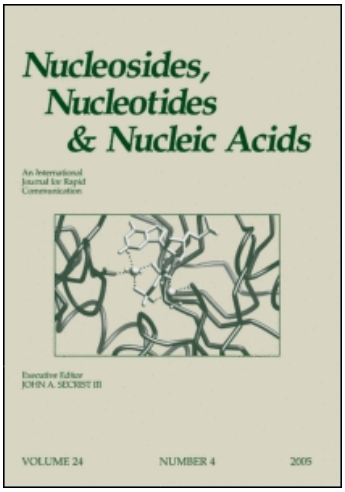


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# Derivatives of L-Adenosine and L-Guanosine as Substrates for Human Deoxycytidine Kinase

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# DERIVATIVES OF L-ADENOSINE AND L-GUANOSINE AS SUBSTRATES FOR HUMAN DEOXYCYTIDINE KINASE

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**ABSTRACT**: A series of analogues of L-adenosine and of L-guanosine, including ß-L-dA, ß-L-Ado, ß-L-araA, and ß-L-dG, have been shown to be substrates of human deoxycytidine kinase thus demonstrating the complete lack of enantioselectivity of this enzyme.



The recent discovery of the antiviral or anticancer properties of some L-nucleoside analogues has prompted a considerable interest for these compounds. Indeed, they present same order activities as the corresponding D-enantiomers and also have lower short-term cytotoxicities[1,2]. To be active, L-nucleosides have to be enzymatically converted to the corresponding 5'-triphosphate derivatives which are the entities able to inhibit viral DNA polymerases. Consequently, the activity of a L-nucleoside analogue depends primarily on the enantioselectivity of the activation enzymes (kinases), of the deactivation enzymes (deaminases, phosphorylases) and of the viral or cellular polymerases. We are currently studying the correlation between the antiviral activities of L-nucleoside analogues and the properties of these enzymes, especially the nucleoside kinases which catalyze the first phosphorylation step of the nucleosides. This step is often considered as the most critical in the transformation of the nucleoside to the 5'-triphosphate. Thus, the anti-HIV or anti-HBV activities of several analogues of L-

cytidine have been explained by the considerably relaxed enantioselectivity of human deoxycytidine kinase (dCK) for these compounds[3,4]. Owing to the fact that, not only ß-D-2'-deoxycytidine but also β-D-2'-deoxyadenosine and β-D-2'-deoxyguanosine are substrates of dCK[5], we have examined the properties of several derivatives of ß-L-adenosine[6] or ß-L-guanosine with respect to the human enzyme and we report here some of the results of this study.

## MATERIALS AND METHODS

Recombinant human dCK was purified using the human dCK cDNA sequence cloned into the pET19b and pET9d vector[7]. Both types of preparations contained a histidine tag sequence and were more than 90% pure with very similar properties.

The derivatives of L-adenosine or L-guanosine were stereospecifically synthesized according to previously published procedures or using new methods from commercially available L-sugars. The details of synthesis as well as the results of their antiviral evaluation will be reported elsewhere.

In kinetic studies, the reaction medium contained 50 mM Tris HCl pH 7.5, 5 mM ATP, 5 mM $MgCl_2$, 1 mM dithiothreitol, appropriate amounts of substrate and the enzyme (2.4 to 160 µg/ml, depending on the substrate). Analyses of the reaction mixture were performed by HPLC on a Hypersil C-18 3µm column under the following conditions: 5 min of isocratic elution using eluent A (phosphate buffer 50 mM pH 5.9) followed by 80 min linear gradient from eluent A to eluent B (phosphate buffer 50 mM pH 5.9 and 40% acetonitrile) with a flow rate of 1 $mL.min^{-1}$. The identifications of the products were achieved through coinjection of authentic samples or from the UV spectra of the eluted compounds. The kinetic curves were determined at 37°C by at least three measurements of substrate transformation as a function of time. The apparent kinetic parameters were obtained using the Grafit program (Erithacus Software, 1992) according to the Lineweaver-Burk method.

## RESULTS AND DISCUSSION

The kinetic parameters of the studied nucleoside analogues are presented in Table 1.

**TABLE 1**. Substrate properties of the studied nucleoside analogues *vs.* dCK

| Compound | Km(μM) | Vm (μmol/min/mg) | Compound | Km (μM) | Vm (μmol/min/mg) |
|---|---|---|---|---|---|
| ß-D-dA | 230 | 0.90 | ß-L-dA | 40 | 0.20 |
| ß-D-Ado | a | a | ß-L-Ado | 200 | 0.006 |
| ß-D-araA | 640 | 0.098 | ß-L-araA | 820 | 0.26 |
| ß-D-dG | 130 | 0.47 | ß-L-dG | 220 | 0.16 |
| ß-D-3'dG | a | a | ß-L-3'dG | 1600 | 0.003 |

[a]Reaction too slow to allow the determination of kinetic parameters.



It is remarkable that the most efficient substrate studied (as calculated from Km/Vm values) is an unnatural enantiomer, namely ß-L-dA. The D-enantiomers ß-D-dA and ß-D-dG were slightly less efficient and ß-L-dG was 5 times less effective than its D-enantiomer. Both enantiomers of ß-araA were average substrates of the enzyme compared to ß-D-dA. Finally, the 2',3'-dideoxynucleosides in the guanosine and in the adenosine series[6], were poor substrates of human dCK regardless of enantiomerism. Our results demonstrated that the lack of enantioselectivity of dCK for cytidine derivatives[4] could be extended to the adenosine or guanosine series, thus increasing the field of potentially active L-nucleoside analogues in antiviral therapy.

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