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Specificity of Uracil Uptake in *Neurospora crassa*

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The specificity of uracil uptake was investigated in germinating wild-type conidia of *Neurospora crassa*. From comparative inhibition studies, several generalizations concerning the specificity of uracil uptake can be made. (i) The tautomeric forms of uracil analogs is an important determinant of recognition by the uptake system. (ii) Substituents at the 5 position of the pyrimidine ring may impose steric constraints on binding. (iii) The presence of a negative charge results in the loss of recognition. (iv) The double bond between the 5 and 6 carbons appears to be important for recognition. (v) Purine bases do not inhibit uracil uptake. Crude extracts of the transport-deficient mutant strain *uc-5 pyr-1* were shown to have uridine 5'-monophosphate pyrophosphorylase activity comparable to that of the wild-type strain, suggesting that uracil uptake in *Neurospora* does not occur by a group translocation mechanism involving phosphoribosylation. Specificity studies of uridine 5'-monophosphate pyrophosphorylase indicated that phosphoribosylation was not an important determinant of the specificity of uracil uptake.

Despite the use of many pyrimidine-base analogs as mutagenic or chemotherapeutic agents (e.g., 5-fluorouracil, 2-thiouracil, and 5-azathymine), little information is available on the specificity of pyrimidine-base transport systems. Specificity is readily determined by measuring the ability of structural analogs to inhibit transport of the substrate. In several organisms, cytosine has been shown to inhibit uracil transport (1, 5); thymine and 5-fluorouracil (1, 5) also inhibit in some cases. Cytosine has also been shown to inhibit uracil uptake in wild-type strains of *Saccharomyces cerevisiae* and *Candida albicans*, although evidence has been found which indicates that cytosine is taken up in these species by a common system for cytosine, adenine, and hypoxanthine which is independent of a uracil, 5-fluorouracil system (9). However, no study has determined the structural features of the pyrimidine base required for binding to the transport system.

The mechanism of uracil transport has been studied by Hochstadt and Stadtman (6) in *Escherichia coli* vesicles and is reported to proceed by a group translocation mechanism in which uracil is condensed with 5'-phosphoribosyl-1'-pyrophosphate (PRPP) by UMP pyrophosphorylase (EC 2.4.2.9) to yield UMP within the cell. Although it is very difficult to distinguish group translocation from uptake followed by rapid metabolic conversion, other recent evidence (4) showing that allosteric regulation of UMP pyrophosphorylase activity in *E. coli* vesicles directly affected uracil transport supports a group translocation mechanism. However, UMP pyrophosphorylase does not appear to be involved in uracil uptake in mammalian systems (5, 17).

Uracil uptake in *Neurospora crassa* is an energy-dependent process which appears to involve two separate transport systems or a single system with negative cooperativity (7). Strains carrying the *uc-5* mutation lack the ability to transport uracil (7, 16). In this paper we report the results of studies which show that UMP pyrophosphorylase activity is present in the uracil transport-deficient mutant strain, *uc-5 pyr-1*. The specificity of uracil uptake in germinated wild-type conidia has been investigated and compared to the specificity of UMP pyrophosphorylase activity in crude extracts of germinated *uc-5 pyr-1* conidia.

MATERIALS AND METHODS

Chemicals. [2-^{14}C]uracil and Omnifluor scintillation fluid were purchased from New England Nuclear Corp. 4(3H)-pyrimidone (3-deazuracil) was obtained from Aldrich Chemical Co. Uracil and adenine were purchased from Nutritional Biochemical Co. The remaining analogs were obtained from Sigma Chemical Co., as were PRPP and ATP.

Strains. The wild-type strain 74A and the mutant strain *uc-5 pyr-1* used in these experiments were obtained from the Fungal Genetics Stock Center, Humboldt University, Arcata, Calif. Strains with the *uc-5* mutation lack the ability to transport uracil (7, 16), and *pyr-1* strains lack dihydroorotate dehydrogenase activity (3).

