Phosphorylation of Uridine and Cytidine Nucleoside Analogs by Two Human Uridine-Cytidine Kinases

AN R. VAN ROMPAY, AMELI NORDA, KARIN LINDÉN, MAGNUS JOHANSSON, and ANNA KARLSSON

Division of Clinical Virology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden

This paper is available online at http://molpharm.aspetjournals.org

ABSTRACT

Uridine-cytidine kinases (UCK) have important roles for the phosphorylation of nucleoside analogs that are being investigated for possible use in chemotherapy of cancer. We have cloned the cDNA of two human UCKs. The ≈30-kDa proteins, named UCK1 and UCK2, were expressed in *Escherichia coli* and shown to catalyze the phosphorylation of Urd and Cyd. The enzymes did not phosphorylate deoxyribonucleosides or purine ribonucleosides. UCK1 mRNA was detected as two isoforms of ≈1.8 and ≈2.7 kb. The 2.7-kb band was ubiquitously expressed in the investigated tissues. The band of ≈1.8 kb was present in skeletal muscle, heart, liver, and kidney. The two isoforms of UCK2 mRNA of 1.2 and 2.0 kb were only detected in placenta among the investigated tissues. The genes encoding UCK1 and UCK2 were mapped to chromosome 9q34.2-9q34.3 and 1q22-1q23.2, respectively. We tested 28 cytidine and uridine nucleoside analogs as possible substrates of the enzymes. The enzymes phosphorylated several of the analogs, such as 6-azauridine, 5-fluorouridine, 4-thiouridine, 5-bromouridine, N^4-acetylcytidine, N^4-benzoylcytidine, 5-fluorocytidine, 2-thiocytidine, 5-methylcytidine, and N^4-anisoylcytidine. The cloning and recombinant expression of the two human UCKs will be important for development of novel pyrimidine ribonucleoside analogs and the characterization of their pharmacological activation.

Uridine-cytidine kinase (UCK) (EC 2.7.1.48) is a pyrimidine ribonucleoside kinase that catalyzes the phosphorylation of uridine and cytidine to UMP and CMP. The enzyme also catalyzes the phosphorylation of several cytotoxic ribonucleoside analogs that have been investigated for possible use as chemotherapeutic agents for treatment of cancer. The nucleoside analogs are dependent on phosphorylation for their pharmacological activity. Once phosphorylated, the compounds interfere with vital cellular processes such as DNA or RNA synthesis or inhibit enzymes involved in nucleotide synthesis (Cihak and Rada, 1976). The ribonucleoside analogs phosphorylated by UCK include 5-fluorouridine, 5-azacytidine, and cyclopentenyl cytosine/-uracil as well as the recently developed 1-(3-*C*-ethynyl-β-D-ribo-pentofuranosyl)-cytosine/-uracil (Sköld, 1960; Lee et al., 1974; Vesely, 1985; Kang et al., 1989; Hattori et al., 1996; Tabata et al., 1997; Takatori et al., 1999; Verschuur et al., 2000). In addition to the ribonucleoside analogs, UCK may also be important for the pharmacological activation of uridine and cytidine base analogs, such as the clinically used analog 5-fluorouracil. This compound may be converted to 5-fluorouridine by uridine phosphorylase that subsequently will

be dependent on UCK catalyzed phosphorylation (Reichard and Sköld, 1957; Reichard and Sköld, 1958).

Most of the nucleoside analogs are dependent on phosphorylation to their triphosphate form for pharmacological activity. After the first phosphorylation catalyzed by UCK, pyrimidine ribonucleoside analogs are further phosphorylated by UMP-CMP kinase (Van Rompay et al., 1999) and nucleoside diphosphate kinases (Parks and Agarwal, 1973). However, the first phosphorylation step catalyzed by UCK is considered rate-limiting and the level of UCK activity may be correlated with the cellular sensitivity to the nucleoside analogs (Reichard and Sköld, 1958; Anderson and Brockman, 1964). Loss of UCK activity is also seen in cells resistant to the nucleoside analogs (Reichard et al., 1959; Vesely et al., 1971; Greenberg et al., 1977). Interestingly, several studies suggest that UCK activity may increase in tumor cells compared with normal tissues (Reichard et al., 1959; Herzfeld and Raper, 1979; Shen et al., 1998).

Mammalian UCKs have been purified from several different tissues (Reichard and Sköld, 1957; Krystal and Webb, 1971; Anderson, 1973; Cihak and Rada, 1976; Absil et al., 1980). However, the only mammalian UCK cDNA yet cloned is a cDNA isolated from mouse (Ropp and Traut, 1996). We decided to identify and clone human uridine-cytidine kinases to study these enzymes for the pharmacological activation of nucleoside analogs. Our findings show that a UCK enzyme

---

ABBREVIATIONS: UCK, uridine-cytidine kinase; kb, kilobase pairs(s); bp, base pair(s); BSA, bovine serum albumin.

family with at least two members exists in humans cells. We have further expressed the two enzymes recombinantly and have investigated their ability to catalyze the phosphorylation of several pyrimidine nucleoside analogs.

