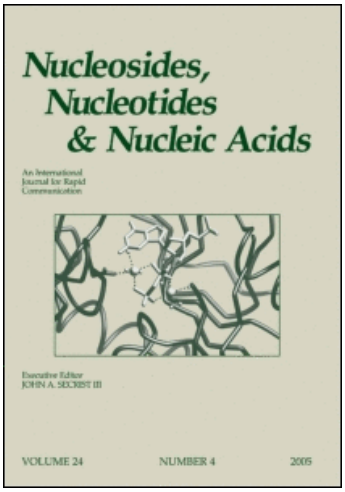

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Phosphorylation of Anti-HIV Nucleoside Analogs by Nucleoside Diphosphate Kinase

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PHOSPHORYLATION OF ANTI-HIV NUCLEOSIDE ANALOGS BY NUCLEOSIDE DIPHOSPHATE KINASE

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ABSTRACT : The reaction of NDP kinase with antiviral nucleoside triphosphates used in antiviral therapies was studied at the presteady state by fluorescence stopped-flow and compared with the steady-state parameters. The affinity of the analogs was determined by fluorescence titration of a mutated enzyme with an inserted Trp in the binding site. The lack of the 3' hydroxyl in analogs is shown to decrease the k_{cat} more than the K_D .

Nucleoside analogues like AZT (3'deoxy-3'azidothymidine) and ddN (dideoxy-nucleosides) are widely used as antiviral drugs targeted at the HIV reverse transcriptase. The nucleoside analogues must be phosphorylated by cellular kinases. The last step in the pathway leading to the triP-derivative is catalyzed by nucleoside diphosphate (NDP) kinase which is believed to present little specificity towards the nucleobase. The reaction involves the formation of a phosphorylated intermediate according to a ping-pong mechanism. Human and *Dictyostelium* NDP kinases have been resolved by X-ray crystallography and have similar folding with a highly conserved active site[1].

The presteady-state reaction of *Dictyostelium* NDP kinase with ddNTP was studied by quenching of protein fluorescence after manual mixing or by stopped-flow. The fluorescence signal, which is correlated with the phosphorylation state of the catalytic histidine in the enzyme active site[2], decreases upon ddNTP addition according to a

monoexponential time course. The pseudo-first order rate constant was determined for different concentrations of the various ddNTPs and was found to be saturable. The data are compatible with a two-step reaction scheme where fast association of the enzyme with the dideoxynucleotide is followed by a rate-limiting phosphorylation step. The rate constants and dissociation equilibrium constants determined for each dideoxynucleotide were correlated with the steady-state kinetic parameters measured in the enzymatic assay in the presence of the two substrates[3]. It is shown that ddNTPs are poor substrates for NDP kinase with a rate of phosphate transfer of 0.02 to 3.5 s^{-1} and a K_S of 1 to 5 mM. The dissociation constants for ADP, GDP, ddADP and ddGDP were also determined by fluorescence titration of a mutant F64W NDP kinase where the introduction of W at the nucleotide binding site provides a direct spectroscopic probe. The lack of the 3'OH in ddNTP causes a ten fold increase in K_D. Contrary to « natural » NTPs, NDP kinase discriminates between various ddNTPs, with ddGTP the more efficient and ddCTP the least efficient substrate within a range of 100 in k_{cat} values[4].

3'phosphorylated nucleotides are structural analogs of the bound nucleotide to NDP kinase and should be efficient competitif inhibitors. It was shown by fluorescence titration that pApS, a natural nucleotide involved in sulphur metabolism, binds to NDP kinase with a high affinity (K_D = 10 μM) and indeed inhibits the enzyme activity. The structure of the cocrystal shows a different mode of binding of the nucleotide to the enzyme[5]. PApS is the best NDP kinase inhibitor known so far.

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