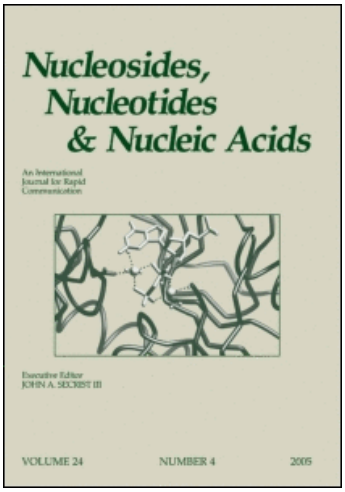

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Enantioselectivity of the Cellular Deoxynucleoside Kinases

Jianghai Wang[a]; Jyoti Chattopadhyaya[b]; Staffan Eriksson[a]
[a] Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, The Biomedical Center, Uppsala, Sweden [b] Department of Bioorganic Chemistry, Uppsala University, The Biomedical Center, Uppsala, Sweden

To cite this Article Wang, Jianghai , Chattopadhyaya, Jyoti and Eriksson, Staffan(1999) 'The Enantioselectivity of the Cellular Deoxynucleoside Kinases', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 807 — 810
To link to this Article: DOI: 10.1080/15257779908041567
URL: http://dx.doi.org/10.1080/15257779908041567

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 807–810 (1999)

THE ENANTIOSELECTIVITY OF THE CELLULAR DEOXYNUCLEOSIDE KINASES.

Jianghai Wang[1], Jyoti Chattopadhyaya[2] and Staffan Eriksson[1]*

[1]Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, The Biomedical Center, Box 575, S-75123 Uppsala, Sweden
[2]Department of Bioorganic Chemistry, Uppsala University, The Biomedical Center, Box 581, S-75123 Uppsala, Sweden

ABSTRACT: Cytosolic thymidine kinase (TK1) and deoxycytidine kinase (dCK) and the mitochondrial thymidine kinase (TK2) and deoxyguanosine kinase (dGK) phosphorylate deoxynucleosides and their analogs. Recombinant human TK1 only phosphorylated β-D Thd, but recombinant TK2, dCK and dGK all phosphorylated equally well β-D and β-L as well as to some extent α-D and α-L deoxynucleosides.

One of the most used anti-HIV nucleosides is β-L-2',3'-dideoxy-3'-thiocytidine (3TC), which has to be phosphorylated by cellular nucleoside and nucleotide kinases to be transformed into the active triphosphate inhibiting HIV-reverse transcriptase[1,2]. It was shown that deoxycytidine kinase (dCK) can carry out the initial phosphorylation of 3TC and later studies have demonstrated that this enzyme has a relaxed enantioselectivity with several substrates[3-6]. Recently, the two mitochondrial deoxynucleoside kinases, TK2 and dGK has been cloned and shown to have high sequence homology to the primary sequence of dCK[7-10]. The Herpes Simplex 1 thymidine kinase, the 3D-structure of which is known[11], also have regions with high similarity to these three cellular enzymes. However, cytosolic thymidine kinase (TK1) does not belong to this enzyme family but instead show homology to Pox virus thymidine kinases[7].

The enzymatic properties of these enzymes are different in that dCK, TK2 and dGK all are active as dimers and they have a broad substrate specificity. These enzymes are also expressed in most tissues but the dCK levels are much higher in lymphocytic cells as compared to other cells. TK1, which is only found in proliferating cells, is activated by the presence of ATP and forms a tetramer with a rather limited specificity for thymine and uridine 2´-deoxyribonucleosides[7,12].

All the four cellular kinases have been expressed and isolated in pure and highly active form from recombinant bacteria and the objective of this investigation was to determine

their activity with the β-D, β-L, α-D and α-L enantiomer of the four natural 2´-deoxynucleosides.

MATERIALS AND METHODS

The β-D deoxynucleosides were obtained from Sigma and the β-L, α-D and α-L enantiomer of the 2'-deoxynucleosides were prepared in J. Chattopadhyaya's laboratory by procedures described in reference 13 and references cited therein. The purity of the nucleoside preparations were more than 99.5% as determined by NMR spectroscopy.

Recombinant TK1 and TK2 were prepared from *E. coli* BL21 (DE3) lysates after induction using the pET-14b vector, where the entire coding sequence of human TK1 and in case of TK2 the sequence starting with amino acid 18 to the C-terminal end were present[7-9]. Recombinant dCK and dGK were prepared from induced bacteria using the pET-9d vector and the coding sequences for human dCK and dGK and in all cases the recombinant histidine tagged enzymes were purified by metal affinity chromatography as described[10,14]. The proteins were more than 95% pure and had high specific activities.

The phosphoryl transfer assay were performed as described[10] with 100 μM γ-^{32}P-ATP, 100 μM deoxynuclesoides and approximately 50 ng enzyme for 30 min at 37°C in buffers containing 50 mM Tris-HCL pH 7.6, 5 mM $MgCL_2$ 125 mM KCL and 0.5 mg/ml bovine serum albumin. The reaction products were separated and quantified by thin layer chromatography.

RESULTS AND DISCUSSION

The activity of the four recombinant cellular deoxynucleoside kinases, prepared by a one step affinity purification procedure was tested with a fixed concentration of the various deoxynucleoside enantiomers in parallel assays. The results are shown in Table 1.