Experimental Procedures

Cloning and Expression of Human UCK1 and UCK2 cDNA. We searched the expressed sequence tag library of the GenBank database at the National Institute for Biotechnology Information (*http://www.ncbi.nlm.nih.gov/*) with the Basic Local Alignment Search Tool (BLAST) to identify human cDNA clones that encoded protein homologs to the cloned mouse UCK (Ropp and Traut, 1996). The expressed sequence tag cDNA clones were obtained from Research Genetics Inc (Huntsville, AL). The DNA sequences of the plasmids were determined with the ABI Prism 310 Genetic Analyzer (PerkinElmer/Applied Biosystems, Norwalk, CT) and with the automatic laser fluorescent sequencer (Amersham Pharmacia Biotech, Piscataway, NJ).

The full-length human UCK1 was amplified using polymerase chain reaction (5′-CGAGATGGCTTCGGCGGGGAG and 5′-GTGTCCCAG-CAAGTTGAGTCTG-AGTG) from a fetal brain cDNA library (CLONTECH, Palo Alto, CA). The cDNAs were expressed with an *N*-terminal polyhistidine tag in the pET-15b vector (Novagen, Madison, WI). Oligonucleotide primers that flanked the open reading frame of the human UCK1 (5′-ACATATGGCTTCGGCGGGGAGGCG and 5′-CTCTCGAGT-CAGTGGGG-TCTGCTGCTGGA) and UCK2 (5′-AACATATGGC-CGGGGACAGCGAGCAG   and   5′-CTCTCGAGTCAA-TGCCGGCCTGCTGCTGGA) cDNAs were designed with engineered *Nde*I and *Xho*I restriction enzyme sites. The polymerase chain reaction-amplified DNA fragments were cloned in the *Nde*I-*Xho*I sites of the pET-15b vector.

The expression plasmid vector were transformed into the *Escherichia coli* strain BL21(DE3) (Novagen). A transformed colony was inoculated in LB medium supplemented with 100 μg/ml ampicillin. Protein expression was induced with 1 mM isopropylthio-β-D-galactoside at $A_{595} = 0.9$ for 4 h. The bacteria were harvested by centrifugation at 5,000g for 10 min and resuspended in a buffer containing 50 mM NaH$_2$PO$_4$ and 300 mM NaCl. The bacteria were lysed by the addition of 1 mg/ml lysozyme and by sonication. The protein extracts were cleared by centrifugation at 12,000g for 20 min. The crude extracts were loaded onto a Talon Metal affinity resin column (CLONTECH). The purified recombinant proteins were eluted in a 50 mM NaH$_2$PO$_4$/300 mM NaCl buffer, pH 7.0, supplemented with 150 mM imidazole (Sigma, St. Louis, MO). The size and purity of the recombinant proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PhastSystem; Amersham Pharmacia Biotech). The protein concentrations were determined with Bradford Protein Assay (Bio-Rad, Hercules, CA). Bovine serum albumin (BSA) was used as the concentration standard. The proteins were aliquoted and stored at −80°C with 10 mM dithiothreitol and 0.5 mg/ml BSA.

Enzyme Assays. The nucleosides, nucleoside analogs, nucleoside triphosphates, and deoxynucleoside triphosphates were obtained from Sigma and ICN Biomedicals Inc (Costa Mesa, CA). Tetrahydrouridine was obtained from CN Biosciences, Inc. (San Diego, CA). [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Amersham Pharmacia Biotech. Enzyme assays were performed on fresh aliquots of UCK1 and UCK2. For substrate screening, the nucleosides and their analogs were added at a final concentration of 100 μM in a 10-μl reaction mixture containing 50 mM Tris-HCl, pH 7.6, 5 mM MgCl$_2$, 1 mM unlabeled ATP, 1 μCi of [γ-^{32}P]ATP and 5 ng of enzyme. The reaction mixtures were incubated 30 min at 37°C. Two μl of the reaction mixtures were spotted on poly(ethyleneimine)-cellulose F chromatography sheets (Merck Inc., Whitehouse Station, NJ) and the nucleosides were separated in a buffer containing NH$_4$OH/isobuturic acid/distilled H$_2$O (1:66:33, v/v). The thin-layer chromatogra-

phy sheets were autoradiographed and quantified using a FujiX BAS 1000 Bio-imaging Analyzer System (Fuji, Tokyo, Japan).

UCK1 and UCK2 activity was measured using a radiochemical method (Ives et al., 1969; Ahmed, 1981). Briefly, the assays were performed in 50 mM Tris, pH 7.6, 100 mM KCl, 5 mM MgCl$_2$, 15 mM NaF, 5 mM dithiothreitol, 0.5 mg/ml BSA, 5 mM ATP and 1 μM [5-^{3}H]Cyd (21.5 Ci/mmol; PerkinElmer Life Sciences, Boston, MA) or 1 μM [5-^{3}H]Urd (26 Ci/mmol; Amersham Pharmacia Biotech). Nucleoside and enzyme were added to a total reaction volume of 50 μl. The amount of enzyme added to the reaction was adjusted so that no more than 20% of the substrate was consumed during the incubation period. The nucleoside concentration ranged from 1 μM to 2 mM. At 0, 10, 20, and 30 min of incubation at 37°C, 10 μl of the reaction mixtures were spotted on Whatman DE-81 filters. The filters were dried and washed three times in 5 mM ammonium formate, once in distilled water, and once with 95% ethanol. The filter bound monophosphates were eluted with 0.1 M KCl and 0.1 M HCl. Radioactivity was determined using a scintillation counter. All assays were at least performed in triplicate. Donor specificity of UCK1 and UCK2 was determined with the radiochemical method as described above. The NTP concentrations were 5 mM and substrate concentrations were at the K_m values of Cyd and Urd.

