYMIDINE PHOSPHORYLASE OF *E. COLI*: ROLE OF HYDROXYL GROUPS

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□ *Substrate specificity of* E. coli *thymidine phosphorylase to pyrimidine nucleoside modified at 5′-, 3′-, and 2′-positions of sugar moiety has been studied. Equilibrium (K_{eq}) and kinetics constants of phosphorolysis reaction of nucleosides were measured. The most important hydrogen bonds in enzyme-substrate complex have been determined.*

Keywords Thymidine phosphorlase; substrate specificity; thymidine derivatives

INTRODUCTION

Thymidine phosphorylase (TP) belongs to a class of nucleoside phosphorylases, which are involved in the processes of nucleoside methabolism in cell. It catalyzes the reversible reaction of phosphorolysis of 2′-deoxypyrimidines to heterocyclic base and 2′-deoxy-α-D-ribose-1-phosphate and normally the equilibrium shifts to nucleoside:[1,2] Thd + Pi ↔ Thy + dRib-P.

Thus TP participates in the salvage way of nucleoside synthesis and maintains the thymidine pool in cell. TPs were found in all live organisms and it appeared that their homology is nearly 40%.[1] The three-dimensional structures of TPs from different sources were determined and a key role for highly conserved amino acids and their hydrogen bonds with pyrimidine base was found.[3] On the bases of the structure of bacterial TP and molecular dynamic simulations it has been shown that substrate binding starts a

Received 2 November 2006; accepted 27 March 2008.
This manuscript was presented at the 2006 Round Table Conference, XVII International Roundtable, Bern, Switzerland, September 3–7, 2006.
Financial support of the Russian Foundation for Basic Research is acknowledged.
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domain movement, which leads to the "closed" conformation of the active site and initiates phosphorolysis.[4] The level of nucleoside phosphorylases is higher in numerous cancers than in normal cells.[5] Efficient inhibitors of these enzymes are promising as potential anticancer drugs, suitable not only for direct treatment of cancer, but also for prolonging the effect of other drugs.

RESULTS AND DISCUSSION

Nucleoside phosphorylases are widely used in industry for synthesis of drugs and commercially important nucleosides.[6,7] The aim of this work was to study substrate and inhibitory properties of thymidine derivatives in the reactions catalyzed by recombinant *E. coli* TP.[8] Substrate properties of a series of sugar modified nucleosides were investigated (Figure 1). 5′-Deoxythymidine (**2**) showed good substrate properties according to its kinetic constants, while replacement of the 5′-OH with bulky substituents (**3–6**) or an amino group (**7**), or the introduction of methyl (**8, 9**) or methylene (**10**) groups did not hinder binding but led to a significant reduction in the reaction rate (Table 1).

Modification of the 3′-OH dramatically influenced the binding of nucleosides to TP. Nucleosides **11–13** were not substrates but inhibited TP activity with a K_I close to the K_M of the natural substrate. On the bases of the "closed structure" of active site (model structure was obtained using molecular dynamic simulation)[9] we concluded that the 3′-hydroxyl group

TABLE 1 Kinetic constants[a] of modified nucleosides in phosphorolysis reaction catalyzed by TP

Nucleoside	$K_{eq}(1/K_{eq})$	$K_M(K_I) \cdot 10^{-6}$ M	k_{cat}, s^{-1}	k_{cat}/K_M
1	0.07(14.3)	300	198	0.66
2	0.05 (20)	400	260	0.64
3	0.07(14.3)	300	1.7	0.006
4		(400)		
5		(400)		
6		(>1000)		
7		400	0.58	0.0015
8	0.14 (7)	330	0.1	0.0003
9	0.10 (10)	350	0.45	0.0013
10	0.068(14.6)	300	0.6	0.002
11		(850)		
12		(600)		
13		(450)		
14	ND	350	0.4	0.0011

[a]The kinetic parameters for phosphorolysis of thymidine analogs in the presence of TP were determined spectrophotometrically.[8] The equilibrium constants were measure using HPLC at pH 6.5 and 37°C.[8]

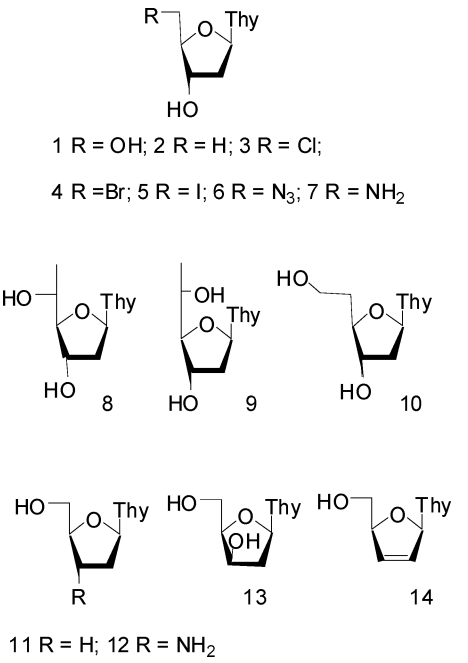

FIGURE 1 Structure of thymidine derivatives.

formed a hydrogen bond with Thr123 and due to this bond nucleoside was moved into a position suitable for nucleophilic attack by phosphate.

In conclusion, the absence of a 5′-hydroxyl group leads to no significant differences in binding constants and reaction rate, but introduction of bulky groups can hinder the domain movement because of their close proximity to the Gly88 residue, thereby lessening the reaction rate, while the 3′-hydroxyl group is essential for phosphorolysis reaction.

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