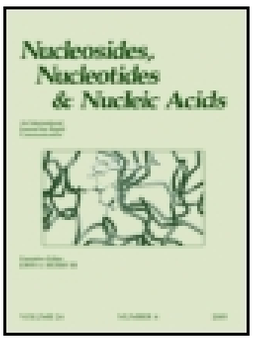

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Substrate Specificity of *E. Coli* Uridine Phosphorylase. Further Evidences of High-*syn* Conformation of the Substrate in Uridine Phosphorolysis

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ABSTRACT

Twenty five uridine analogues have been tested and compared with uridine with respect to their potency to bind to *E. coli* uridine phosphorylase. The kinetic constants of the phosphorolysis reaction of uridine derivatives modified at 2′-, 3′- and 5′-positions of the sugar moiety and 2-, 4-, 5- and 6-positions of the heterocyclic base were determined. The absence of the 2′- or 5′-hydroxyl group is not crucial for the successful binding and phosphorolysis. On the other hand, the absence of both the 2′- and 5′-hydroxyl groups leads to the loss of substrate binding to the enzyme. The same effect was observed when the 3′-hydroxyl group is absent, thus underlining the key role of this group. Our data shed some light on the mechanism of ribo- and 2′-deoxyribonucleoside discrimination by *E. coli* uridine phosphorylase and *E. coli* thymidine phosphorylase. A comparison of the kinetic results obtained in the present study with the available X-ray structures and analysis of hydrogen bonding in the enzyme-substrate complex demonstrates that uridine adopts an unusual high-*syn* conformation in the active site of uridine phosphorylase.

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KEYWORDS
Uridine phosphorylase; substrate specificity; modified uridines; conformation of uridine

Introduction

Nucleoside phosphorylases are involved in salvage pathways of nucleoside biosynthesis and catalyze the reversible phosphorolysis of nucleoside to a free heterocyclic base with the formation of α-D-ribose-1-phosphate (Sug-p), and the equilibrium of this reaction is shifted towards the nucleoside.[1, 2] Thus, uridine phosphorylase (UP) catalyzes the following reaction[2] depicted in Scheme 1. This class of enzymes includes thymidine phosphorylase (TP; EC 2.4.2.4), UP (EC 2.4.2.3), and purine nucleoside phosphorylase (PNP; EC 2.4.2.1). These enzymes were found in virtually all organisms and share similar primary structures. The most specific enzyme is TP, whose substrate is thymidine. UP catalyzes the phosphorolysis of both thymidine

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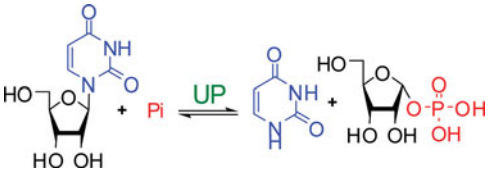


Scheme 1. Reversible uridine phosphorolysis catalyzed by UP.

and uridine, whereas cytidine is not a substrate for both enzymes. PNP may cleave the N-glycosidic bond in purine ribo(and 2′-deoxyribo)nucleosides.[1,2]

UP activity is usually elevated in various tumor tissues, including breast cancer, compared to matched normal tissues and this induction appears to contribute to the therapeutic efficacy of fluoropyrimidines in cancer patients.[3] Bacterial uridine phosphorylase functions as a dimer composed of identical subunits that have a molecular mass most commonly reported equal to 27.5 kDa.[1]

Nucleoside phosphorylases are used in industry for the synthesis of drugs (Cladribin, Fludarabine, and Nelarabin) and practically important nucleosides.[4–7] The ability of UP and PNP to cleave 2′-deoxynucleosides, β-D-arabinofuranosyl nucleosides and to catalyze their formation[6,8] is widely used. In these cases, enzymatic methods for creating the N-glycosidic bond can compete with the chemical synthesis. In general, this transglycosylation reaction is shown in Scheme 2.

