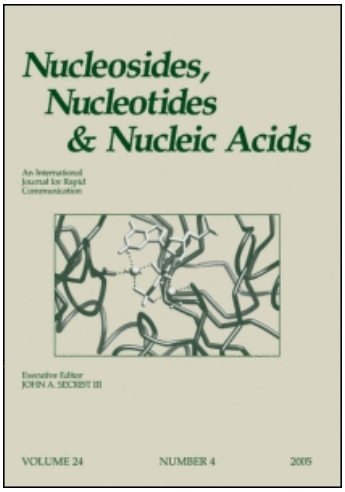

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Uridine Recognition Motifs of Human Equilibrative Nucleoside Transporters 1 and 2 Produced in *Saccharomyces cerevisiae*†

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ABSTRACT

The sugar moiety of nucleosides has been shown to play a major role in permeant-transporter interaction with human equilibrative nucleoside transporters 1 and 2 (hENT1 and hENT2). To better understand the structural requirements for interactions with hENT1 and hENT2, a series of uridine analogs with sugar modifications were subjected to an assay that tested their abilities to inhibit [^{3}H]uridine transport mediated by recom1binant hENT1 and hENT2 produced in *Saccharomyces cerevisiae*. hENT1

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.
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displayed higher affinity for uridine than hENT2. Both transporters barely tolerated modifications or inversion of configuration at C(3′). The C(2′)-OH at uridine was a structural determinant for uridine-hENT1, but not for uridine-hENT2, interactions. Both transporters were sensitive to modifications at C(5′) and hENT2 displayed more tolerance to removal of C(5′)-OH than hENT1; addition of an O-methyl group at C(5′) greatly reduced interaction with either hENT1 or hENT2. The changes in binding energies between transporter proteins and the different uridine analogs suggested that hENT1 formed strong interactions with C(3′)-OH and moderate interactions with C(2′)-OH and C(5′)-OH of uridine, whereas hENT2 formed strong interactions with C(3′)-OH, weak interactions with C(5′)-OH, and no interaction with C(2′)-OH.

Key Words: Uridine recognition motifs; *Saccharomyces cerevisiae*; hENT1; hENT2.

INTRODUCTION

Nucleoside transporters (NTs) are integral membrane proteins that mediate uptake and release of physiologic and synthetic nucleosides (reviewed in.[1–3] NT-mediated salvage of physiologic nucleosides is an energetically favorable alternative to de novo synthesis. Additionally, NT-mediated cellular uptake is a critical determinant of metabolism and pharmacological actions of anticancer and antiviral nucleosides. NTs also affect the surface concentrations of adenosine, a signaling molecule that regulates vasodilation, neuromodulation and platelet aggregation.[4,5] Two distinct NT families, the equilibrative nucleoside transporters (ENTs) and the concentrative nucleoside transporters (CNTs) have been identified by molecular cloning and functional expression. The ENTs are facilitative transporters that mediate transport of nucleosides across membranes according to nucleoside concentration gradients and have a broad tissue distribution, whereas the CNTs mediate cation-dependent transport of nucleosides against concentration gradients and are found primarily in specialized cells such as intestinal and renal epithelia.

Human ENT1 and ENT2 (hENT1 and hENT2) share 50% amino acid identity and have a common membrane architecture. hENT1 and 2 are functionally distinguished by their differential sensitivities to nitrobenzylmercaptopurine ribonucleoside (NBMPR) and have therefore been assigned the functional designations equilibrative *s*ensitive (*es*) and equilibrative *i*nsensitive (*ei*), respectively.[6–8] The two proteins differ in that transport by hENT1 is inhibited by nanomolar concentrations of vasodilators such as dilazep and dipyridamole, whereas hENT2 is two to three orders of magnitude less sensitive. The *es* and *ei* NTs are under independent genetic control and are often produced together in various cell types and tissues. Both are broadly selective for purine and pyrimidine nucleosides, and hENT2 transports most naturally occurring nucleosides with lower affinities and can be further distinguished by its ability to transport nucleobases.[9] The different abilities of hENT1 and hENT2 to interact with the compounds tested as well as permeants suggest that the two proteins might have different structural determinants for binding of inhibitors and permeants. The involvement of individual amino acid residues, such as M33, and of structural domains, such as transmembrane domains 3 to 6 in the interaction of hENT1 with tight-binding inhibitors has been demonstrated.[10,11] Determination of permeant structures that are involved in interactions with NTs is needed to rationally design nucleoside analogs for anticancer and antiviral chemotherapy.