Northern Blot. cDNA probes of the human UCK1 (bp 5–836) and UCK2 (bp 19–802) were labeled with [α-^{32}P]dCTP (6000 Ci/mmol, QuickPrime; Amersham Pharmacia Biotech). The labeled probes were hybridized to a human multiple tissue Northern blot (CLONTECH) with poly(A)$^+$ RNA of 12 different human tissues using ExpressHyb hybridization solution (CLONTECH) as described in the manufacturer's protocol.

Chromosome Mapping. We searched the sequence tagged sites library of the GenBank database at the National Institute for Biotechnology Information (*http://www.ncbi.nlm.nih.gov/*) with the Basic Local Alignment Search Tool (BLAST) to identify gene markers for the human UCK1 and UCK2 sequences. The cytogenetic locations of UCK1 and UCK2 gene markers were obtained from the Genome database (*http://www.gdb.org*).

Results

cDNA Cloning of Two Human UCK. Human and mouse expressed sequence tag cDNA clones homologs to the cloned mouse UCK cDNA (Ropp and Traut, 1996) were identified in GenBank. Two distinct clusters of cDNA sequences were identified as two separate enzymes present in both human and mouse cDNA libraries. The cDNAs most similar to the cloned mouse enzyme were named UCK1 (I.M.A.G.E. clone ID 2783059, 1401966, 877609, and 486302) and the remaining cDNA clones were named UCK2 (I.M.A.G.E. clone ID 1340011, 1160750, 1713319, 2178111, 642042, 1225388, 350272, and 846324) (Lennon et al., 1996). The longest open reading frame of UCK1 encoded a 277-amino-acid protein with a predicted molecular mass of 31 kDa. The enzyme was 92% identical to the cloned mouse cDNA at the amino acid level. The longest open reading frame of human UCK2 encoded a 261-amino-acid protein with a predicted molecular mass of 29 kDa. The mouse homolog of UCK2 was 98% identical to the human sequence at the amino acid level. We aligned the sequences of human and mouse UCK1 and UCK2 with the UCKs of *Caenorhabditis elegans* and *Escherichia coli* (Fig. 1). Human UCK1 and UCK2 were 72% similar, and the enzymes were ≈37 and ≈28% similar to UCK of *C. elegans* and *E. coli*, respectively.

Expression and Characterization of Recombinant Human UCK. We expressed the human UCK1 and UCK2 cDNA in *E. coli* and purified the recombinant enzymes. SDS-

polyacrylamide gel electrophoresis showed a major band of ≈30 kDa for both enzymes (Fig. 2). To verify the enzymatic activity of the recombinant enzymes, we tested the naturally occurring ribo- and deoxyribonucleosides (Fig. 3). We used ATP as the phosphate donor in the assays, because studies on tissue purified UCK indicate that it is the preferred donor (Anderson, 1973; Cihak and Rada, 1976; Ropp and Traut, 1998). Both enzymes efficiently phosphorylated Urd and Cyd. No phosphorylation of Ado, Guo, or any deoxyribonucleosides was detected for either enzyme.

Urd and Cyd phosphorylation followed Michael-Menten kinetics (data not shown). UCK1 exhibited a K_m value of 0.3 mM for both Urd and Cyd, whereas the K_m values for these substrates was 4- to 6-fold lower for UCK2 (Table 1). UCK2 exhibited a V_{max} value severalfold higher than that of UCK1. The V_{max} value for Cyd was 2-fold higher than the V_{max} for Urd for both UCK1 and UCK2. The efficiency of the different substrates, calculated as k_{cat}/K_m, showed that Urd and Cyd were more efficient substrates for UCK2 compared with UCK1.

We also tested different phosphate donors for activity in UCK1 and UCK2 catalyzed phosphorylation. At 5 mM, both ATP and GTP were efficient phosphate donors, whereas no activity was detected with CTP or UTP (data not shown).

Phosphorylation of Nucleoside Analogs. In addition to the natural substrates, we also studied the phosphorylation of cytidine and uridine nucleoside analogs (Fig. 4). We tested 14 uridine and 14 cytidine base-substituted nucleoside analogs for phosphorylation activity at 100 μM concentration. The phosphorylation activity and a map of the base substituents are shown in Table 2. We considered nucleoside analogs that phosphorylated more than 5% of uridine to be substrates of UCK.