Media and culture conditions. Since NH_4^+ has been shown to depress the uptake of uracil in *Neurospora* (7), all media used contained NO_3^- instead of NH_4^+ as the N source. Westergaard and Mitchell medium at pH 6.5 (15) and NH_4^+-free Vogel medium (pH 6.0), a variation of Vogel minimal medium (14) in which NH_4NO_3 is replaced by 0.3% $NaNO_3$, were used. Wild-type strain (74A) was grown on Westergaard-Mitchell or NH_4^+-free Vogel medium solidified with 1.5% agar for 5 to 10 days at 30°C. The mutant strain *uc-5 pyr-1* was grown on Westergaard-Mitchell or NH_4^+-free Vogel medium with 1.5% agar, supplemented with 20 mg of uridine per 100 ml for 5 to 10 days at 30°C.

Transport assays. For the inhibition studies (see Table 1), conidia from the wild-type strain 74A were harvested from 5- to 7-day cultures and germinated in NH_4^+-free Vogel medium for 5 to 7 h at 30°C with stirring. The conidia were then centrifuged and suspended in NH_4^+-free Vogel medium to 4×10^6 conidia per ml. The assays were initiated by adding 2 ml of the conidial suspension (8×10^6 conidia) to tubes containing [2-^{14}C]uracil and NH_4^+-free Vogel minimal medium (total volume, 4 ml). Inhibitor concentrations were 1 mM, with the exception of guanine which was 0.25 mM. The incubation time was 6 min, which is on the linear portion of the time course curve (7). Uptake was stopped by rapid filtration through a glass-fiber filter (Reeve-Angel, division of Whatman, Inc.), immediately followed by a cold wash of 10 ml of NH_4-free Vogel minimal medium. After air drying, the filters were added to vials containing 10 ml of Omnifluor scintillation fluid and counted with a Beckman liquid scintillation counter.

UMP pyrophosphorylase assays. For the inhibition studies (Table 1), conidia from the mutant strain *uc-5 pyr-1* were harvested from 5- to 10-day cultures and germinated for 5 to 6 h in NH_4^+-free Vogel medium supplemented with 0.02% uridine. The conidia were collected on Metricel GA-6 membrane filters (0.45-μm pore size, 47 mm in diameter; Gelman), washed with 25 to 50 ml of NH_4^+-free Vogel medium, and resuspended in NH_4^+-free Vogel medium for an additional 1 to 2 h to deplete pyrimidine pools. The conidia were then collected by vacuum filtration on a Metricel filter, and a crude homogenate was prepared by making 35 passes of a motor-driven pestle in a 10-ml Potter-Elvehjem homogenizer containing 1 ml of 50 mM Tris-hydrochloride (pH 7.2) per 0.25 g (wet weight) of conidia. Extracts were freshly prepared for each experiment, because virtually all enzyme activity was lost when stored overnight at −20 or 4°C.

The assay used is a modification of the assay of Sabina et al. (11). Final concentrations in the assay mix were: [2-^{14}C]uracil, 20 μM (14 μCi/μmol); PRPP, 1.0 mM; ATP, 1.0 mM; $MgCl_2$, 2.0 mM; and Tris-hydrochloride (pH 7.2), 50 mM. Inhibitor concentrations were 1.0 mM. Assays were initiated by adding 20 μl of the homogenate to a final assay volume of 150 μl in 5-ml Pyrex brand conical centrifuge tubes. The assay mix was incubated at 30°C for 6 min in a shaking water bath. The reaction was stopped by gently boiling for 10 to 15 s in an open flame. The tubes were then placed on ice. After centrifugation in a clinical centrifuge at 2,000 × *g*, 100 μl of the supernatant was placed on double-thickness, 2.3-cm diameter DEAE-cellulose filter circles (Whatman, DE-81 circles). Nonphosphoribosylated [2-^{14}C]uracil was removed from the DEAE circles by washing with 10 ml of 1.0 mM ammonium formate followed by 10 ml of distilled water and 5.0 ml of 95% ethanol. After drying, the filters were added to 7.5 ml of Omnifluor scintillation fluid and counted on a Beckman liquid scintillation counter.