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The examination of individual NTs is hindered by the presence of multiple NTs in a given tissue or cell type. The availability of model expression systems in which the endogenous nucleoside transport processes are known has facilitated characterization of individual recombinant NTs. Several model expression systems have been used to functionally produce both ENTs and CNTs including oocytes of *Xenopus laevis*,[12–16] cultured cells[17,18] and *Saccharomyces cerevisiae*.[9,10,19–23] The identification of endogenous NTs of *S. cerevisiae* allowed the generation of a yeast strain (fui1::Trp1) lacking the endogenous uridine (Urd) permease (Fui1).[20] A variety of base and/or sugar-modified Urd analogues were used to undertake structure-activity relationship studies with recombinant hENT1 and hENT2 produced in fui1::Trp1 yeasts.[22] This work suggested that the sugar moiety of Urd plays a major role in its interactions with the transporter.[22]

We describe here the use of synthetic Urd analogs with individual modifications in the sugar moiety to determine the interactions of C(2′), C(3′) and C(5′) of Urd with recombinant hENT1 and hETN2. A new method was used for measurement of NT-mediated uptake in *S. cerevisiae* in which nucleoside uptake into yeast was conducted in 96-well plates and yeast cells were collected using an automated 96-well harvester. This ''plate-method'' allowed high-throughput low-volume assays to quantify the inhibitory effects of Urd analogs. The inhibition data yielded K_i (inhibitory constant) values that were used to compare binding energies for interaction of Urd analogs with the nucleoside-binding sites of hENT1 and hENT2.

MATERIALS AND METHODS

Strains and Media. fui1::TRP1 (MATα, gal, ura3-52, trp1, lys2, ade2, hisd2000, Δ*fui*::TRP1) was the host yeast strain used throughout.[20] The hENT1 and hENT2 cDNAs were inserted into the yeast expression vector pYPGE15 (under control of the constitutive PGK promoter) and the resulting plasmids, pYPhENT1 and pYPhENT2, were transformed into fui1::TRP1 yeast using a lithium acetate method.[24] Characterization of recombinant hENT1 and hENT2 produced in fui1::TRPP1 yeast has been described previously.[10,22] Yeast were maintained in complete minimal media (CMM) containing 0.67% (w/v) yeast nitrogen base (Difco, Detroit, MI), amino acids (as required to maintain auxotrophic selection) and 2% (w/v) glucose (CMM/GLU). Agar plates contained CMM with various supplements and 2% (w/v) agar (Difco, Detroit, MI). Plasmids were propagated in the *Escherichia coli* (*E. coli*) strain TOP10F' (Invitrogen, Carlsbad, CA) and maintained in Luria broth with ampicillin (50 μg/ml).

Nucleoside Uptake in Yeast. Uridine transport rates by recombinant hENT1 and hENT2 produced in fui1::TRP1 yeast were determined for all subsequent experiments using incubation intervals of 20 min, which was previously shown to yield initial rates of uptake of uridine into both pYPhENT1-and pYPhENT2-containing yeast.[10] The extended linear time courses are the result of efficient trapping of uridine by its conversion to UMP, thereby maintaining the inwardly directed uridine concentration gradient. Transporter-mediated uptake of [^{3}H]-uridine (Moravek Biochemicals, Brea, CA) by logarithmically proliferating yeast was measured using a cell-harvester based approach. Briefly, yeast cells were grown in CMM/GLU to an OD_{600} of 0.8–1.5, washed three times with fresh CMM/GLU (pH7.4), and restenosis-suspended to an

OD_{600} of 4 in CMM/GLU (pH7.4). [^{3}H]Urd at 2 μM and a test compound (if present) at a desired concentration in CMM/GLU (pH 7.4) were preloaded into a 96-well plate. The transport assays were initiated by adding an equal volume of yeast suspension at OD_{600} = 4 to the individual wells of a preloaded 96-well plate. Transport assays were terminated by harvesting the yeast cells of each plate onto binding enhanced filtermats (Molecular Devices, Ca) using a Micro96 Harvester (Molecular Devices, Ca) with continuous washing by distilled, deionized water to remove unincorporated permeant. The filter discs with yeast cells corresponding to each transport assay were placed into individual scintillation counting vials (Fisher) to which 5 ml of scintillation counting fluid (EcoLite) was added. Scintillation vials were allowed to sit at room temperature overnight with shaking before counting.