The knowledge of the substrate specificity and equilibrium constants will help in predicting and optimizing the yield of the desired Nuc^2. For example, the use of a lower concentration of inorganic phosphate and an excess of $Base^2$ will shift the equilibrium towards the Nuc^2 formation. In these reactions one nucleoside phosphorylase may be used to catalyze the formation of pyrimidine or purine Nuc^2 starting from the appropriate Nuc^1 and pyrimidine or purine ($Base^2$). In the second variant both UP and PNP may be used to transform pyrimidine Nuc^1 to purine Nuc^2. This approach is very popular because the chemical transformation of the sugar moiety of pyrimidine nucleosides, for example, the synthesis of 1-β-D-arabinofuranosyluracil is, in general, more efficient and less costly compared to purine nucleosides. Moreover, the equilibrium of PNP phosphorolysis is shifted to the nucleoside to a much higher extent compared with UP and may be considered as a driving force for the total transglycosylation process. Some other approaches were employed to improve the yields of transglycosylation reactions. For example, α-D-arabinose-1-phosphate was successfully used for the preparation of β-D-arabinofuranosyl nucleosides.[8] 7-Methylguanosine derivatives as a source of the ribose moiety have important advantages because their phospholysis with PNP is shifted towards the formation of α-D-ribose-1-phosphate.[9–12]

$$Nuc^1 + Base^2 + p_i \leftrightarrow Sug\text{-}p + Base^1 + Base^2 \leftrightarrow Nuc^2 + Base^1 + p_i$$

Scheme 2. Nucleoside transglycosylation reaction (transfer of the sugar moiety from one heterocyclic base to another *via* α-D-sugar-1-phosphate, Sug-p).

Bacterial nucleoside phosphorylases are rather thermostable and may be utilized at elevated temperatures (50°–60°C), thus providing higher solubility of substrates and, consequently, their higher concentration in the reaction mixture. This is especially important for purine heterocyclic bases and nucleosides poorly soluble in aqueous buffers. The particular conditions of the transglycosylation reaction should be optimized to ensure the maximum yields.

The substrate specificity of these enzymes has not been fully investigated, and the role of the functional groups of nucleosides in the formation of the productive enzyme-substrate complex is unknown. This is due to the lack of convenient methods for the determination of kinetic parameters of the phosphorolysis reaction. Therefore, knowledge of the substrate specificity and the architecture of the active site of these enzymes is of considerable interest. The investigation of the substrate properties and determination of the kinetic parameters is an important step towards the creation of enzymes with improved characteristics. It should be noted that the kinetic parameters of this enzyme have been measured predominantly for natural ribo- and 2′-deoxynucleosides[13, 14] and studies of the properties of modified nucleosides were limited to the determination of their inhibitory properties.[15–17]

The aim of this work is to study the substrate and inhibitory properties of uridine derivatives in the reactions catalyzed by *E. coli* UP in order to shed some light on the conformation of the substrate in the productive complex with the enzyme.

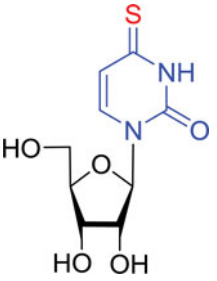
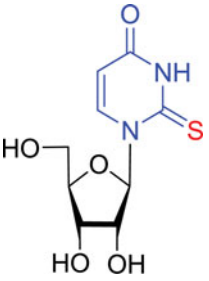
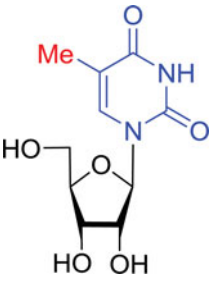
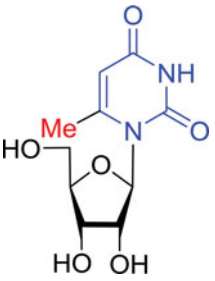
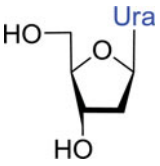
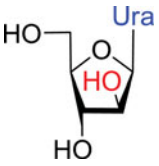
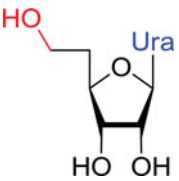
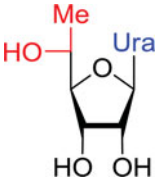
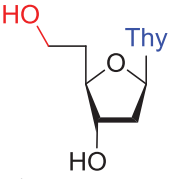
Results and discussion