Crude extracts of germinating conidia from the *uc-5 pyr-1* mutant strain were used for investigating the specificity of UMP pyrophosphorylase because preparations from the wild-type strain could not be used to differentiate between phosphoribosylation of uracil alone and phosphoribosylation of uracil subsequent to uracil transport. Crude extracts of wild-type conidia contain whole cells or vesicles or both, which are capable of accumulating [2-^{14}C]uracil. When boiled, the cells lyse and the contents are released into the supernatant. Since the specificity of phosphoribosylation subsequent to transport is necessarily influenced by the specificity of the transport system in whole cells, extracts of the transport-deficient mutant strain *uc-5 pyr-1* were used to insure that the specificity observed could be attributed solely to UMP pyrophosphorylase.

Under the conditions described for Fig. 1, the phosphoribosylation of uracil is only linear for approximately 4 min, with less than 2% conversion of uracil to UMP. However, the addition of an inhibitor of 5'nucleotidase activity such as ADP or ATP was found to increase the linearity to at least 10 min with about 20% conversion of the substrate. Therefore, ATP was added to the incubation mixture for specificity studies to prevent loss of linearity, presumably by inhibiting 5'-nucleotidase activity and by stimulating nucleotide kinases. ATP alone did not stimulate the conversion of uracil to UMP in the absence of PRPP.

RESULTS

To determine whether phosphoribosylation was involved in uracil uptake in *Neurospora*, as it appears to be in *E. coli*, crude extracts of the transport-deficient mutant strain *uc-5 pyr-1* and the wild-type strain 74A were assayed for UMP pyrophosphorylase activity. Comparable UMP pyrophosphorylase activities were demonstrated in the wild-type and the *uc-5 pyr-1* mutant strain as shown in Fig. 1a. However, the *uc-5 pyr-1* mutant strain only accumulated about 2% of the amount of [2-^{14}C]uracil accumulated by the wild-type strain in 12 min.

The specificity of uracil uptake in germinating wild-type conidia of *Neurospora* was investigated by determining the ability of structural analogs of uracil to inhibit the accumulation of ^{14}C-labeled uracil by whole cells and compared to the specificity of UMP pyrophosphorylase from crude extracts of germinating conidia of the transport-deficient mutant strain *uc-5 pyr-1*. The specificity of UMP pyrophosphorylase was