Several of the nucleoside analogs tested in the present study were substrates of both UCK1 and UCK2. Among the aza-substituted analogs, 6-azauridine was a good substrate for both UCK1 and UCK2, whereas 6-azacytidine was phos-

phorylated only by UCK2. 5-Azacytidine was not a substrate of either UCK1 or UCK2. For Cyd nucleoside analogs, substituents at N4-position were generally well-tolerated for phosphorylation catalyzed by both UCK1 and UCK2. Also, analogs with bulky substituents, such as a benzoyl group, at this position were efficiently phosphorylated. Similarly, a thio substitution at the 4-position on Urd was well tolerated. Halogenated substituents at the 5-position of the uridine and
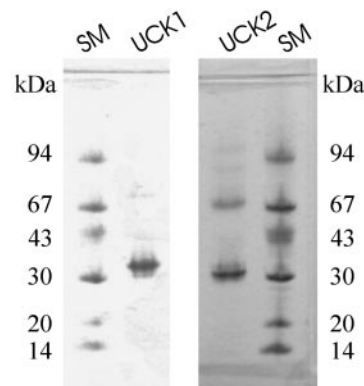

Fig. 2. SDS-polyacrylamide gel electrophoresis of purified recombinant human UCK1 and UCK2. SM, size marker.
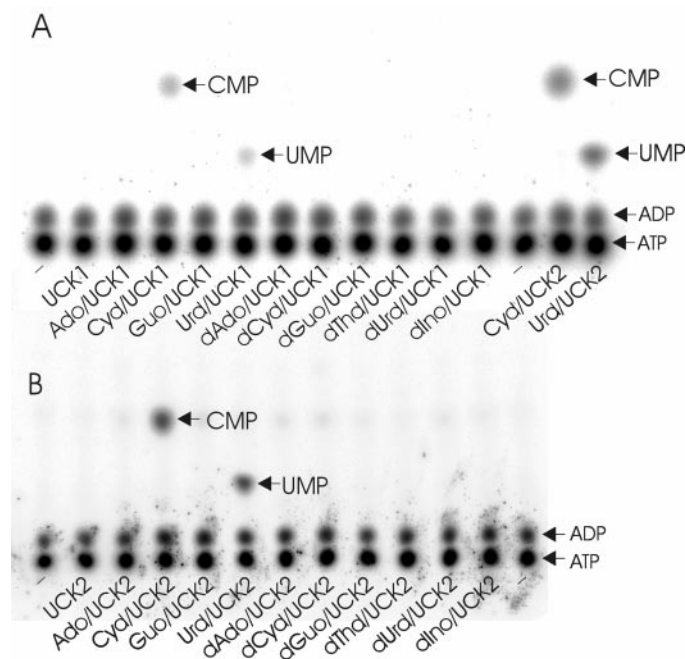

Fig. 3. Screening of substrate specificity of human recombinant UCK1 (A) and UCK2 (B). The substrate concentration was 100 μM and ATP was used as phosphate donor. −, negative control, mixture containing no enzyme and no substrate. UCK1/UCK2, mixture containing enzyme but no substrate.
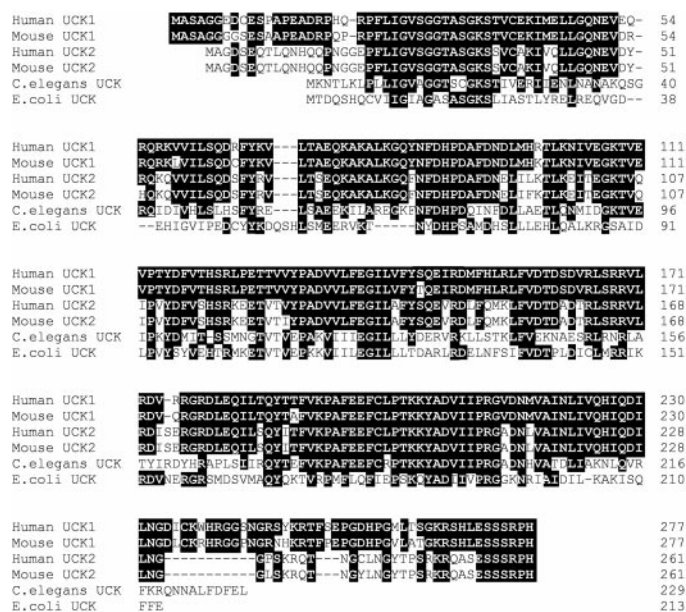

Fig. 1. Alignment of the predicted amino acid sequences of human UCK1, mouse UCK1, human UCK2, mouse UCK2, *E. coli* UCK, and *Saccharomyces cerevisiae* UCK. Black boxes indicate conserved amino acid residues compared with human UCK1.

TABLE 1
Kinetic properties of the recombinant UCK1 and UCK2 with ATP as donor. k_{cat} was calculated using a molecular mass of 31 kDa for UCK1 and 29 kDa for UCK2.