Table 1. K_i and ΔG° values for the inhibition of hENT1- or hENT2-mediated [^{3}H]Urd transport by uridine and its analogs.

Urd compounds	hENT1		hENT2	
	K_i (μM)	ΔG°(kJ/mol)	K_i (μM)	ΔG°(kJ/mol)
uridine (Urd)	37 ± 7	25.3	184 ± 22	21.3
2′-modifications				
2′-deoxyuridine (2′dUrd)	171 ± 19	21.3	155 ± 11	21.7
1-(β-D-arabinofuranosyl)uracil (araU)	227 ± 19	20.8	227 ± 9	20.8
2′-azido-2′-deoxyuridine (2′AzdUrd)	13 ± 1	27.8	169 ± 14	21.5
2′-*O*-methyluridine (2′OMeUrd)	471 ± 80	19.0	1571 ± 124	16.0
3′-modifications				
3′-deoxyuridine (3′dUrd)	> 2000		> 2000	
1-(β-D-xylofuranosyl)uracil (xyloU)	ND, 3000		ND, 3000	
3′-azido-3′-deoxyuridine (3′AzdUrd)	ND, 3000		ND, 3000	
3′-*O*-methyluridine (3′OMeUrd)	ND, 3000		ND, 3000	
2′,3′-dideoxyuridine (2′,3′ddUrd)	ND, 3000		ND, 3000	
3′,5′-dideoxyuridine (3′,5′ddUrd)	ND, 3000		ND, 3000	
2′,3′-*O*-isopropylideneuridine (iPUrd)	ND, 3000		ND, 3000	
5′-modifications				
5′-deoxyuridine (5′dUrd)	273 ± 29	20.3	702 ± 68	18.0
5′-*O*-methyluridine (5′OMeUrd)	> 2000		> 2000	
5′-azido-5′-deoxyuridine (5′AzdUrd)	> 2000		> 2000	
2′,5′-dideoxyuridine (2′,5′ddUrd)	430 ± 54	19.2	606 ± 135	18.4
5′-chloro-5′-deoxyuridine (5′CldUrd)	382 ± 11.5	19.5	232 ± 40	20.7

The uptake of 1 μM [^{3}H]uridine into fui1::TRP yeast that contained either pYPhENT1 or pYPhENT2 in the presence or absence of graded concentrations of test compound is described in Materials and Methods. Average K_i values (mean ± S.E.) were converted from average IC_{50} values (mean ± S.E., n = 3) by the Cheng–Prushoff equation[23] using K_m values (mean ± S.E.) of 42.6 ± 3.3 and 194.5 ± 34.3 μM for hENT1 and hENT2, respectively. The Gibbs free energy was determined from $\Delta G^\circ = -RT\ln(K_i)$. ND indicates that IC_{50} values could not be determined at the indicated highest concentration tested because only partial inhibition of Urd transport mediated by hENT1 or hENT2 was observed.

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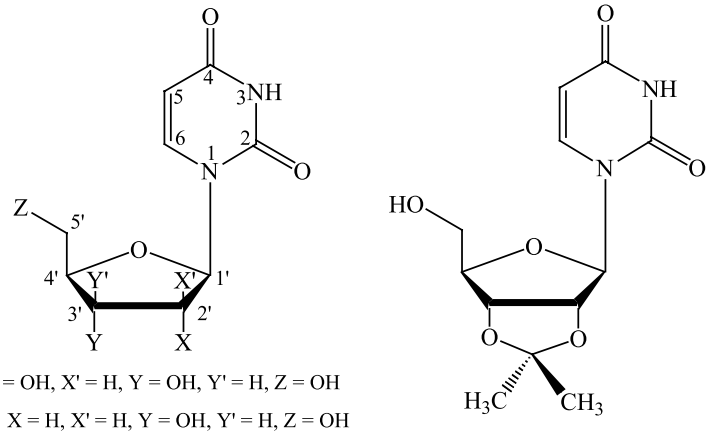
Data analysis. All experiments were carried out in quadruplicate. The amount of [^{3}H]Urd associated with yeast in the presence of 10 mM Urd was determined as nonspecifically associated radioactivity and was subtracted from total radioactivity for each transport assay. Data were fitted to theoretical inhibition curves by nonlinear regression to obtain IC_{50} values (concentration that inhibited reactions by 50%). Apparent K_i values were calculated from the Cheng and Prusoff equation,[23] in which $K_i = IC_{50}/[1 + (L/K_m)]$ and L = [^{3}H]Urd concentration, which was always 1 μM. Gibbs free energy (ΔG°) was determined from $\Delta G^\circ = -RT\ln(K_i)$, in which R is the gas constant and T is the absolute temperature. The thermodynamic stability of transporter-inhibitor complexes was estimated from ΔG° as described elsewhere.[25,26]