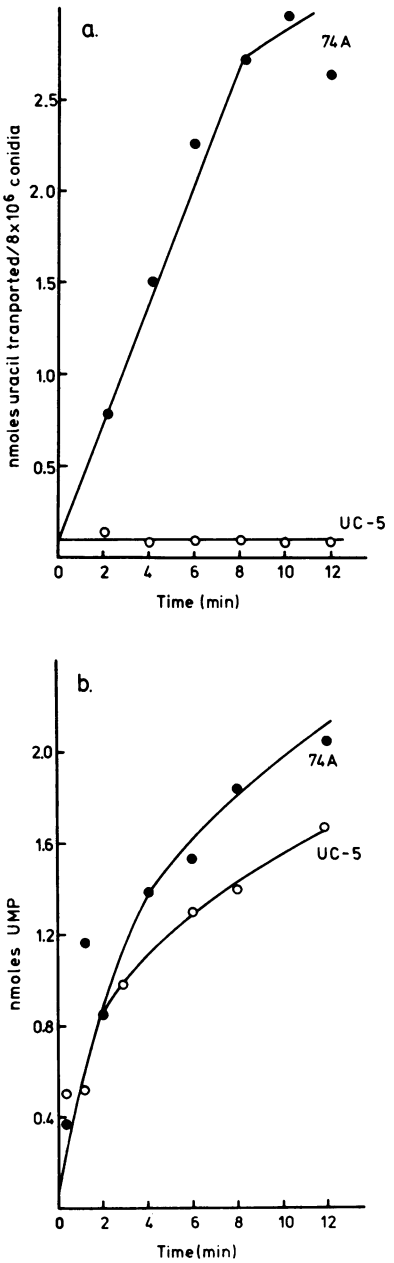


FIG. 1. *Comparison of UMP pyrophosphorylase activity and uracil uptake in germinating conidia of wild-type and uc-5 pyr-1 mutant strains of N. crassa. Wild-type 74A conidia were germinated for 7 h at 30°C in Westergaard-Mitchell medium (15). Conidia from the mutant strain uc-5 pyr-1 were germinated for 5.5 h in Westergaard-Mitchell medium (15) supplemented with 0.02% uridine followed by 1.5 h of germination in unsupplemented Westergaard-Mitchell medium. (a) Uracil uptake in wild-type (●) and the uc-5 pyr-1 mutant strain (○). Uptake was measured as described in the text, with the exception that Westergaard-Mitchell medium was used. Similar results are found by using NH_4^+-free Vogel medium. (b) UMP pyrophosphorylase activity in crude extracts of wild-type (●) and the uc-5 pyr-1 mutant strain (○). Homogenates contained 6.7×10^7 conidia: 1 ml Tris-hydrochloride (pH 7.3). The assay was conducted by incubating 10 μl of the extract in a final volume of 150 μl containing 20 μM [2-^{14}C]uracil (56 μCi/μmol), 1.33 mM $MgCl_2$, 1.33 mM PRPP, and 50 mM Tris-hydrochloride (pH 7.2). The reaction was stopped by gently boiling each tube in an open flame. [2-^{14}C]-UMP formed was determined as described in the text.*

investigated by determining the ability of structural analogs of uracil to inhibit the PRPP-dependent conversion of [2-^{14}C]uracil to [2-^{14}C]-UMP. Because uracil uptake does not exhibit Michaelis-Menten kinetics (7), true K_i values could not be calculated for the base analogs used as inhibitors of uracil transport. For this reason, the degree of inhibition of [2-^{14}C]uracil uptake by these compounds is expressed as percent inhibition. For comparative purposes, the degree of inhibition of UMP pyrophosphorylase is also expressed as percent inhibition. The relationship between inhibitor concentration, substrate concentration, K_i, K_m, and percent inhibition is described by Segel (12). The results of the inhibition studies of uracil transport and UMP pyrophosphorylase are shown in Table 1. Inhibition greater than 70% was considered to be strong, 40 to 70% was moderate, and 20 to 40% was weak. Unlabeled uracil (1 mM) gave 88% inhibition of [2-^{14}C]uracil (20 μM) uptake and 98% inhibition of UMP pyrophosphorylase activity under the described conditions. In addition to the pyrimidine analogs listed in Table 1, the purine bases adenine, xanthine, hypoxanthine (1 mM), and guanine (0.25 mM) were found to have no effect on uracil transport.

DISCUSSION

Hochstadt-Ozer and Stadtman (6) have shown that transport of uracil into *E. coli* vesicles occurs by a group translocation mechanism involving UMP pyrophosphorylase. Fast and Skold (4) recently showed that allosteric regulation of UMP pyrophosphorylase by guanine nucleotides directly affected uracil uptake, providing more evidence for a group translocation mechanism for uracil uptake in *E. coli*. However, the demonstration of UMP pyrophosphorylase activity (see Fig. 1) in the transport-deficient mutant strain of *N. crassa* strongly suggests that uracil transport in *Neurospora* is not mediated by a group translocation mechanism such as that found in *E. coli*.

Because the specificity of uracil uptake in germinating wild-type conidia of *Neurospora*

TABLE 1. *Effect of uracil analogs on uracil uptake and UMP pyrophosphorylase activity in N. crassa*[a]

