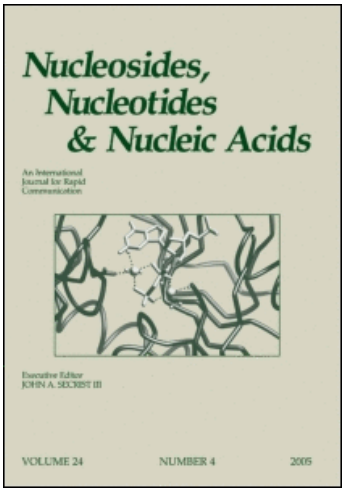

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Substrate Specificities of Mitochondrial Thymidine Kinase and Cytosolic Deoxycytidine Kinase Against 5-Aryl Substituted Pyrimidine-2'-deoxyribose Analogues

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To cite this Article Eriksson, Staffan , Wang, Jianghai , Gronowitz, Salo and Johansson, Nils Gunnar(1995) 'Substrate Specificities of Mitochondrial Thymidine Kinase and Cytosolic Deoxycytidine Kinase Against 5-Aryl Substituted Pyrimidine-2'-deoxyribose Analogues', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 507 — 510
To link to this Article: DOI: 10.1080/15257779508012414
URL: http://dx.doi.org/10.1080/15257779508012414

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 507-510 (1995)

SUBSTRATE SPECIFICITIES OF MITOCHONDRIAL THYMIDINE KINASE AND CYTOSOLIC DEOXYCYTIDINE KINASE AGAINST 5-ARYL SUBSTITUTED PYRIMIDINE-2'-DEOXYRIBOSE ANALOGUES

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Abstract: Some 5-aryl-2'-deoxyuridine and -deoxycytidine analogues, many with known antiviral activity, were evaluated as substrates for pure deoxycytidine kinase (dCK) and pure mitochondrial thymidine kinase (TK2). Some of the deoxyuridine compounds were also tested with pure cytosolic thymidine kinase (TK1). TK2 showed the highest activity with this type of analogues.

A number of 5-substituted pyrimidine nucleosides show high anti-herpes virus activity and low cellular toxicity. 5-(2-bromovinyl)-2'-deoxyuridine (BVDU) is one of the most active agents against herpes simplex type 1 (HSV-1) and varicella-zoster virus replication and it does not affect the growth of uninfected cells[1]. 5-(2-Thienyl)- and 5-(2-furanyl)-2'-deoxyuridine have been shown to have similar antiviral properties[2]. Also a series of 5-(thienyl)- and 5-(furyl)-2'-deoxycytidine compounds have shown anti-herpes activity as well as enhanced resistance to degradation[3]. A high capacity of virus encoded thymidine kinase to phosphorylate this type of analogues is primarily responsible for the antiviral selectivity observed[3]. However, although earlier studies have shown that the cytosolic and cell growth related thymidine kinase (TK1) can not use this class of nucleoside substrates, it has been reported that mitochondrial thymidine kinase (TK2) can phosphorylate BVDU[4,5].

Cellular deoxycytidine kinase (dCK) has a broad substrate specificity[5] and there is significant sequence homology between dCK and HSV1-thymidine kinase[6]. Purification protocolls for the three cellular kinases have been developed[7,8] and pure preparations of these enzymes are available in one of our laboratories. We therefore decided to determine the capacity of human dCK and human (or bovine) TK2 to phosphorylate some of these 5-substitutued dUrd and dCyd analogues. The activity with human TK1 was also tested with some of the compounds.

Chemicals: ^{32}P-ATP was obtained from Radiochemical Center Amersham, UK and the 5-substituted nucleosides were prepared by N.G. Johansson, T. Persson, D. Peters and C. Sahlberg[9,10].

Enzyme preparations: dCK, TK1 and TK2 were prepared from human leukemic spleen as described[7,8]. For the majority of the experiments TK2 was prepared from bovine brain, purified as in ref 8. Recombinant dCK, expressed in *E. coli* [8] were purified from bacterial extracts to homogeneity by two high resolution Memsep DEAE (Milipore) chromatographies. The structure and specificity's of bovine TK2 and recombinant dCK were very similar to those of the human spleen enzymes[7,8].