Urd analogs. Abbreviations and structures of uridine and the uridine analogs are given in Table 1 and Figure 1. Uridine and 2′-deoxyuridine were obtained from Sigma-Aldrich (Oakville, Ont.); 3′-*O*-methyluridine, 2′,3′-*O*-isopropylideneuridine,



Urd: X = OH, X' = H, Y = OH, Y' = H, Z = OH

2'dUrd: X = H, X' = H, Y = OH, Y' = H, Z = OH

araU: X = H, X' = OH, Y = OH, Y' = H, Z = OH

2'AzdUrd: X = N_3, X' = H, Y = OH, Y' = H, Z = OH

2'OMeUrd: X = OCH_3, X' = H, Y = OH, Y' = H, Z = OH

3'dUrd: X = OH, X' = H, Y = H, Y' = H, Z = OH

xyloU: X = OH, X' = H, Y = H, Y' = OH, Z = OH

3'AzdUrd: X = OH, X' = H, Y = N_3, Y' = H, Z = OH

3'OMeUrd: X = OH, X' = H, Y = OCH_3, Y' = H, Z = OH

2',3'ddUrd: X = H, X' = H, Y = H, Y' = H, Z = OH

3',5'ddUrd: X = OH, X' = H, Y = H, Y' = H, Z = H

5'dUrd: X = OH, X' = H, Y = OH, Y' = H, Z = H

5'OMeUrd: X = OH, X' = H, Y = OH, Y' = H, Z = OCH_3

5'AzdUrd: X = OH, X' = H, Y = OH, Y' = H, Z = N_3

2',5'ddUrd: X = H, X' = H, Y = OH, Y' = H, Z = H

5'ClduRd: X = OH, X' = H, Y = OH, Y' = H, Z = Cl

Figure 1. Structures of Urd and Urd analogs. Numbering for Urd is indicated.

2′,3′-dideoxyuridine, and 2′,5′-dideoxyuridine were obtained from R. I. Chemical, Inc. (Orange, CA). 1-(β-D-Arabinofuranosyl)uracil, 2′-azido-2′-deoxyuridine, 2′-*O*-methyluridine, 1-(β-D-xylofuranosyl)uracil, 3′-deoxyuridine, 3′-azido-3′-deoxyuridine, 5′-deoxyuridine, 5′-chloro-5′-deoxyuridine, 5′-azido-5′-deoxyuridine, 5′-*O*-methyluridine and 3′,5′-dideoxyuridine were prepared as described elsewhere.[27]

RESULTS AND DISCUSSION

In the current work we examined the ability of recombinant hENT1 and hENT2 to interact with a series of uridine analogs with sugar modifications by assessing the concentration dependence of inhibition of transport of 1 μM Urd. The inhibition of Urd

