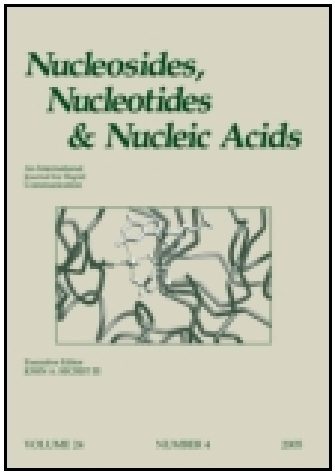

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Interaction of β-L-Adenosine-5′-triphosphate (L-ATP) with Human Deoxycytidine Kinase, Human DNA Primase and T4 DNA Ligase: Does the Chance Direct Enzymatic Enantioselectivity?

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INTERACTION OF β-L-ADENOSINE-5'-TRIPHOSPHATE (L-ATP) WITH HUMAN DEOXYCYTIDINE KINASE, HUMAN DNA PRIMASE AND T4 DNA LIGASE: DOES THE CHANCE DIRECT ENZYMATIC ENANTIOSELECTIVITY?

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ABSTRACT: We demonstrate that L-ATP: 1) as well as its natural D-enantiomer, acts as a phosphate donor in the reaction catalysed by human deoxycytidine kinase; 2) inhibits human DNA-primase and the ATP-dependent T4 DNA ligase. Thus, the lack of enantioselectivity of the enzymes is more frequent than it was believed a few years ago and we suggest that it would depend on chance more than on an evolutionary strategy.

Enzymes are thought to be able to bind effectively to only one enantiomer of a chiral substrate. However, some exceptions to this rule have recently been found among enzymes involved in the synthesis and polymerisation of deoxyribonucleotides[1]. In fact, we have demonstrated that herpes virus thymidine kinase (TK) human mitochondrial TK, cellular deoxycytidine kinase (dCK), some cellular and viral DNA polymerases (such as DNA polymerase α, HIV-1 reverse transcriptase, etc) are able to recognise and metabolise L-nucleosides or L-nucleotides[2-4]. The lack of enantioselectivity of HSV TK and human dCK represents the molecular basis for the development of L-thymidine and L-deoxycytidine analogues as antiviral and anticancer drugs[1].

In the present work we studied the enantioselectivity for ATP of some enzymes involved in DNA synthesis such as human dCK, human DNA primase, *E. coli* RNA polymerase and T4 DNA ligase.

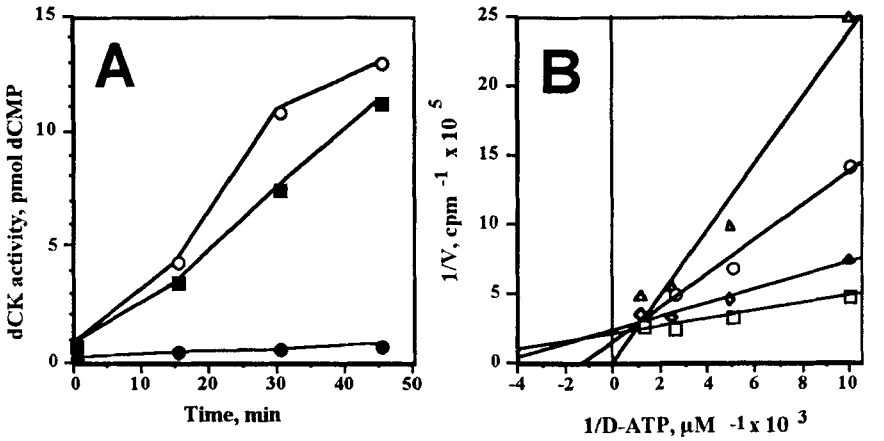

FIG. 1. Effect of L-ATP on human dCK and human DNA primase activity. Panel A: kinetics of human dCK activity in presence of 1 mM D-ATP (B), 1 mM L-ATP (E) and in absence of ATP (J). Panel B: Lineweaver-Burk plot of the effect of L-ATP on the activity of human DNA primase. L-ATP: (G) 0 μM; (A) 100 μM; (E) 200 μM and (C) 400 μM.

Human dCK lacks enantioselectivity not only for the nucleoside substrate, but also for the phosphate donor. In fact, we found that both D-ATP and L-ATP are indifferently used by human dCK to phosphorylate dCyd (**FIG. 1A**), whereas the herpes virus TKs, whose gene sequences suggest a common origin with human dCK, are unable to utilise L-ATP as a phosphate donor (data not shown). Human cytoplasmic TK is strictly enantioselective for both thymidine[2] and ATP (data not shown). L-ATP competitively inhibited human DNA primase activity with a Ki value of 80 μM (**FIG. 1B**). This indicates that the active site of the enzyme is non-enantioselective being able to recognize both enantiomers of ATP with comparable efficiency. On the contrary, *E. coli* RNA polymerase, which, like DNA primase, synthesises RNA chains utilizing ATP as a substrate, is strictly enantioselective and does not recognize L-ATP at all. L-ATP inhibits the adenylation reaction of T4 DNA ligase with an estimated IC_{50} value of 4.2 μM. We have previously demonstrated that T4 DNA ligase can perform two different kind of complex with DNA, depending on its adenylation state: a stable complex in the absence of ATP and a transient complex in the presence of ATP. Only in the absence of ATP the DNA/enzyme complex is sufficiently stable to allow the DNA retardation in a electromobility-shift assay (EMSA). Therefore we challenged L-ATP in comparison with

D-ATP in this assay and we found that no band retardation occurred in the presence of L-ATP, indicating that L-ATP was able to modulate the binding of DNA ligase to DNA in the same manner of D-ATP (data not shown). This result suggests that L-ATP can be used for the adenylation step of the enzyme reaction. Although able to adenylate the enzyme, L-ATP is not effective as a cofactor in the joining reaction of poly-d(AT). However, it competitively inhibits the D-ATP dependent joining reaction with an estimated Ki value of 24 μM.

Since the emergence of an enzymatic behavior depends on natural selection, we think that enantioselectivity could have been very important at the origin of life when different choices were possible. But now, in a world made up only by D-nucleosides and L-aminoacids, the enantioselectivity of the enzymes may be purely a side effect and not a vital choice, thus depending more on chance than on evolutionary strategies. From a structural point of view, our findings on substrate/enzyme interactions could be interpreted by an extension of the Koshland's "hand-in-glove" metaphor. In fact, rather than by the "hand-in-glove", it could be better represented by a "hand-in-mitten" metaphor [5], in which the thumb would be the aminoacid pocket interacting with the adenine moiety of ATP, while the rest of the mitten would fit the sugar-triphosphate moiety as well as it would fit the right or the left hand.

Acknowledgments

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