We studied the *E. coli* UP-catalyzed phosphorolysis of uridine and its 25 derivatives modified in the heterocyclic base and the sugar moiety. The kinetic constants (K_M, K_I, k_{cat}) of the phosphorolysis reaction of uridines modified at 2-, 4-, 5- and 6-positions of the heterocyclic base and 2′-, 3′- and 5′-positions of the sugar moiety were determined. These data are summarized in Table 1.

6-Methyluridine is slowly converted into 6-methyluracil, and the substrate binding is significantly weaker compared to the 5-methyl derivative. These results are in agreement with those reported previously.[18] The replacement of oxygen with sulfur (the van der Waals radii are O, 1.40 Å; S, 1.85 Å[19]) in position four has no appreciable effect on the substrate affinity for the enzyme (Table 1). By contrast, 2-thiouridine is not a substrate for UP. The substrate properties of UP are summarized in Figure 1.

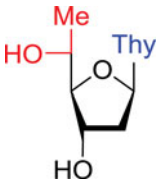
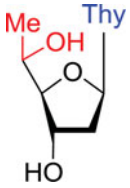
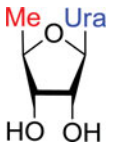
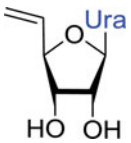
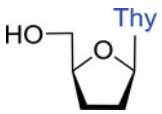
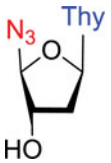
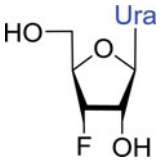
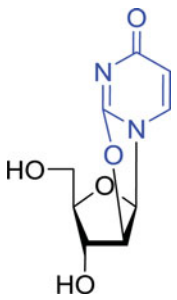
2′-Deoxyuridine and 5′-deoxyuridine show rather good substrate properties, as follows from their kinetic constants (Table 1). Therefore, we can assume that the absence of the 2′- or 5′-hydroxyl group is not crucial for successful binding and phosphorolysis. On the other hand, the absence of both the 2′- and 5′-hydroxyl groups results in the inability of the substrate to bind to the enzyme, as in the case of 5′-deoxythymidine, 5′-deoxy-5′-chlorothymidine and other 5′-deoxy derivatives. The lack of substrate properties of 3′-fluoro-3′-deoxyuridine and 3′-deoxythymidine clearly demonstrates the key role of the substrate 3′-hydroxyl group. Our data provide evidence that hydroxyl groups of the ribose moiety of

Table 1. Kinetic constants and equilibrium constants (K_{eq}) for uridine and its analogs in phosphorolysis reaction catalyzed by *E. coli* UP at pH 7.5 and 37°C.

Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M $sec^{-1}/\mu M$	Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M $sec^{-1}/\mu M$
Urd	80	98	1.22	Thd	270	5	0.02
 4-thiouridine	130	49	0.38	 2-thiouridine	*	**	—
 5-methyluridine	65	4.8	0.074	 6-methyluridine	1030	24	0.023
 2′-deoxyuridine	133	14	0.1	 1-β-D-arabino-furanosyluracil	500	2	0.004
 5′-homouridine	174	97	0.56	 5′-C-methyluridine (D-allo)	400	45	0.11
 5′-C-methyluridine (L-talo)	280	360	1.28	 5′-homothymidine	*	**	—

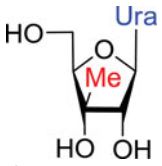
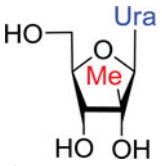
(*Continued on next page*)

Table 1. (Continued).

Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M $sec^{-1}/\mu M$	Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M $sec^{-1}/\mu M$
 5′-C-methylthymidine (D-ribo)	(280)	—	—	 5′-C-methylthymidine (L-lyxo)	500	1.5	0.003
 5′-deoxyuridine	180	11	0.1	 5′-deoxythymidine	*	**	—
 5′-deoxy-5′-methyleneuridine	500	3	0.006	 3′-deoxythymidine	—	—	—
 5′-deoxy-5′-chlorothymidine	*	**	—	 5′-deoxy-5′-azidothymidine	*	**	—
 5′-deoxy-5′-bromothymidine	*	**	—	 5′-deoxy-5′-iodothymidine	*	**	—
 3′-fluoro-3′-deoxyuridine	(770)	—	—	 2,2′-O-anhydrouridine	(10)	—	—

(*Continued on next page*)

Table 1. (Continued).

Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M sec^{-1} /μM	Substrate	K_M (K_I) μM	k_{cat} sec^{-1}	k_{cat}/K_M sec^{-1} /μM
 3′-C-methyluridine	*	**	—	 2′-C-methyluridine	*	**	—

[a]The accuracy of the estimation is better than 20% for K_m and k_{cat}.
*K_m >1000 μM; **the reaction rate was smaller than 0.1% of that for the natural substrate; For the preliminary account of this work see Ref [21].

the substrate are involved in hydrogen bonding, thus providing its binding to the enzyme to form the UP substrate complex.

Our data shed some light on the mechanism of discrimination between ribo- and 2′-deoxyribonucleosides by UP and TP. The most specific enzyme is TP, whose substrates are thymidine and 2′-deoxyuridine. UP catalyzes the phosphorolysis of both thymidine and uridine. Uridine and 5-methyluridine are not *E. coli* TP substrates, but they effectively inhibit the enzyme activity.[20] On the other hand, UP catalyzes the phosphorolysis of uridine 20 times more effectively than 5-methyluridine (*cf.* the k_{cat}/K_M values). This difference between 2′-deoxyuridine and thymidine is substantially smaller (5 times) (Table 1). Meanwhile, UP catalyzes the phosphorolysis of uridine 60 times more effectively compared to thymidine. The above pairwise comparison provides evidence that the presence of the 2′-hydroxyl group with the simultaneous absence of the bulky 5-methyl group is crucial for the formation of the productive UP substrate complex.

The efficiency of phosphorolysis of 1-β-D-arabinofuranosyluracil is 300 times lower than that of uridine but only 25 times lower as compared to 2′-deoxyuridine. Meanwhile, 2,2′-O-anhydrouridine, a nucleoside with a fixed orientation around the glycosidic bond, binds to *E. coli* UP more effectively than uridine. The same situation has been observed earlier for UPs of different origin.[15–17]

Let us consider the effect of substituents on the nucleoside conformation. It is generally believed that nucleosides are rather flexible molecules, resulting in their

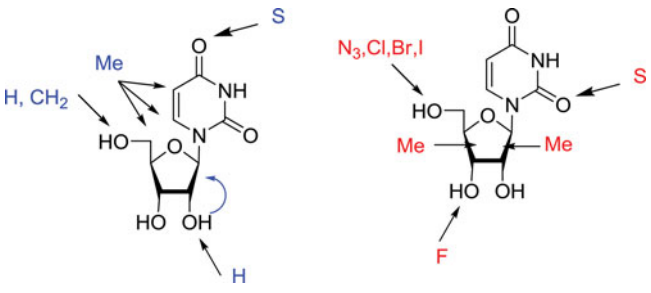


Figure 1. Tolerated (left) and untolerated (right) modifications of uridine.