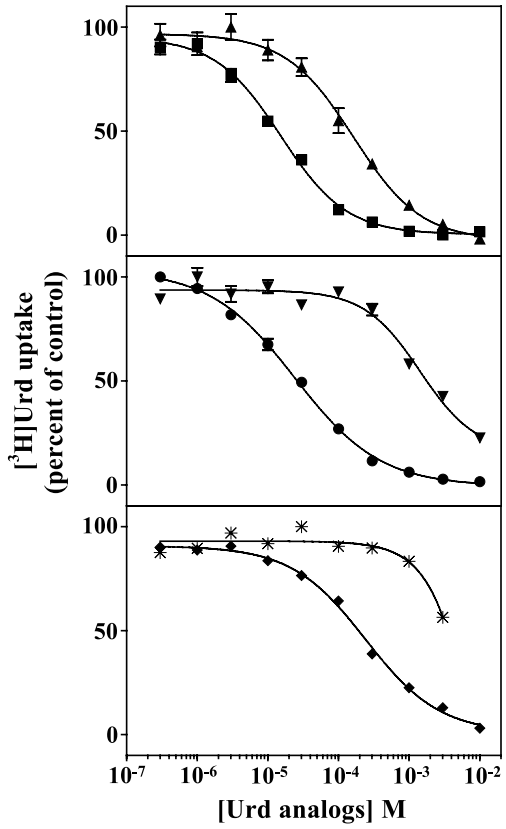


Figure 2. Inhibition of recombinant hENT1-mediated Urd uptake by some Urd analogs. The uptake of 1 μM [^{3}H]Urd into fui1::TRP yeast expressing pYPhENT1 was measured over 20 min in the presence of graded concentrations of test compounds as described in Materials and Methods. The test compounds shown are: Urd (●), 2′dUrd (▲), 2′AzdUrd (■), 2′,5′dUrd (◆), 3′dUrd (▼), and 5′OMedUrd (∗). Uptake values in the presence of Urd compounds are given as the percentage of uptake values in their absence. Each data point represents the means ± S.E. of quadruplicate determinations; error bars are not shown where they are smaller than the symbol. Three or four independent experiments gave similar results and results from representative experiments are shown.

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uptake was assumed to be competitive since 1) the inhibitors tested were close structural analogs of uridine, and 2) the transporter under study was most likely to be the sole route of interaction/entry of the potential inhibitor. The representative concentration-effect curves of some of the Urd analogs for inhibition of hENT1-and hENT2-mediated Urd transport are shown in Figure 2 and Figure 3, respectively. The Hill coefficients determined from Hill plots were-1.0 ± 0.2, indicating a single class of inhibitor-binding sites. The mean K_i values (± S.E.) and the corresponding Gibbs free energy values for all of the compounds tested are listed in Table 1.

Differences in the interaction of recombinant hENT1 and hENT2 with Urd are highlighted by the observation in some systems that hENT1 exhibits higher apparent affinity for Urd transport than hENT2. For example, K_m values of uridine for *es*-and *ei*-mediated transport of uridine by human erythroleukemia (K562) cells exhibited a 5-fold difference (230 versus 1080 μM, 37°C),[28] and the apparent K_m values obtained for recombinant hENT1 and hENT2 produced in the fui1::Trp1 yeast strain were

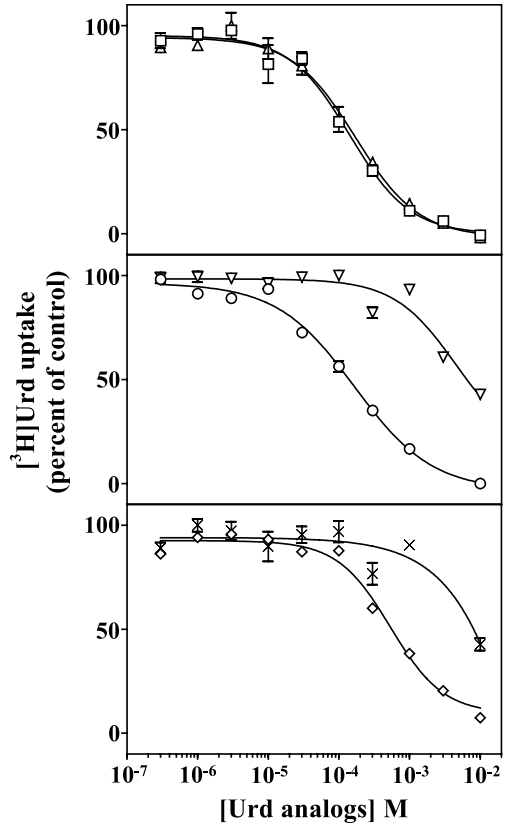

Figure 3. Inhibition of recombinant hENT2-mediated Urd uptake by some Urd analogs. The uptake of 1 μM [^{3}H]Urd into fui1::TRP yeast expressing pYPhENT2 was measured over 20 min in the presence of graded concentrations of test compounds as described in Figure 2. The test compounds shown are: Urd (○), 2′dUrd (△), 2′AzdUrd (□), 5′dUrd (◇), 3′dUrd (▽), and 5′OMedUrd (+).