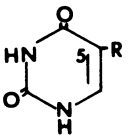
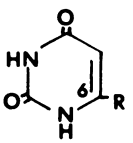
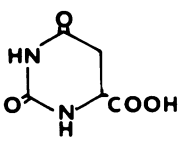
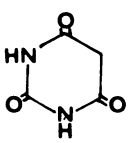
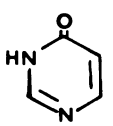
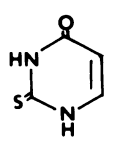
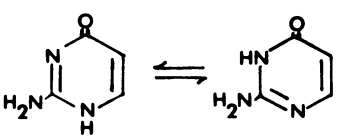
Uracil analog[b]	Structure	% Inhibition of: Uracil uptake by 74A	% Inhibition of: UMP-pyrophosphorylase in *uc-5 pyr-1*
Derived by additions to the 5 position			
	R^c		
Thymine	—CH_3	75 (71 to 79, 4)	14 (9 to 19, 2)
5-Aminouracil	—NH_2	47 (47, 2)	16 (5 to 30, 4)
Isobarbituric acid	—OH	82 (80 to 84, 2)	−8 (−11 to −6, 2)
5-Fluorouracil	—F	84 (82 to 84, 3)	96 (93 to 97, 6)
5-Bromouracil	—Br	62 (60 to 64, 2)	26 (24 to 28, 2)
5-Iodouracil	—I	51 (47 to 55, 2)	4 (0 to 9, 2)
Iso-orotic acid	—COOH	10 (8 to 13, 2)	17 (15 to 18, 2)
Derived by additions to the 6 position			
	R^c		
6-Methyluracil	—CH_3	47 (43 to 50, 2)	7 (7, 2)
6-Aminouracil	—NH_2	83 (82 to 83, 2)	25 (21 to 31, 2)
Orotic acid	—COOH	21 (17 to 24, 4)	
Dihydroorotic acid		3 (3, 2)	9 (5 to 14, 2)
Barbituric acid		12 (10 to 13, 2)	32 (29 to 35, 3)
Derived by substitution at the 2 or 4 position or both			
4-Hydroxypyrimidine [4(3H)pyrimidone]		29 (28 to 29, 2)	17 (15 to 19, 2)
2-Thiouracil		54 (51 to 57, 4)	20 (16 to 23, 3)
Isocytosine		11 (9 to 13, 2)	0 (0, 2)

TABLE 1—*Continued*

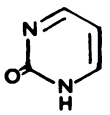
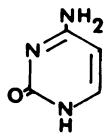
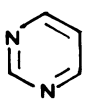
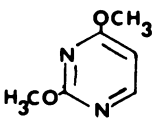
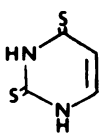
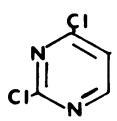
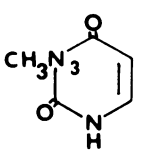
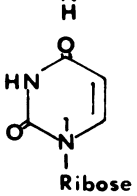
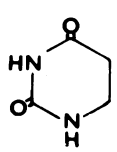
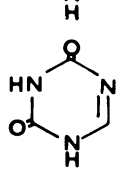
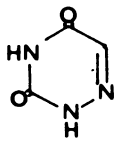
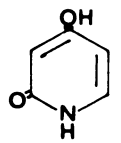
Uracil analog[b]	Structure	% Inhibition of: Uracil uptake by 74A	% Inhibition of: UMP-pyrophosphorylase in *uc-5 pyr-1*
2-Hydroxypyrimidine [2(1H)pyrimidone]		9 (5 to 13, 2)	18 (14 to 22, 2)
Cytosine		36 (35 to 37, 2)	0 (−6 to 0, 3)
Pyrimidine		11 (11, 2)	ND
2,4-Dimethoxypyrimidine		5 (2 to 8, 2)	ND
2,4-Dithiouracil		14 (14, 2)	29 (24 to 34, 2)
2,4-Dichlorouracil		10 (7 to 13, 2)	ND
Derived by additions to the 1 or 3 nitrogen			
3-Methyluracil		2 (1 to 3, 2)	−3 (−3, 2)
Uridine		20 (14 to 23, 4)	12 (12, 2)
Derived by substitution in the pyrimidine ring			
Dihydrouracil		55 (46 to 65, 4)	6 (0 to 12, 2)
5-Azauracil		78 (75 to 80, 2)	82 (73 to 91, 4)

TABLE 1—*Continued*

Uracil analog[b]	Structure	% Inhibition of: Uracil uptake by 74A	% Inhibition of: UMP-pyrophosphorylase in *uc-5 pyr-1*
6-Azauracil		55 (50 to 59, 2)	5 (2 to 8, 2)
2,4-Dihydroxypyridine (3-deazauracil)		3 (2 to 4, 2)	−8 (−32 to 16, 2)

[a] Inhibitor concentrations were 1 mM; substrate concentrations were 20 μM. Mean percent inhibitions are given, followed by the range and number of trials (in parentheses). Uracil at 1 mM causes 88% inhibition of 20 μM [2-^{14}C]uracil uptake and 98% inhibition of UMP pyrophosphorylase activity. The structures presented are in the predominant tautomeric form (2).