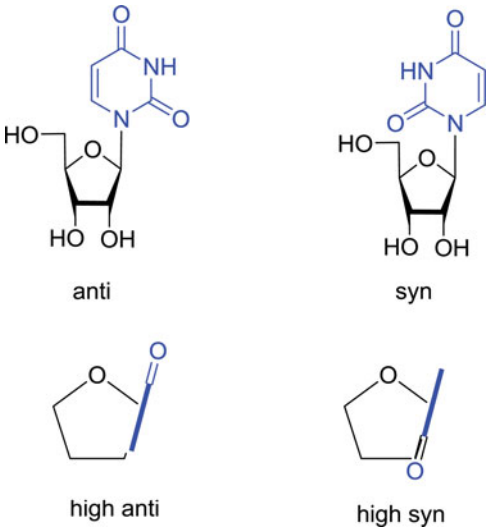


Figure 2. *Anti* and *syn* conformations of uridine (top) and the steric contacts in high-*anti* and high-*syn* conformations with the 2′-proton and the 2′-C-methyl group (bottom).

existence in a manifold of different conformational states separated by low-energy barriers, which can be overcome at room temperature. The rotation around the glycosidic linkage in natural uridine is hindered by steric encumbrance between the base H6 proton and the ribose H2′ proton (high-*anti* conformation) and between the C2 carbonyl group and the H2′ proton (high-*syn* conformation), resulting in two energy barriers. The former barrier is relatively low, whereas the latter one is much higher. The *anti* and *syn* conformations of uridine are shown in Figure 2. In pyrimidine derivatives the barriers to hindered rotation around the N-glycosidic bond, resulting from steric encumbrance between the C2 carbonyl group and the H2′ proton, were estimated by Wagner and Jochims using NMR spectroscopy.[22] Derivatives of 1-(β-D-ribofuranosyl)isocyanuric acids (Figure 3) were used as model compounds. Two methyl groups of l,3-dimethyl-5-(2,3,5-tri-O-acetyl-β-D-ribofurunosyl)-s-triazine-2,4,6(1H,3H,5H)-trione have different chemical shifts due to different environments. Two singlets at low temperatures coalesce to one singlet at elevated temperature. The Gibbs free energies of activation of the rotation around the glycosidic bond in this model compound were estimated at 43–46 kJmoI^{-1}.[22].

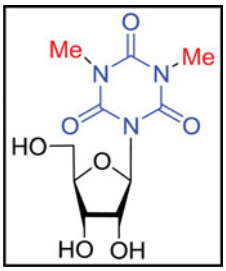


Figure 3. Structure of 1-(β-D-ribofuranosyl)isocyanuric acid.

In nucleosides the least favorable conformations of the ribose moiety in terms of steric interactions are W (04′-exo) and E (04′-endo) conformations, in which the substituents at C2′ and C3′ are eclipsed with the nearly zero C1′-C2′-C3′-C4′ torsion angle. The activation energy barrier was estimated at 20–23 (±0.9) kJ mol^{-1}.[23]

Functionally competent analogues of nucleosides and nucleotides, namely C'-methylnucleosides and their phosphoric esters, are widely used for the study of different enzymes catalyzing the synthesis and decomposition of nucleic acids.[24–26] The presence of bulky methyl groups can give rise to intramolecular and intermolecular steric contacts when fixing the substrate in a particular conformation of the enzyme-substrate complex, thus preventing substrate binding. Conformational maps (the influence of the pseudorotation angle P and the glycoside angle χ on the potential energy E) were calculated for nucleosides and C'-methylnucleosides in order to detect intramolecular contacts between the methyl group and the heterocyclic base.[24–26] Energetically forbidden conformations were observed in 3′-C-methylnucleosides only in the case of N-conformers. The restricted conformations of 5′-C-methylnucleosides were observed only if the methyl group lies above the furanose ring. The introduction of the bulky methyl group in the 2′-position leads to a substantial increase in the energy barrier to the *syn-anti* conversion due to intramolecular contacts of this group with the 2-keto group and the proton in 6-position of pyrimidines.[24–26]. These steric interactions should be taken into account in the discussion of the substrate properties of C'-methylnucleosides.