42.6 ± 3.3 and 194.5 ± 34.3 μM, respectively (Visser F. and Cass C.E., manuscript in preparation). The apparent K_m values of recombinant hENT1 and hENT2 expressed in yeast were lower than those obtained in cultured human cells[28] or in *Xenopus laevis* oocytes,[7,8] presumably reflecting differences in the various expression systems in post-translational modifications. This difference between hENT1 and hENT2 was also evident in the apparent K_i values (mean ± S.E.) of 37.2 ± 7.1 and 183.7 ± 21.7 μM for Urd inhibition of hENT1-or hENT2-mediated [^{3}H]Urd uptake, respectively (Table 1, Figure 2 and Figure 3). The corresponding Gibbs free energy values (ΔG°) for hENT1 and hENT2 were 25.3 and 21.3 kJ/mol, respectively.

2′ MODIFICATIONS

There were apparent interactions through hydrogen bonding between hENT1 and C(2′)-OH since removal or inversion of the orientation of the 2′-hydroxyl group resulted in a decrease of 4 to 5 kJ/mol in ΔG° with a 5-fold difference in K_i values. The addition of an O-methyl group to the 2′ position (2′OMeUrd) produced a further loss in ΔG° of 2 kJ/mol, indicating that C(2′)-OH is part of the structural region of Urd that interacts with hENT1 (Figure 2). However, the presence of C(2′)-OH was not an absolute requirement for high-affinity interaction of hENT1 to a Urd analog since replacement of the 2′-hydroxyl group by an azido group (2′AzdUrd) produced an analog that displayed higher affinity (K_i = 13.2 ± 1.2 μM) than Urd itself for hENT1 (t-test, $p < 0.05$) (Figure 2). The increase of 2.5 and 6.5 kJmol^{-1} in ΔG° for 2′AzdUrd relative to Urd and to 2'dUrd, respectively, might be due to new hydrogen bonding and/or hydrophobic interactions between the azido group and the transporter.

hENT2 differed from hENT1 in that removal of the 2′-hydroxyl group to create 2′dUrd did not significantly affect its sensitivity to inhibition of Urd transport (Figure 3). Consistent with this observation, inversion of the orientation of C(2′)-OH (araU) or substitution of the 2′-hydroxyl group with an azido (2′AzdUrd) resulted in no change in inhibitory effect on hENT2-mediated Urd uptake (Figure 3). However, addition at the 2′ position of an O-methyl group produced an analog with an 8.5-fold increase in the $K_i$value for hENT2. The lost binding energy ($\delta(\Delta G^\circ)$ = 5.3 kJ/mol, relative to ΔG° of Urd) of 2′OMeUrd for hENT2 was probably due to steric effects.

Taken together, these findings suggested that the 2′-hydroxyl group played a more important role in interaction with hENT1 than with hENT2. Removal or inversion of configuration resulted in moderate changes in K_i values for hENT1 and negligible changes in K_i values for hENT2. The minimal changes observed in ΔG° values were inconsistent with interactions through hydrogen bonding between Urd and the transporters.

3′ MODIFICATIONS

Previous studies have pointed out the importance of the C(3′) position of uridine and cytidine for interaction with hENTs.[22,29,30] Two uridine analogs with modifications at C(3′) [3′-azido-2′-deoxyuridine and 3′-azido-2′-deoxy-5-methyluridine (AZT)]

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do not inhibit hENT1-or hENT2-mediated [^{3}H]uridine uptake[22] and recombinant hENT2, but not recombinant hENT1, transports AZT.[30] To further examine the role of the 3′-position of uridine, several of the 3′-modified uridine analogs were assessed for their ability to interact with both hENT1 and hENT2 (Table 1). Compounds with modifications at C(3′) had little, or no, effect on either transporter. 3'dUrd had no effect on hENT1 or hENT2 at concentrations higher than 2 mM (Figures 2 and 3), indicating that C(3′)-OH is an important structural determinant for binding of Urd. The estimated loss of more than 15 kJ in ΔG° for 3′Urd relative to that of Urd indicated a possible loss of hydrogen bonding between hENT proteins and C(3′)-OH. Further modifications at C(3′), including removal of both hydroxyl groups (2′,3′ddUrd and 3′,5′ddUrd), inversion of the configuration of the 3′-hydroxyl group (xyloU), or replacement of the 3′-hydroxyl group with bulky groups (3′AzdUrd and 3′OMeUrd) virtually eliminated binding of the Urd analogs to hENT1 and 2. Additionally, iPUrd did not inhibit Urd transport. The 3′-hydroxyl group is also the most critical functional group of Urd for binding to human CNTs[27] and to fui1, which is a uridine permease of *Saccharomyces cerevisiae* with no sequence similarity to mammalian nucleoside transporters (Zhang J. and Cass C.E., manuscript in preparation). The requirement for the 3′-hydroxyl group suggests a strong evolutionary pressure to maintain a permeant binding site for C(3′)-OH.