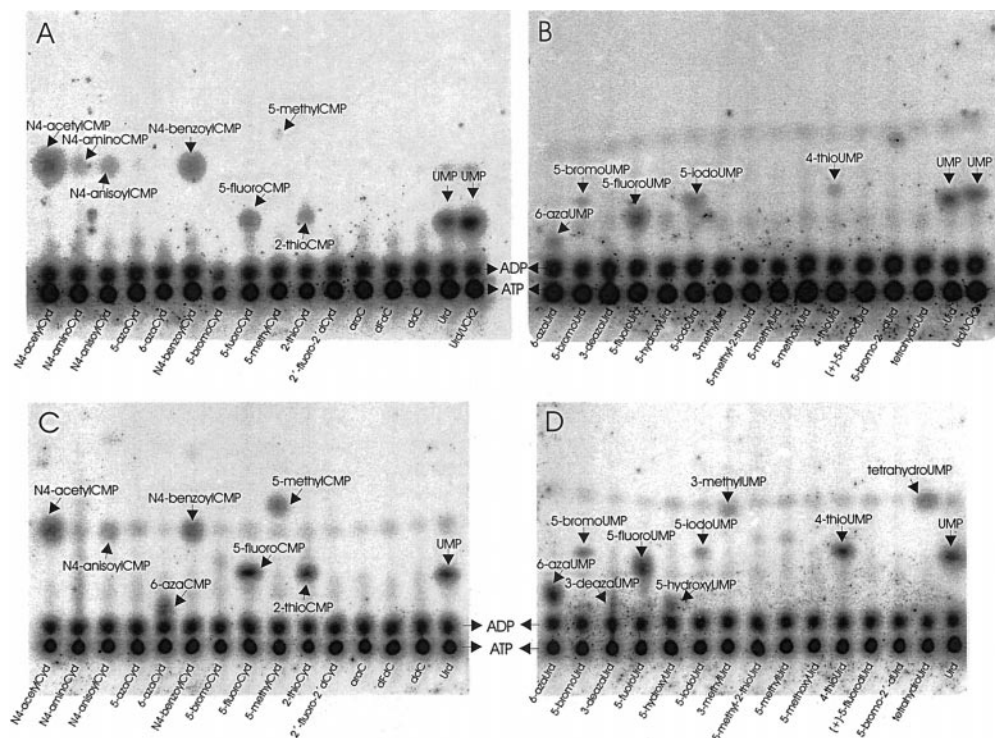| Substrate	K_m		V_{max}		k_{cat}/K_m	
	UCK1	UCK2	UCK1	UCK2	UCK1	UCK2
	μM		$\mu mol/mg/min$		$s^{-1} M^{-1}$	
Urd	268	50	0.6	2.7	1.2×10^3	26×10^3
Cyd	291	86	1.1	6.5	2.0×10^3	37×10^3

Fig. 4. Screening of nucleoside analog specificity of human recombinant UCK1 and UCK2 with cytidine analogs (A and C, respectively) and with uridine analogs (B and D, respectively). The substrate concentration was 100 μM and ATP was used as phosphate donor. The analogs from A and B are incubated with UCK1, except in the last lane, we performed Urd with UCK2 (Urd/UCK2) as control. An analog phosphorylated less than 5% of uridine phosphorylation was not labeled. araC, 1-β-D-arabinofuranosylcytosine; dFdC, 2′,2′-difluorodeoxycytidine; ddC, 2′,3′-dideoxycytidine.

cytidine base also resulted in efficient phosphorylation. However, some substitutions at this position resulted in a different activity pattern depending on whether the substitutions were on Cyd or Urd. A 5-bromo substitution of cytidine reduced the phosphorylation, whereas 5-bromouridine was efficiently phosphorylated. In contrast, 5-methyl substituted cytidine retained its activity, but not 5-methyl substituted uridine. With 5-OH or 5-I substituents on uridine, the UCKs kept their activity, but the activity was decreased with a 5-methoxy substitution. The sugar-modified cytidine nucleoside analogs 1-β-D-arabinofuranosylcytosine, 2′,2′-difluorodeoxycytidine and 2′,3′-dideoxycytidine were not substrates of either enzyme.

Northern Blot Analysis. We used a multiple tissue Northern blot to study the expression pattern of human UCK1 and UCK2 mRNAs (Fig. 5). UCK1 mRNA was detected as two isoforms of $\approx$1.8 and $\approx$2.7 kb. The 2.7-kb band was ubiquitously expressed in the investigated tissues, with high level of expression in liver, kidney, skeletal muscle, and heart, whereas low levels were present in brain, placenta, small intestine, and spleen. The band of $\approx$1.8 kb was detected in skeletal muscle, heart, liver, and kidney. In contrast, UCK2 mRNA was only detected in placenta as two transcripts of $\approx$1.2 and $\approx$2.0 kb. We were not able to detect UCK2 mRNA in any other tissue even after extended exposure times (data not shown).