[b] Positions refer to pyrimidine ring.

[c] Substituent.

was investigated by determining the ability of structural analogs of uracil to inhibit the accumulation of ^{14}C-labeled uracil in whole cells (see Table 1), the inhibition of uracil uptake observed does not distinguish between inhibition of uptake occurring at the transport step and inhibition occurring at some subsequent step in the metabolism of uracil. By comparing the specificity of uracil uptake in wild-type conidia with the specificity of UMP pyrophosphorylase from crude extracts of the transport deficient mutant strain *uc-5 pyr-1*, it is possible to eliminate phosphoribosylation as an important determinant of the specificity of uracil uptake in *Neurospora*, since a much broader specificity was observed for uracil uptake than was observed for UMP pyrophosphorylase. Thymine, isobarbituric acid, 5-fluorouracil, 6-aminouracil, and 5-azauracil all strongly inhibited uracil uptake. Of these, only 5-fluorouracil and 5-azauracil strongly inhibited UMP pyrophosphorylase activity. 6-Aminouracil is only a weak inhibitor of UMP pyrophosphorylase, and thymine and isobarbituric acid did not significantly affect UMP pyrophosphorylase activity. In addition, of the analogs which moderately inhibited uracil uptake (5-aminouracil, 5-bromouracil, 5-iodouracil, 6-methyluracil, 2-thiouracil, dihydrouracil, and 6-azauracil), only 5-bromouracil and 2-thiouracil are even weak inhibitors of UMP pyrophosphorylase. Therefore, phosphoribosylation can be eliminated as a major determinant of the specificity of the system responsible for uracil accumulation in whole cells.

Strong and moderate inhibitors of uracil uptake exist in the same predominant tautomeric form (2) as uracil (Table 1). Since the 1- and 3-amino groups can serve as hydrogen donors and the 2- and 4-keto groups as hydrogen acceptors, the proper tautomeric form of a uracil analog may be critical in the recognition process, due to the formation of hydrogen bonds at an active site. Analogs which have these groups available and are weak or poor inhibitors have either a negative charge (iso-orotate, $pK_a = 2.4$; orotate, $pK_a = 2.4$; barbiturate, $pK_a = 3.9$) or a large, bulky group associated with the molecule (xanthine, iso-orotate, orotate). Although 2-thiouracil, a moderate inhibitor of uracil uptake, does not have the 2-keto group, the 2-thione is capable of forming weak hydrogen bonds with a hydrogen donor and could therefore substitute for the keto group in binding.

There is evidence to indicate that the 3 and 4 positions may play a greater role in recognition that do the 1 and 2 positions. 4-(3H)-pyrimidone, which exists predominantly as 1,6-dihydro-6-oxopyrimidine (2) and therefore differs from uracil in the absence of the oxygen at the C2 and a resultant loss of the hydrogen donor at the N1 position, demonstrates weak inhibition (32%) of uptake. (In aqueous solution, there is also a contribution from 1,4-dihydro-4-oxopyrimidine [2].) However, in the case of 2-(1H)-pyrimidone, which lacks the 4-keto group and one of the amino groups, only poor (9%) inhibition is exhibited. 3-Deazauracil which, in addition to a substitution at the 3 position, has the O4 in the enol form, causes little inhibitory effect (3%) on uracil uptake, even though the 1 and 2 positions

are in the amino and keto tautomeric forms, respectively. The lack of inhibition could be due to the inability of the enol group (a hydrogen donor) to form appropriate hydrogen bonds at an active site. The moderate inhibition observed for 2-thiouracil (53%), as opposed to the poor inhibition (14%) by 2,4-dithiouracil, also lends support to the significance of the 4-keto group in recognition. The contrast between the poor inhibition (3%) obtained with 3-methyluracil and the weak inhibition (20%) by uridine (1-ribouracil) may be due to the presence of the hydrogen at the N3 in uridine. However, the lack of commercially available uracil analogs in which only the N1 or N3 are modified limited further elucidation of the relative roles of the 1 and 3 positions.