The replacement of protons with a methyl group at the 2′, 3′ or 5′ positions of the nucleoside gives nucleoside analogs containing all functional groups of natural compounds, for example, all possible binding sites for the complex formation with the enzyme. As a result, comparable binding constants for analogs and natural compounds should be observed. Two extreme cases of the applicability of the proposed[24–26] approach may be considered: (1) the enzyme efficiently binds and transforms the analog; therefore, the condition $E_{(\text{analog})} \sim E_{(\text{natural substance})}$ is fulfilled. (2) the enzyme does not bind the analog; the substrate conformation may be located in the regions where $E_{(\text{analog})} >> E_{(\text{natural substance})}$ provided that the introduction of a bulky methyl group results in intramolecular rather than intermolecular steric hindrance. This concept can explain the conformation of the substrate during its transformation by several enzymes of nucleic acid biosynthesis.[24–26]

Both 5′-C-methyluridines are substrates for UP, and the efficiency of phosphorolysis of 5′-C in the L-talo configuration is nearly the same as for uridine. Another diastereomer in the D-allo configuration reacts with phosphate an order of magnitude slower. The same configurational preferences may be seen for 5′-C-methylthymidine (L-talo) but the phosphorolysis is much slower. 2′-C-Methyluridine and 3′-C-methyluridine are not substrates for UP and virtually do not bind to the enzyme.

Molecular modeling of the binding of 2′-C-methyluridine and 3′-C-methyluridine to UP was performed based on the X-ray structure of the complex of uridine with UP.[27] When complexed with UP, 3′-C-methyluridine forms a close steric contact (2.45 Å) between the 3′-C-methyl group and sulfur of methionine

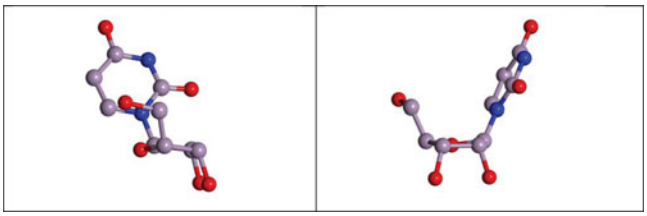


Figure 4. High-*syn* and C4′-*exo* conformation of uridine complexed with a sulfate ion and UP.[27] The views from different sides.

194. By contrast, the additional methyl group of 2′-C-methyluridine in the complex with UP does not form contacts with the enzyme but causes intramolecular steric hindrance.

The obtained kinetic results may be best explained by the binding of UP to uridine in the high-*syn* conformation. There is the following evidence for this unusual conformation of the uridine complexed with UP when the carbonyl is located near the 2′-proton. The efficiency of phosphorolysis of 1-β-D-arabinofuranosyluracil is 25 times lower as compared to 2′-deoxyuridine evidently due to the steric hindrance caused by the 2′-hydroxyl group and the 2-keto group. The tendency to lose substrate properties is observed with an increase in the van der Waals radius of the 2′-substituent (H, 1.30Å; O, 1.40Å; Me, 2.00Å[19]). Meanwhile, 2,2′-O-anhydrouridine that models the high-*syn* conformation of uridine binds to UP better than uridine, despite the absence of a proton in the 3-position of the heterocyclic base, thus playing an important role in the substrate binding.[1] In this case, the orientation of the heterocycle substantially outweighs the contribution of hydrogen bonding by the 2′-hydroxyl and 3-NH groups in the active site of UP. This strongly suggests that the 2-keto group is located quite close to 2′-H in the substrate-UP complex. This is in line with the lack of substrate properties of 2-thiouridine.