Chromosome Mapping and Gene Structure. Human sequence-tagged site sequence markers identical to human UCK1 and UCK2 cDNAs were identified. The marker for UCK1 (SHGC-10187) was localized to chromosome 9q34.2-9q34.3. The UCK2 marker (SHGC-35183) was localized to chromosome 1q22-1q23.2. We also identified partially sequenced human genomic clones in the GenBank database, which contained the UCK1 and UCK2 genes (clone RP11–40A7 and RP11–7G12, RP11–525G13). Sequence analysis

showed that the UCK1 gene was divided into seven exons distributed over $\approx$7 kb (data not shown). The coding sequence of UCK2, from bp 116 of the cDNA sequence, was similarly divided into seven exons distributed over $\approx$19 kb (data not shown). The genomic sequence corresponding to bp 1–116 of the UCK2 cDNA sequence was, however, not available in the GenBank database.
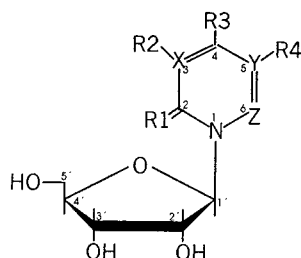
Discussion

We have cloned and recombinantly expressed the cDNA of two human uridine-cytidine kinases and characterized the enzymes for phosphorylation of nucleosides and nucleoside analogs. The kinetic properties of both UCK1 and UCK2 for Cyd and Urd phosphorylation are similar to those reported for enzymes purified from several sources (Sköld, 1960; Anderson, 1973; Cihak and Rada, 1976), and we are therefore convinced that the recombinant enzymes are useful tools to study the phosphorylation of nucleoside analogs in vitro.

UCK activity has been detected in most investigated tissues (Herzfeld and Raper, 1979; Shen et al., 1998). These data are in agreement with ubiquitous expression of human UCK1 mRNA. In contrast to the expression pattern of UCK1, we only detected UCK2 mRNA in placenta. Interestingly, a fragment of human UCK2 cDNA has previously been cloned in a study using differential display to isolate testis-specific transcripts (Ozaki et al., 1996). A cDNA sequence similar to UCK2 has also been shown to detect mRNA expression in tissue of rat brain (Yuh et al., 1999), although we were unable to detect UCK2 expression in human brain. We do not know the physiological importance of the tissue-restricted expression of UCK2 or of the differences in expression reported for different species. It is possible that UCKs may be differentially expressed at different stages of development or that other regulatory mechanisms influence the expression.

TABLE 2

Phosphorylation of uridine and cytidine nucleoside analogs by UCK1 and UCK2. For the analogs, only the substituents are indicated. The phosphorylation efficiency (%) of the analogs are correlated to the efficiency of uridine phosphorylation catalyzed by UCK1 and UCK2, respectively. Analogs that phosphorylated less than 5% of uridine phosphorylation were not considered substrates of UCKs.

Analogs	Substituents							% Phosphorylation	
	X	Y	Z	R1	R2	R3	R4	UCK1	UCK2
Uridine	N	C	C	O	H	O	H	100	100
3-Deazauridine	C					OH		<5	49
6-Azauridine			N					38	148
5-Methyl-2-thiouridine				S			CH_3	<5	<5
3-Methyluridine					CH_3			<5	8
3-,4-,5-,6-TetrahydroUrd						OH + H	H + H	<5	18
4-Thiouridine						S		13	81
5-Fluorouridine							F	122	125
5-Hydroxyuridine							OH	<5	89
5-Bromouridine							Br	14	12
5-Iodouridine							I	50	6
5-Methyluridine							CH_3	<5	<5
5-Methoxyuridine							OCH_3	<5	<5
Cytidine	N	C	C	O		NH_2	H	122	92
5-Azacytidine		N						<5	<5
6-Azacytidine			N					<5	75
2-Thiocytidine				S				20	86
N^4-Acetylcytidine						$NHCOCH_3$		142	84
N^4-Benzoylcytidine						$NHCOC_6H_5$		68	51
N^4-Anisoylcytidine						$NHCOC_6H_4OCH_3$		20	12
N^4-Aminocytidine						$NHNH_2$		20	<5
5-Fluorocytidine							F	37	184
5-Methylcytidine							CH_3	9	30
5-Bromocytidine							Br	<5	<5

Kinetic properties have been reported for UCK purified from several sources (Sköld, 1960; Anderson, 1973; Cihak and Rada, 1976). Most studies have been performed on murine ascites tumors, calf thymus, human colon, and murine liver

Fig. 5. Northern blot analysis of UCK1 and UCK2 mRNA expression in human tissues. The Northern blot analysis showed that two mRNA isoforms of UCK1 were detected at ≈1.8 and ≈2.7 kb. The ≈2.7 kb band was ubiquitously expressed in human tissues (A). The UCK2 cDNA probe hybridized with two mRNA species at ≈1.2 and 2.0 kb present only in placenta (B). PBL, peripheral blood leukocyte.

and kidney; to our knowledge, however, no study has been performed on such UCK2-expressing tissues as placenta and testis (Sköld, 1960; Lee et al., 1974; Fulchignoni-Lataud et al., 1976; Ahmed, 1984).

Several of the nucleoside analogs tested in the present study were substrates of both UCK1 and UCK2, although for some of the compounds, there was a difference in phosphorylation efficiency between UCK1 and UCK2. 3-Deazauridine, 5-hydroxyuridine, and 6-azacytidine were phosphorylated >10-fold more efficiently by UCK2 compared with UCK1. This would indicate a higher toxicity for these compounds in tissues expressing UCK2 but, to our knowledge, there has been no report of nucleoside analog selectivity for a specific tissue expressing either UCK1 or UCK2.

