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Breanna Wentz
Concordia University - Portland

Jaclyn Einspahr
Concordia University - Portland

Michael H. Godsey
Concordia University - Portland, mgodsey@cu-portland.edu

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KINETIC ANALYSIS OF PRODRUG ACTIVATION AND ATP/UTP SUBSTRATE PREFERENCE OF NINE HUMAN DEOXYCYTIDINE KINASE MUTANTS

Breanna Wentz*, Jaclyn Einspahr*, Michael Godsey Ph.D

Math and Science Department, Concordia University, Portland, OR 97211. *Authors contributed equally to this work.

Abstract / Introduction

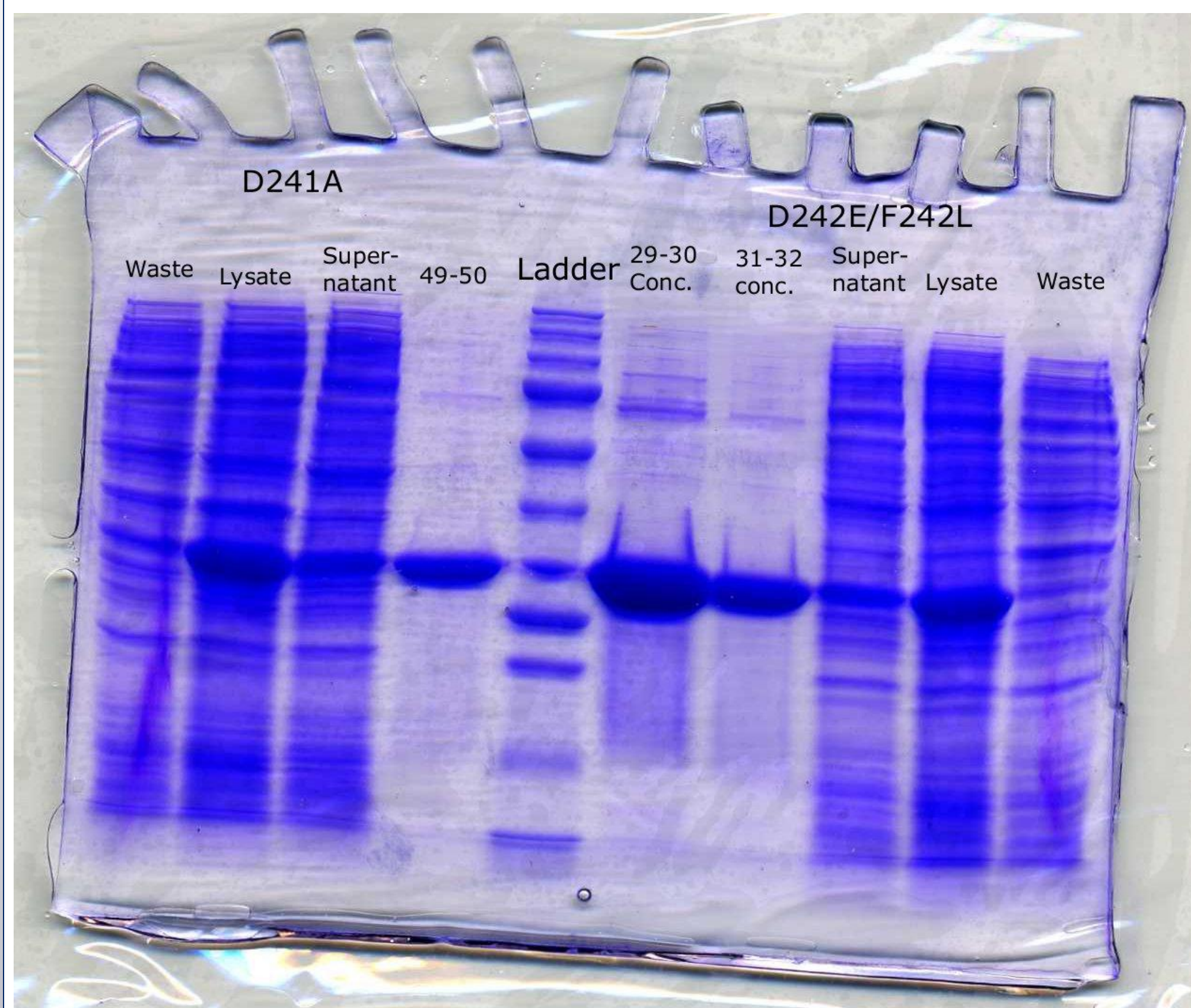
Deoxynucleoside analogues are prodrugs that can function as inhibitors of both viral and cellular DNA replication processes. They are important in anti-cancer therapy because they hinder DNA synthesis and cellular mitosis. Within the cell, deoxyribonucleotides are synthesized using the salvage pathways by converting the unphosphorylated nucleosides to their mono, di- and tri-phosphate forms using a phosphoryl donor: ATP or UTP. Human deoxycytidine kinase (dCK) is the first and rate-limiting enzyme in this process. The dCK protein uses nucleotide triphosphates to phosphorylate several clinically important nucleoside analogue prodrugs in addition to its natural substrates. The preferred physiological phosphoryl donor for dCK is UTP although it is less prevalent in the human body than ATP. Our objective is to improve the understanding of the phosphate-donor binding loop of dCK by kinetic analysis of a series of mutants of Asp241 and Phe242. These mutants were designed in an attempt to improve the activity of dCK with phosphate donors. Results show several mutants with improved kinetics and some with an ATP donor preference over UTP.