It is interesting to compare the obtained results with the X-ray diffraction data for the complexes of uridine with UP. The high-resolution structure of *Shewanella oneidensis* MR-1 uridine phosphorylase complexed with uridine and sulfate[27] is best suited for this purpose because the active site region of the enzyme in this complex is well resolved and all contacts (hydrogen bonds and hydrophobic contacts) are reliably identified. Besides, the presence of sulfate, which mimics phosphate, is crucial for the formation of the active site.

This enzyme is homologous to *E. coli* UP and the active sites of both enzymes are virtually identical. The equilibrium constants and the kinetic characteristics of these enzymes with respect to uridine, thymidine and 5-methyluridine are nearly the same.[28]

The unusual high-*syn* conformation of the substrate was unequivocally established by X-ray crystallographic analysis of *Shewanella oneidensis* UP complexed with sulfate and uridine (1.75 Å resolution).[27] This structure is characterized by the torsion angle C2-N1-C1′-C2′= 13.5° and the N1-C1′ glycosidic bond lengths of 1.49 Å (Figure 4). The ribose residue adopts the least favorable C4′-*exo* conformation in terms of steric interactions, in which the C2′ and C3′ hydroxyl groups are

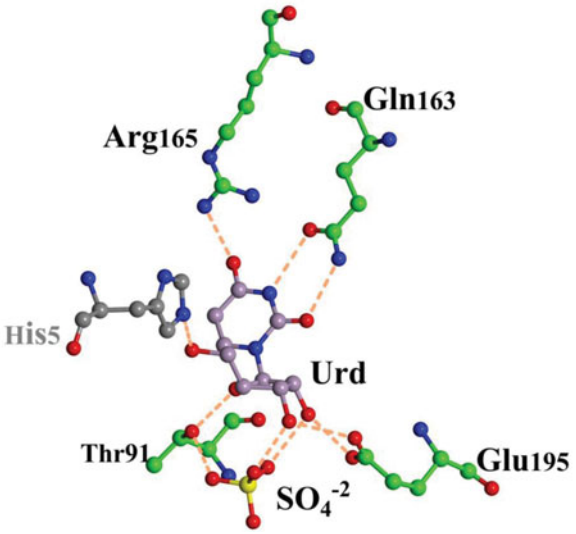


Figure 5. The active site containing uridine and a sulfate ion. His5 belongs to another subunit. Hydrogen bonds are shown as orange broken lines.

eclipsed and both the CH_2OH group and the heterocyclic base (bulky substituents) are maximally axial (Figure 4). Additionally, the ribose ring is highly flattened, as can be seen from the torsion angles (C1′-C2′-C3′-C4′ is −2°). There is also a very short contact between the O2′ and O3′ atoms (2.25 Å). The distance between the carbonyl O2 atom and the C2′ atom of the ribose is 2.92 Å (the distance between H2′ and O2 can be estimated at 1.8 Å).[27]

All functional groups of the uridine molecule are involved in an extensive hydrogen bond network with UP (see Figure 5). Thus, the O4 atom of uridine forms a hydrogen bond with the NH2 atom of Arg165, the O2 atom forms a hydrogen bond with Gln163, and the latter residue forms a hydrogen bond with the N3 atom of uridine. The ribose residue forms several hydrogen bonds: O4′ and Thr91, O2′ and Glu195, O3′ and Glu195, O5′ and His5.[27]

Conclusions

The combined kinetic and structural data provide clear evidence that UP binds uridine in the most energetically unfavorable conformation, which, to the best of our knowledge, has no precedents in the enzymes of nucleic acid metabolism. This is possible due to multiple interactions between the substrate and the protein environment (active site residues) mainly through hydrogen bonds. These results are important for understanding the mechanism of action of this class of enzymes.

An analysis of the conformations of nucleosides in solution and rotational barriers suggests that the energy difference between the ground state of uridine and uridine complexed with UP may be as high as 63–69 kJ mol^{-1}. The binding in a high-energy conformation results in the weakening of the glycosidic bond. The

observed conformation of uridine complexed with sulfate (mimetic of phosphate) may be very similar to its conformation in the transient state.