Enzyme assays: The nucleoside substrates were tested as phosphate acceptors with 100 uM ^{32}P -ATP as phosphate donor, 50 mM Tris-HCL, pH 7.6, 0.5 mM $MgCl_2$, 100 mM KCL and and 0.5 mg/ml bovine serum albumine at 37°C for 10 to 30 min. The products were analysed by PEI-cellulose TLC, quantitated and the kinetic constants determined as described[5].

Results and Dicussion: Table 1 shows the capacity of TK1, TK2 and dCK to phosphorylate a series of 5-substituted dUrd and dCyd nucleosides. TK1 showed, as expected from earlier studies[3,4], no activity with the tested analogues, while TK2 was able to phosphorylate this type of 5-heteroaryl compounds and in several case with activities close to those observed with Thd or dCyd. The substrate efficiency, i.e. the apparent Vmax /Km values, for TK2 with BVDU and 5-(2-thienyl)-dUrd or -dCyd were 10 and 40 times lower than that with Thd (Table 2). 5-(2-furyl)-dUrd and 5-(3-furyl)-dCyd showed similar activty as the 5-thienyl- analogues (Table 2). Earlier results with HSV1 TK showed a similar pattern of substrate preference for the viral enzyme[3]. Thus, it appears that with respect to 5-aryl pyrimidines the mitochondrial thymidine kinase has overlapping specificity with herpes thymidine kinase. If this fact is of importance in relation to any mitochondrial or

TABLE 1. Phosphorylation of nucleosides by TK1, TK2 and dCK, using 100 μM γ^{32}P-ATP as phosphate donor. The relative activity with 100 μM nucleoside as substrate is shown. The values are the means of at least two different determinations and the SD is approx ±15%. nd=not determined.

	TK1	TK2	dCK
Thd	1.0	1.0	0.02
dUrd	1.0	1.0	0.06
dCyd	≤0.01	0.70	1.0
AraU	≤0.01	0.20	≤0.01
5-(2-bromovinyl)-2'-deoxyuridine	≤0.01	0.25	0.02
5-(2-thienyl)-2'-deoxyuridine	≤0.01	0.12	nd
5-(3-thienyl)-2'-deoxyuridine	nd	0.04	nd
5-(2-furanyl)-2'-deoxyuridine	nd	0.25	nd
5-(2-thienyl)-2'-deoxycytidine	nd	0.30	0.02
5-(3-thienyl)-2'-deoxycytidine	nd	0.34	≤0.01
5-(3-furanyl)-2'-deoxycytidine	nd	0.50	≤0.01
5-(2-selenienyl)-2'-deoxyuridine	nd	0.16	≤0.01
5-(4-pyridyl)-2'-deoxycytidine	nd	0.02	0.05
5-(3-pyridyl)-2'-deoxycytidine	nd	≤0.01	0.04
5-(2-pyridyl)-2'-deoxycytidine	nd	0.07	≤0.01
5-(2-thienyl)-uracil arabinoside	≤0.01	0.06	nd

TABLE 2. Kinetic parameters for some 5-aryl substituted analogues with TK2, using the phospho-transfer assay

	Km (μM)	Vmax (nmoles/min/mg)	Vmax/Km
Thd	1	200	200
BVDU	2	40	20
5-(2-thienyl)-2'-deoxyuridine	9	44	4.9
5-(2-furyl)-2'-deoxyuridine	6	16	2.6
5-(2-thienyl)-2'-deoxycytidine	8	54	6.8
5-(3-furyl)-2'-deoxycytidine	21	130	6.1

other cellular toxicicity remains to be determined. Interestingly, many of these dCyd compounds were phosphorylated by TK2 but not by dCK.

However, the activity of TK2 is not directly predictive of the anabolism of an analogue, e.g. the triphosphate of 5-(2-thienyl)-deoxyuridine is a potent inhibitor of HIV-reverse transcriptase[9,10], yet there is no anti-HIV activty of the nucleoside in cell culture[10]. Since this analogue is a good TK2 substrate and thus the monophosphate should be formed there is most likely a block in the further phosphorylation and/or transport of the nucleotide in the cells.

Acknowledgement: This work was supported by grants from The Swedish Medical Research Council, The Swedish Natural Science Research Council, Medivir AB, Huddinge, Sweden and The Swedish Technical Board of Development.

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