KINETIC MODELLING OF DEOXYTHYMIDINE KINASE OF *Escherichia coli*

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Deoxythymidine kinase (TDK), EC 2.7.1.21, of Escherichia coli catalyzes the ATP-dependent phosphorylation of deoxythymidine, or thymidine, as well as deoxyuridine in pyrimidine nucleotides biosynthesis:

ATP + deoxythymidine = ADP + deoxythymidine-5'-phosphate;

ATP + deoxyuridine = ADP + deoxyuridine-5'-phosphate.

Subsequently, the enzyme named deoxythymidylate kinase catalyses the conversion of dTMP to dTTP. In turn, dTTP serves as one of the substrates (additionally to dATP, dCTP, and dGTP) for DNA polymerase in DNA synthesis.

TDK is important enzyme in pyrimidines biosynthesis pathway, it attracts many investigator's attention. Nevertheless, we have not found developed kinetic models of TDK in literature. The TDK action mechanism is of high complexity, therefore the choice of its specificity is crucial for modelling. We applied the steady-state and quasi-equilibrium approaches, elaborating the model.

Kinetic model of TDK catalytic cycle is elaborated based on the experimental data for Escherichia coli.

Literature available experimental data relevant to the kinetic properties of this enzyme (catalytic constants, Michaelis constants, and others) are not sufficient for reliable kinetic model development; therefore additional parameters had been obtained by fitting model curves against experimental data. Values of 70 parameters overall were obtained using DBSolve7.01 software. Parameters were fitted consequently, in groups corresponding to the data for different experiments, described in [1, 2]. The expression for reaction rate dependence on pH was also obtained.

Using the elaborated model it is possible to extrapolate the action of the deoxythymidine kinase to wide range of pH, as well as to predict the action of the enzyme in presence of different phosphate donors, activators and effectors in various combinations.

Using the elaborated kinetic model of TDK we can solve next practical tasks, which are important for microbiology and biomedicine:

1. Prediction of TDK behavior in the cell under different conditions.
2. Prediction of switching activities of TDK regarding to different phosphate donors *in vivo* and *in situ*.

***References*:**

1. Okazaki, R., and Kornberg, A. 1964. Deoxythymidine Kinase of Escherichia coli. I. Purification and some properties of the enzyme. *J. Biol. Chem.* 239: 269-274.

2. Okazaki, R., and Kornberg, A. 1964. Deoxythymidine Kinase of Escherichia coli. II. Kinetic and feedback control. *J. Biol. Chem.* 239: 275-284.