The ability of *E. coli* UP to cleave and catalyze the formation of β-D-ribo-(2-deoxyribo- and arabino-)furanosyl nucleosides is widely used in enzymatic transglycosylation reactions, in which a sugar residue is transferred from a pyrimidine nucleoside to the purine heterocyclic base.

Experimental part

The following reagents were used in this work: thymidine, uridine, 5-methyluridine, 2′-deoxyuridine, 1-β-*D*-arabinofuranosyluracil, 2,2′-*O*-anhydrouridine, (Sigma, USA). Kinetic constants were measured using a Cary 300 spectrophotometer (Varian, Australia). High performance liquid chromatography was performed on a Gilson chromatographer (USA) using a 4.6 × 150 mm column (5 μm, Nucleosil C18).

The following nucleosides were obtained according to the previously published protocols: 4-thiouridine[29], 5′-homouridine[30], 5′-C-methyluridines (D-allo and L-talo)[31,32], 5′-homothymidine[33], 5′-C-methylthymidines (D-ribo and L-lyxo)[33], 5′-deoxyuridine[34], 5′-deoxythymidine[35], 1-(5,6-dideoxy-β-D-ribo-hex-5-enofuranosyl)adenine (5′-deoxy-5′-methyleneuridine)[36], 3′-fluoro-3′-deoxyuridine[37], 3′-C-methyluridine[38], 2′-C-methyluridine[39]. 2-Thiouridine and 6-methyluridine were the gift of Dr. A.Holy. 5′-Chloro-5′-deoxythymidine, 5′-bromo-5′-deoxythymidine, 5′-iodo-5′-deoxythymidine, 5′-azido-5′-deoxythymidine were the gift of Dr. A.M. Kritzyn. (Chemical structures of modified nucleosides are shown in Table 1.)

Enzymic activity assays for E. coli UP

The UP activity was assayed spectrophotometrically at 25°C and estimated from the difference in A_{280} for the nucleoside and the heterocyclic base resulting from its enzymatic cleavage. Calculations were performed assuming $\Delta\varepsilon_{280}$= 2030 $M^{-1}\cdot cm^{-1}$ for the uridine/uracil pair. Measurements were taken in 50 mM potassium-phosphate (pH 7.5). The reaction mixture (1 mL) contained 0.5 μmol of uridine and 0.18 units of UP. *E. coli* UP was obtained according to the literature procedure[40] and was supplied by Dr. R.S. Esipov from Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences.

Estimation of the Michaelis constant (K_M) for phosphorolysis of uridine and its derivatives

The constant K_M of phosphorolysis of uridine by UP was determined from the spectrophotometric estimation of the reaction rate at 280 nm. The substrate concentration varied in the range of 10−200 μM for uridine derivatives at a constant phosphate concentration (50 mM), pH 7.5, at 25°C. The reactions were initiated by adding 0.03 units of the enzyme; the rate was measured for 5 min. To obtain K_M, we

used nonlinear regression analysis of the Michaelis–Menten and Lineweaver–Burk plots.

Estimation of the inhibition constant (K_I)

K_I of uridine derivatives were measured spectrophotometrically[29]. An enzyme was preincubated at various concentrations of an inhibitor at 25°C for 15 min, and the initial rate was measured for phosphorolysis of the natural substrate or its thio derivative at 280 and 365 nm in the case of uridine and 4-thiouridine, respectively. Substrates were used at 0.5 or 1 K_M. The estimation of K_I followed the Dixon method.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Author contributions

Conceived and designed the experiments: S.N. Mikhailov. Performed the kinetic experiments: C.S. Alexeev. Analyzed the data: S.N. Mikhailov. Analysis of crystallographic data: T. N. Safonova. Synthesis of 3′-deoxythymidine and 3′-fluoro-3′-deoxyuridine: G.G.Sivets. Wrote the paper: S.N. Mikhailov.

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