5′-MODIFICATIONS

Both hENT1 and hENT2 displayed changed affinities for analogs with modifications at C(5′) of Urd, with hENT1 showing somewhat greater sensitivity. Removal of the 5′-hydroxyl (5′dUrd) produced a decrease of 5 kJmol^{-1} in ΔG° with a 7.3-fold increase in K_i values (Figure 2), suggesting that the 5′-hydroxyl forms weak hydrogen bonds with hENT1. The additional removal of the 2′-hydroxyl group (2′,5′ddUrd) further decreased the binding energy for hENT1 ($\delta(\Delta G^\circ)$ = 1.1 kJ/mol, relative to ΔG° of 5′dUrd). Removal of the 5′-hydroxyl group also reduced the affinity of hENT2 with a 4-fold difference in K_i values for Urd and 5′dUrd and a loss of 3 kJ/mol in ΔG° (Figure 3). Since hENT2 showed no difference in its interaction with Urd and 2′dUrd, the decreased affinity for 2′,5′ddUrd was due to the removal of C(5′)-OH alone.

The substantial decrease in K_i value for hENT1 upon substitution of chloride for the hydroxyl group at C(5′) further indicated its involvement in the hENT1-Urd interactions. hENT2 exhibited an affinity for 5′CldUrd similar to that for Urd. Although removal of the 5′-hydroxyl group shifted the concentration-effect curves for both transporters to the right (Figure 2), high concentrations of 5′dUrd were able to fully inhibit Urd transport mediated by hENT1 and hENT2. Addition of a bulkier group, such as an azido or O-methyl, at C(5′) greatly reduced the interactions with either hENT1 or hENT2 (K_i > 3 mM for 5′AzdUrd and 5′OMeUrd, Figures 2 and 3). A bulky charged group at C(5′) of Urd (uridine monophosphate) was not transported by recombinant hENT1 produced in *Xenopus* oocytes.[29] These finding suggested direct interactions between hENT proteins and the 5′-hydroxl group and steric limitations near C(5′) for binding.

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CONCLUSIONS

Recombinant hENT1 and hENT2 produced in *S. cerevisiae* displayed similarities as well as differences in the binding requirements for uridine-transporter interactions. As observed previously[6,22] and in this work, hENT1 exhibited a higher affinity than hENT2 for uridine, although it should be noted that recombinant hENT1 and hENT2 exhibited similar affinities for uridine when produced in *Xenopus* oocytes.[7,8] The 3′-hydroxyl group was a critical requirement for Urd binding to recombinant hENT1 and hENT2 in yeast. Any perturbation of this group greatly reduced interactions with hENT1 or hENT2. The 2′ and 5′-hydroxyl groups played lesser roles than the 3′-hydroxyl group in interactions with the transporters. Modifications at C(2′) were moderately tolerated by hENT1 and well tolerated by hENT2. Although hENT1 was sensitive to the removal or inversion of the 2′-hydroxyl group, and to a much greater extent than hENT2, its replacement with an azido group resulted in an analog (2′AzdUrd) with higher affinity. Removal of the 5′-hydroxyl group or its replacement with chloride reduced the affinities of both hENT1 and hENT2. However, both 5′dUrd and 5′CldUrd fully inhibited Urd uptake by hENT1 or hENT2 at high concentrations. Larger substituents (azido or O-methyl) at C(5′) abolished the interactions with both hENT1 and hENT2.

These and continued analyses of structure-activity relationships of recombinant NT proteins produced in *S. cerevisiae* will aid in the rational design of nucleoside-based drugs and prodrugs. Ideally, this approach will allow the design of uridine anticancer drugs with cytotoxic substituent(s) that are differentially transported by hENT1 and/or hENT2.

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