Several pyrimidine ribonucleoside analogs like 6-azauridine (Pasternak et al., 1961; Vesely and Cihak, 1973), 5-azacytidine (Cihak and Broucek, 1972; Vesely and Cihak, 1973), 5-hydroxyuridine (Smith and Visser, 1965), and 4-thiouridine (Lindsay and Yu, 1974) exert the pharmacological effects in their monophosphate forms by inhibiting OMP decarboxylase, in addition to their incorporation into RNA or DNA (Cihak and Rada, 1976). In contrast, most deoxyribonucleoside analogs are predominantly dependent on phosphorylation to their triphosphate form, and incorporation
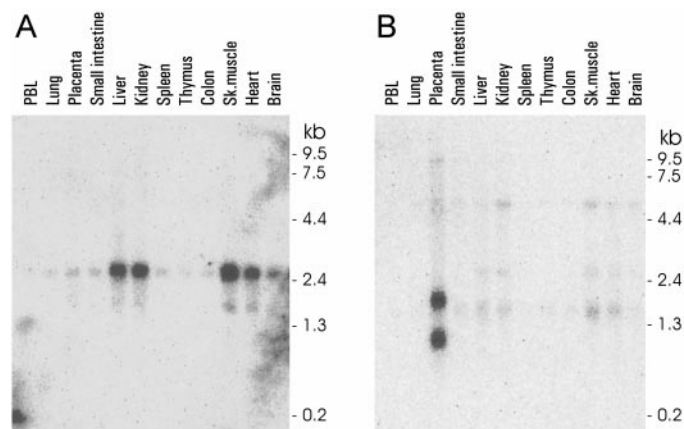
into DNA, for pharmacological activity. However, the deoxyribonucleoside analog 5-fluorodeoxyuridine is also active as a monophosphate derivative through its inhibition of thymidylate synthase, a target that subsequently causes inhibition of DNA synthesis. In conclusion, the targets for deoxyribonucleoside analogs mainly affect DNA synthesis and are therefore different compared with the molecular targets for ribonucleoside analogs. The different mode of action of ribonucleoside analogs makes this group of compounds interesting for further studies to develop clinically useful drugs. Promising results in animal tumor models have recently been shown for 1-(3-C-ethynyl-β-D-ribo-pentofuranosyl)-cytosine and -uracil (Takatori et al., 1999). Cyclopentyl cytosine is another interesting compound with anti-tumor activity that presently is evaluated in clinical trials (Verschuur et al., 2000). Characterization of the UCKs involved in the pharmacological activation of the pyrimidine ribonucleoside analogs will be important for further development of this therapeutic strategy.

References

Absil J, Tuilie M and Roux JM (1980) Electrophoretically distinct forms of uridine kinase in rat. Tissue distribution and age-depenence. *Biochem J* **185:**273–276.

Ahmed NK (1981) Determination of pyrimidine phosphoribosyl transferase and uridine kinase activities by an assay with DEAE paper discs. *J Biochem Biophys Methods* **4:**123–130.

Ahmed NK (1984) Enzymes of the de novo and salvage pathways for pyrimidine biosynthesis in normal colon, colon carcinoma and xenografts. *Cancer* **54:**1370–1373.

Anderson EP and Brockman RW (1964) Feedback inhibition of uridine kinase by cytidine triphosphate and uridine triphosphate. *Biochim Biophys Acta* **91:**380–386.

Anderson EP (1973) Nucleoside and nucleotide kinases, in *The Enzymes*, 3rd ed, vol 9 (Boyer PD ed) pp 49–96, Academic Press, New York.

Cihak A and Broucek J (1972) Dual effect of 5-azacytidine on the synthesis of liver RNA. Lack of the relationship between metabolic transformation of orotic acid *in vitro* and its incorporation *in vivo*. *Biochem Pharmacol* **21:**2497–2507.

Cihak A and Rada B (1976) Uridine Kinase: Properties, biological significance and chemotherapeutic aspects. *Neoplasma* **23:**233–257.

Fulchignoni-Lataud M-C, Tuilie M and Roux J-M(1976) Uridine-cytidine Kinase from foetal and adult rat liver: purification and study of some properties. *Eur J Biochem* **69:**217–222.

Greenberg M, Shum DE and Wee TE (1977) Uridine kinase activities and pyrimidine nucleoside phosphorylation in fluoropyrimidine-sensitive and -resistant cell lines of the Novikoff hepatoma. *Biochem J* **164:**379–387.

Hattori H, Tanaka M, Fukushima M, Sasaki T and Matsuda A (1996) Nucleosides and nucleotides. 158. 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)-cytosine, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil, and their nucleobase analogues as new potential multifunctional antitumor nucleosides with a broad spectrum of activity. *J Med Chem* **39:**5005–5011.

Herzfeld A and Raper SM (1979) Uridine kinase activities in developing, adult and neoplastic rat tissues. *Biochem J* **182:**771–778.

Ives DH, Durham JP and Tucker VS (1969) Rapid determination of nucleoside kinase and nucleotidase activities with tritium labeled substrates. *Anal Biochem* **28:**192–205.

Kang GJ, Cooney DA, moyer JD, Kelley JA, Kim HY, Marquez VE and Johns DG (1989) Cyclopentenylcytosine triphosphate. *J Biol Chem* **264:**713–718.