Cytosine, which has a hydrogen donor (NH_2) at the 4 position in lieu of a hydrogen acceptor, shows weak inhibition of uptake. In the unsubstituted pyrimidine ring, the 4 and 6 positions are electronically equivalent. Therefore, cytosine can be considered as 4-amino-1,2-dihydro-2-oxopyrimidine or as 6-amino-2,3-dihydro-2-oxopyrimidine. When considered as the latter, it has the 2 and 3 positions in the proper form with no group present at the 4 position and an amino group in the 6 position (6-aminouracil demonstrates 82% inhibition). An orientation of the molecule with the amino group in the 6 position could account for the weak inhibition (36%) demonstrated with cytosine. Pyrimidine, 2,4-dimethoxypyrimidine, and 2,4-dichlorouracil, aromatic molecules which lack all four of the H-bonding groups of uracil, have little inhibitory effect on uracil uptake.

Steric constraints appear to be important in inhibition by analogs derived by addition to the 5 position of the pyrimidine ring. In general, the degree of inhibition tends to decrease as the size of the constituent at the 5 position increases. This is best illustrated by the degree of inhibition exhibited by the 5-halo derivatives. (See Table 1).

The presence of a double bond between the 5 and 6 position of the ring also appears to be important in specificity of uptake. Dihydrouracil (1.0 mM) only inhibits 20 μM [2-^{14}C]uracil uptake 55%, as opposed to 88% inhibition by 1.0 mM unlabeled uracil. Additionally, orotate, which contains the 5 to 6 double bond, is a weak inhibitor (21% inhibition), whereas dihydroorotate, in which the 5 to 6 double bond is reduced, only causes 3% inhibition.

Hypoxanthine, xanthine, adenine (all 1 mM), and guanine (0.25 mM) had little inhibitory effect on uracil uptake in germinating conidia of *N. crassa* (see Table 1). In contrast, the uracil transport system in rat intestine (13) was inhibited by hypoxanthine and xanthine as well as by 5-halo derivatives and, to a lesser degree, by guanine and uric acid. Inhibition of uracil uptake by purines was also demonstrated in *Hymenolepis* (8). However, purine bases do not inhibit uracil uptake in *E. coli* (10) and have little or no effect on uracil transport in cultured Novikoff cells (17).

It is difficult to draw analogies between the specificity of uracil uptake in *Neurospora* and uracil uptake in other organisms because few extensive studies concerning the specificity of uracil uptake in other systems have been carried out. In systems in which their effects have been investigated, 5-halo derivatives have generally been found to be good inhibitors of uracil uptake.

The present study indicates that uracil uptake in *Neurospora* occurs via a specific system which recognizes certain molecular features of uracil. The specificity of uracil uptake is not the same as that of phosphoribosylation. By comparison of the degree of inhibition demonstrated by various analogs, it is possible to make the following generalizations concerning the molecular recognition involved in uracil uptake. (i) The predominant tautomeric form of uracil analogs is an important determinant of specificity. (ii) Steric constraints are imposed by substituents at the 5 position of the pyrimidine ring. (iii) The presence of a negative charge results in the loss of recognition. (iv) The presence of the double bond between C5 and C6 appears to be important for recognition. (v) Purine bases do not inhibit uracil transport.

The presence of UMP pyrophosphorylase in the transport-deficient mutant strain *uc-5 pyr-1* strongly suggests that uracil uptake in *Neurospora* does not occur by a group translocation mechanism involving phosphoribosylation. This is not definitive proof, however, since the lack of transport in the *uc-5 pyr-1* mutant strain could be due to a misorientation of UMP pyrophosphorylase in the cell membrane or a membrane-bound isozyme not detected by our assay. Further elucidation of the utilization of exogenous uracil by *Neurospora* is necessary before a definitive statement of the mechanism of uracil uptake in *Neurospora* can be made.

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