TK1 showed a very limited specificity with significant activity only with β-D Thd, which is in agreement with earlier studies[12]. However, TK2 has a much broader specificity and phosphorylate all the four Thd as well as dCyd enatiomers. The activity with the α-dCyd nucleosides was a least an order of magnitude lower than that of β enantiomers but there was no selectivity against the L pyrimidine nucleosides with TK2. This lack of enantioselectivity for TK2 with regard to both its natural nucleoside substrates has not been described earlier. The capacity of dCK to phosphorylate the different deoxynucleosides is clearly the broadest, since it shows activity with all the enatiomers in case of dCyd, dGuo and dAdo. Generally, the α-nucleosides showed much lower activity, but

TABLE 1 Relative phosphorylation of deoxyribonucleoside enantiomers by recombinant human deoxynucleoside kinases.

Substrate		TK1	TK2	dCK	dGK
Thd	β-D	1	1	0,05	—
	β-L	0,02	0,95	0,20	—
	α-D	0,03	0,22	—	—
	α-L	—	0,25	—	—
dCyd	β-D	—	0,55	1	0,36
	β-L	—	0,90	0,26	2,65
	α-D		0,07	0,21	—
	α-L	—	0,08	0,08	—
dGuo	β-D	—	—	2,99	1
	β-L	—	—	0,44	0,91
	α-L	—	—	0,07	0,35
dAdo	β-D	—	0,01	2,84	2,23
	β-L	—	0,02	1,29	2,33
	α-D	0,003	—	0,05	—
	α-L	—	—	0,10	—

1- All assays were carried out with 100 μM substrate and ^{32}P-ATP.
2- The phosphorylation of substrates are expressed as the ratio of each activity in comparison with the activity of the β-D enantiomer, which was set to 1. The values are based on the mean of at least two determinations.
3- The sign "—" shows that monophosphate product was not detectable.

still there was clearly detectable phosphorylation and this capacity of dCK has to our knowledge not been demonstrated earlier. In case of the β-L-nucleosides they appeared to be 2-3 fold less efficient than the β-D substrates and this result is in accordance with those reported earlier[3-6].

The enantioselectivity of dGK was similar to that of TK2 and dCK although in this case only β-L dGuo and α-dGuo as well as β-L Ado showed high activity, while the α-dAdo nucleoside was not phosphorylated.

The results presented here demonstrate the relaxed enantioselectivity of the cellular enzymes dCK, dGK and TK2, which do belong to the same family and show homology to the Herpes virus thymidine kinase. It is known that also this viral kinase can phosphorylate several nucleosides and nucleoside analogs[11] and thus this family of enzymes most likely has an active site structure capable of interacting with the 5'-ends of many otherwise structurally different nucleosides. Further studies are aimed at defining the molecular details of the active sites of the cellular kinases, but the results presented here already may give important information for the design of new analogs with improved pharmaceutical properties.

Acknowledgement: This work was supported by grant B95-03X-06230-14C from the Swedish Medical Research Council, and a grant BMH4-CT96-0479 from the European Commision and in part from grants from The Swedish Natural Science foundation.

REFERENCES

1. Doong, S.L.; Tsai, C.N.; Schinazi, R. F.; Liotta, D.C.; Cheng, Y.-C. *Proc. Natl. Acad. Sci. USA*, **1991**, 88, 849-8499.
2. Lin, T.-S.; Lou, M.-Z; Liu, M.-C.; Pai, S.B.; Dutschman, G.E.; Cheng Y.-C. *J. Med. Chem.*, **1994**, 37, 798-803.
3. Shewach, D.S.; Liotta, D.C.; Schinazi, R.F. *Biochem Pharmacol.*, **1993**, 45,1540-11543.
4. Pélicano, H.; Pierra, C.; Eriksson, S.; Gosselin, G.; Imbach, J.-L.; Maury, G. *J. Med. Chem.* **40**, 3969-3973, 1997.
5. Shafiee, M.; Griffon,J.-F.; Gosselin, G.; Cambi, A.; Vincenzetti, S.; Vita, A.; Eriksson, S.; Gosselin, G.; Imbach, J.-L.; Maury, G. *Biochem. Pharmacol.*, in press.
6. Gaubert, G.; Gosselin, G.; Imbach, J.-L.; Eriksson, S.; Maury, G. *Nucleosides and Nucleotides,* **1998**, abstract for this issue.
7. Wang, L.; Eriksson, S. *Nucleosides and Nucleotides*, **1997**, 16, 653-659.
8. Johansson, M.; Karlsson, A. *J. Biol. Chem.* **1997**, 272, 8454-8458
9. Wang, L.; Munch-Petersen, B.; Herrström Sjöberg, A.; Bergman, T.; Jörnvall, H.; Hellman, U.; Eriksson, S., submitted.
10. Herrström Sjöberg, A.; Wang, L.; Eriksson, S. *Mol. Pharmacol.* **1998,** 53, 270-273.
11. Brown, D.G.; Visse, R.; Sandhu, G.; Davies; A.; Rizkallah, P.J.; Melitz, C.; Summers, W.C.; Sanderson, M.R. *Nature Struct. Biol.*. **1995,** 2, 876-881.
12. Arnér, E. S. J.; Eriksson, S. *Pharmac. Ther.* **1995**, 67, 155-186.
13. Thibaudeau, C.; Flödesi, A.; Chattopadhyaya, C. *Tetrahedron*, **1997**, 53, 14043-14072.
14. Usova, E.; Eriksson, S. *Eur. J. Biochem.* **248**, 762-766, 1997.