Krystal G and Webb TE (1971) Multiple forms of uridine kinase in normal and neoplastic rat liver. *Biochem J* **124:**943–947.

Lee T, Karon M and Momparler R (1974) Kinetics studies on phosphorylation of 5 azacytidine with purified uridine-cytidine kinase from calf thymus. *Cancer Res* **34:**2482–2488.

Lennon G, Auffray C, Polymeropoulus M and Soares MB (1996) The I. M. A. G. E. consortium: An integrated molecular analysis of genomes and their expression. *Genomics* **33:**151–152.

Lindsay RH and Yu MW (1974) Inhibitory effect of 2-thiouracil and 2-thiouracil metabolism on enzymes involved in the pyrimidine nucleotide biosynthesis. *Biochem Pharmacol* **23:**2273–2281.

Ozaki K, Kuroki T, Hayashi S and Nakamura Y (1996) Isolation of three testis-specific genes (TSA303/TSA806/TSA903) by a differential mRNA display method. *Genomics* **36:**316–319.

Pasternak CA, Fischer GA and Handschumacher RE (1961) Alterations in pyrimidine metabolism in L5178Y leukemia cells resistant to 6-azauridine. *Cancer Res* **21:**110–117.

Parks RE and Agarwal RP (1973) Nucleoside diphosphokinases. *Enzymes* **8:**307–334.

Reichard P and Sköld O (1957) Formation of uridine phosphates from uracil in extracts of Ehrlich ascites tumor. *Acta Chem Scand* **11:**17–23.

Reichard P and Sköld O (1958) Enzymes of uracil metabolism in the Ehrlich ascites tumour and mammalian liver. *Biochim Biophys Acta* **28:**376–385.

Reichard P, Sköld O and Klein G (1959) Possible enzymic mechanism for the development of resistance against fluorouracil in ascites tumours. *Nature (London)* 939–941.

Ropp PA and Traut TW (1996) Cloning and expression of a cDNA encoding uridine kinase from mouse brain. *Arch Biochem Biophys* **336:**105–112.

Ropp PA and Traut TW (1998) Uridine kinase: altered enzyme with decreased affinities for uridine and CTP. *Arch Biochem Biophys* **359:**63–68.

Shen F, Look KY, Yeh YA and Weber G (1998) Increased uridine kinase (ATP:uridine 5′-phosphotransferase (EC 2.7.1.48) activity in human and rat tumors. *Cancer Biochem Biophys* **16:**1–15.

Sköld O (1960) Uridine kinase from Ehrlich ascites tumor: purification and properties. *J Biol Chem* **235:**3273–3279.

Smith DA and Visser DW (1965) Studies on 5-hydroxyuridine. *J Biol Chem* **240:** 446–453.

Tabata S, Tanaka M, Endo Y, Obata T, Matsuda A and Sasaki T (1997) Anti-tumor mechanisms of 3′-ethynyluridine and 3′-ethynylcytidine as RNA synthesis inhibitors: Development and characterization of 3′-ethynyluridine-resistant cells. *Cancer Lett* **116:**225–231.

Takatori S, Kanda H, Takenaka K, Wataya Y, Matsuda A, Fukushima M, Shimamoto Y, Tanaka M and Sasaki T (1999) Antitumor mechanisms and metabolism of the novel antitumor nucleoside analogues, 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)cytosine and 1-(3-C-ethynyl-beta-D-ribo-pentofuranosyl)uracil. *Cancer Chemother Pharmacol* **44:**97–104.

Van Rompay AR, Johansson M and Karlsson A (1999) Phosphorylation of deoxycytidine analog monophosphate by UMP-CMP kinase: molecular characterization of the human enzyme. *Mol Pharmacol* **56:**562–569.

Verschuur AC, Van Gennip AH, Leen R, Muller EJ, Elzinga L, Voute PA and Van Kuilenburg AB (2000) Cyclopentenyl cytosine inhibits cytidine triphosphate synthetase in paediatric acute non-lymphocytic leukaemia: a promising target for chemotherapy. *Eur J Cancer* **36:**627–635.

Vesely J and Cihak A (1973) Resistance of mammalian tumor cells toward pyrimidine analogues. A review. *Oncology* **38:**204–226.

Vesely J (1985) Mode of action and effects of 5-azacytidine and of its derivatives in eukaryotic cells. *Pharmacol Ther* **28:**227–235.

Vesely Y, Dvorak M, Cihak A and Sorm F (1971) Biochemical mechanism of drug resistance- XII Uridine kinase from mouse leukemic cells sensitive and resistance to 5-azacytidine. *Int J Cancer* **8:**310–319.

Yuh I, Yaoi T, Watanabe S, Okajima S, Hirasawa Y and Fushiki S (1999) Up-regulated Uridine kinase gene identified by RLCS in the ventral horn after crush injury to rat sciatic nerves. *Biochem Biophys Res Commun* **266:**104–109.

Send reprint requests to: Dr. Anna Karlsson, Division of Clinical Virology, Karolinska Institute, Huddinge University Hospital, S-141 86 Stockholm, Sweden. E-mail: anna.karlsson@mbb.ki.se