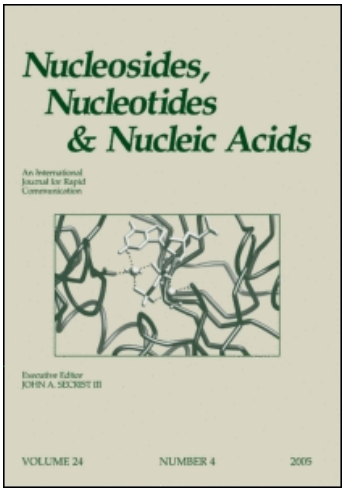

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STUDY OF THE ENANTIOSELECTIVITY OF ENZYMES INVOLVED IN NUCLEOSIDE ANALOGUE METABOLISM: DEOXYCYTIDINE KINASE

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Abstract: The substrate properties of recombinant human deoxycytidine kinase (dCK) with regard to a series of D- or L-enantiomers of cytidine , 2'-deoxycytidine, and 2',3'-dideoxycytidine analogues were studied using HPLC analysis. Our results suggest that dCK has a remarkably relaxed enantioselectivity with respect to a large number of cytidine derivatives in the β configuration.

The recent discovery of the anti-HIV activities of a number of nucleoside analogues having the unnatural L configuration has led to the study of the enantioselectivity of several key enzymes involved in their metabolism and the mechanism of inhibition. First, the target enzyme, *e.i.* HIV-Reverse Transcriptase, has been shown in most examined cases to present a relaxed enantioselectivity and to catalyze the incorporation of both D- and L-nucleoside analogues.[1, 2] The justification of the antiviral activities of β-L-2',3'-dideoxycytidine, β-L-2',3'-dideoxy-5-fluorocytidine, (2R, 5S)-1-[2-(hydroxymethyl)1,3-oxathiolane-5 yl]cytidine (3TC), (2R, 5S)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolane-5 yl]cytidine (FTC), and other L-nucleosides analogues[3-5] has been found in the results of the studies of their interaction with cellular enzymes.[1,3] These results suggest that the cellular enzymes present strict enantioselectivities except deoxycytidine kinase[4,5] and, to a certain extent, nucleotide kinases and nucleoside diphosphate kinases.[1]

In order to establish structure-activity relationships which may be useful for the search of new active analogues, we have studied the substrate properties with respect to deoxycytidine kinase of a series of cytidine derivatives (mostly L-enantiomers) synthesized in our laboratory. This series includes analogues with already known antiviral properties as well as other new cytidine derivatives. The synthesis and characterization of these new analogues will be reported elsewhere.[6]

The enzyme used in our experiments was dimeric recombinant human deoxycytidine kinase (dCK)[7,8] which presented a specific activity of 0.24 μmoles/min.mg with respect to β-D-2'-deoxycytidine (D-dC). The substrate properties of the cytidine analogues were determined from steady state kinetic studies at 37 °C. The reaction medium contained Tris HCl (50 mM) pH 7.5, DTT (1mM), NaF (15 mM), BSA (0.5 mg/ml), ATP (5 mM), $MgCl_2$ (5 mM), the substrate (in the range of 7-100 μM) and dCK. HPLC analysis of the reaction medium was performed using a 3μ Hypersil ODS column and the following elution system: (A) Tetrabutylammonium hydrogenosulfate-phosphoric acid (PIC A-Waters) (5 mM), (B) Buffer A and 50 % acetonitrile (1 ml. min^{-1}). The kinetic parameters were obtained from the Lineweaver-Burk treatment, and the corresponding substrate efficiencies (Vm/Km) were calculated (Table 1).

Our results confirm the data of Van Draanen *et al.* obtained with calf thymus deoxycytidine kinase.[4] These authors also suggest that the enantioselectivity of human deoxycytidine kinase is relaxed with respect to a number of cytidine analogues. In the 2'-deoxy series, our results show that D-dC is the most efficient examined substrate but the enzyme does not markedly discriminate the enantiomers and the substrate efficiencies are remarkably similar. 3TC has a substrate efficiency higher than that already determined using calf thymus dCK. [4] In the series of the 2',3'-dihydroxy analogues, β-D- and β-L-araC are by far the most efficient substrates with the L-enantiomers more active than the D as previously reported in the case of calf thymus dCK.[9] Similarly, β-L-cytidine is a much better substrate than β-D-cytidine. The enzyme is relatively specific of the nature of the pentose in the series of the β-L-enantiomers of the 2',3'-dihydroxy nucleoside analogues with substrate efficiency decreasing according to the sequence: β-L-araC >

TABLE 1 : Substrate properties of D- and L-cytidine analogues with respect to human recombinant deoxycytidine kinase.

Compound	Km (μM)	Vm (μmoles/min.mg)	Vm/Km (Rel.)
2'-Deoxy analogues:			
β-D-2'-Deoxycytidine	9	0.24	1
β-L-2'-Deoxycytidine	8	0.087	0.30
β-D-2',3'-Dideoxycytidine	24	0.10	0.16
β-L-2',3'-Dideoxycytidine	29	0.15	0.20
β-D-2',3'-Dideoxy-5-fluoro-cytidine	63	0.29	0.17
β-L-2',3'-Dideoxy-5-fluoro-cytidine	21	0.12	0.22
3TC	12	0.53	0.60
2',3'-Dihydroxy analogues:			
Cytosine β-D-arabinofuranoside	14	1.04	1
Cytosine β-L-arabinofuranoside	5	0.52	1.4
β-D-Cytidine	60	0.21	0.05
β-L-Cytidine	26	1.30	0.67
Cytosine β-L-lyxofuranoside	77	1.04	0.19
Cytosine β-L-xylofuranoside	59	0.74	0.18
5-Fluoro-cytosine β-L-xylofuranoside	77	0.92	0.16
3'-Deoxy-3'-fluoro-β-L-5-fluorocytidine	62	0.115	0.03

β-L-riboC >> β-L-lyxoC ≅ β-L-xyloC. This seems to indicate that the activity of the β-L-analogues of this series is dependent on the stereochemistry of the 2'-hydroxyl group. In contrast, the corresponding α-L-enantiomers (α-L-araC, α-L-lyxoC, α-L-xyloC, and α-L-riboC) were completely devoid of substrate properties.

In conclusion, our study suggests that a large number of β-L-cytidine analogues may be substrates of dCK and consequently may have a potential to be used in antiviral or anticancer therapy. However, limitations may exist if the enantioselectivities of the other activating enzymes, nucleotide kinases and nucleoside diphosphate kinases, are not favourable.

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