Materials and Methods

Mutated histidine-tagged dCK proteins were grown in *E.coli* and purified by immobilized metal affinity chromatography using Co⁺⁺ resin. Purified proteins were analyzed by SDS-PAGE gel electrophoresis. Protein was >95% pure. Kinetic assays were performed in assay buffer (100mM Tris pH 7.5, 200mM KCl, 5mM MgCl₂, 80μM Phosphoenolpyruvate, 400μM NADH, and 250μM cytosine-β-D-arabinofuranoside (araC)). Helper enzymes lactate dehydrogenase and pyruvate kinase were added to reaction. Phosphoryl donor (ATP or UTP) was added in concentration ranging from 1μM to 200μM. Finally, 0.4μM dCK was added and NADH oxidation was monitored spectrophotometrically by A₃₄₀ decrease over time (Agarwal)

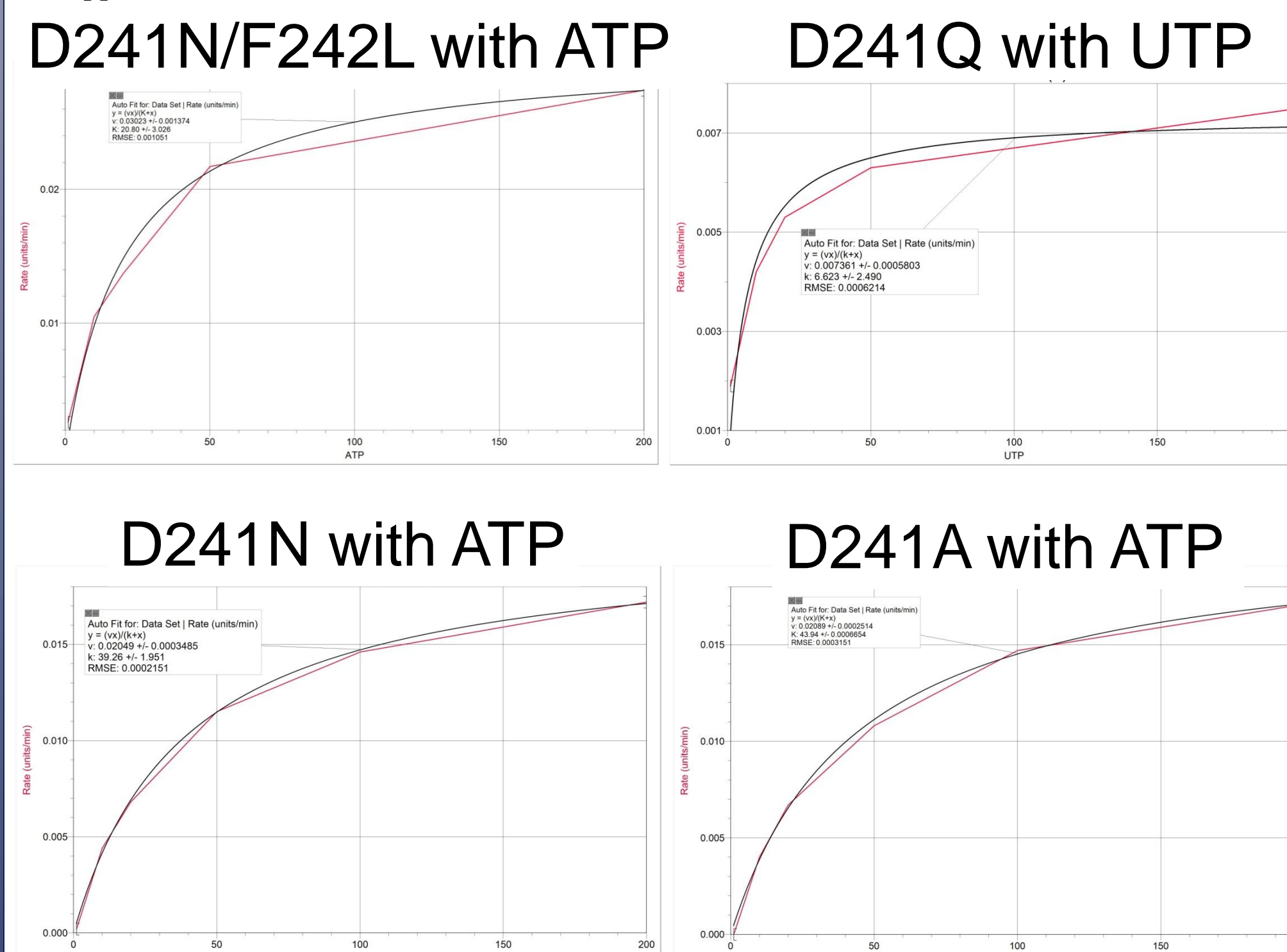
Results

Figure 1:



Gel indicates the presence of the mutant dCK proteins D241A and D241E/F242L after purification. Bands appeared at the expected protein size of 31 kilodaltons as indicated by the ladder. This showed that our purified protein was present in the samples collected. All other samples produced similar results.

Figure 2:



Michaelis-Menten Kinetics: Initial velocity (V_0) calculated using NADH absorbance decrease vs. concentration of the phosphate donor used, either ATP or UTP. Phosphate acceptor araC was 250μM in all reactions. All other experiments produced similar curves.

Results

Table 1: Kinetic Analysis of dCK mutants

Substrate	WT dCK .4μM	D241E dCK .4μM	D241N dCK .4μM
ATP	V_{max} : 3.42×10^{-8} K_m : 52 K_{cat} : 0.085 K_{cat}/K_m : 1.6×10^{-3}	V_{max} : 2.30×10^{-8} K_m : 37 K_{cat} : .057 K_{cat}/K_m : 1.6×10^{-3}	V_{max} : 2.0×10^{-2} K_m : 35 K_{cat} : 0.14 K_{cat}/K_m : 3.9×10^{-3}
UTP	V_{max} : 8.29×10^{-9} K_m : 25 K_{cat} : 0.021 K_{cat}/K_m : 8.4×10^{-4}	V_{max} : 1.26×10^{-8} K_m : 31 K_{cat} : 0.031 K_{cat}/K_m : 1.0×10^{-3}	V_{max} : 5.2×10^{-3} K_m : 40 K_{cat} : .035 K_{cat}/K_m : 8.7×10^{-4}
Substrate	D241Q dCK .4μM	D241E/F242L dCK .4μM	D241N/F242L dCK .4μM
ATP	V_{max} : 2.9×10^{-2} K_m : 30 K_{cat} : 0.19 K_{cat}/K_m : 6.4×10^{-3}	V_{max} : 2.6×10^{-2} K_m : 49 K_{cat} : 0.18 K_{cat}/K_m : 3.6×10^{-3}	V_{max} : 3.1×10^{-2} K_m : 23 K_{cat} : 0.21 K_{cat}/K_m : 9.0×10^{-3}
UTP	V_{max} : 7.9×10^{-3} K_m : 9.6 K_{cat} : 0.053 K_{cat}/K_m : 5.5×10^{-3}	V_{max} : 1.1×10^{-2} K_m : 27 K_{cat} : 0.076 K_{cat}/K_m : 2.8×10^{-3}	V_{max} : 2.0×10^{-2} K_m : 38 K_{cat} : 0.14 K_{cat}/K_m : 3.6×10^{-3}
Substrate	D241Q/F242L dCK .4μM	D241A dCK .4μM	L191Q dCK .4μM
ATP	V_{max} : 2.0×10^{-2} K_m : 20 K_{cat} : 0.14 K_{cat}/K_m : 6.8×10^{-3}	V_{max} : 2.1×10^{-2} K_m : 50 K_{cat} : 0.14 K_{cat}/K_m : 2.8×10^{-3}	V_{max} : 2.3×10^{-2} K_m : 80 K_{cat} : 0.16 K_{cat}/K_m : 1.9×10^{-3}
UTP	V_{max} : 1.2×10^{-2} K_m : 14 K_{cat} : 0.078 K_{cat}/K_m : 5.5×10^{-3}	V_{max} : 6.0×10^{-3} K_m : 30 K_{cat} : 0.041 K_{cat}/K_m : 1.3×10^{-3}	V_{max} : 5.1×10^{-3} K_m : 41 K_{cat} : 0.034 K_{cat}/K_m : 8.2×10^{-4}
Substrate	F242L dCK .4μM	Michaelis-Menten Equation	
ATP	V_{max} : 1.7×10^{-2} K_m : 22 K_{cat} : 0.12 K_{cat}/K_m : 5.3×10^{-3}	$V_0 = \frac{V_{max}[S]}{K_m + [S]}$	
UTP	V_{max} : 1.0×10^{-2} K_m : 24 K_{cat} : 0.067 K_{cat}/K_m : 2.8×10^{-3}		

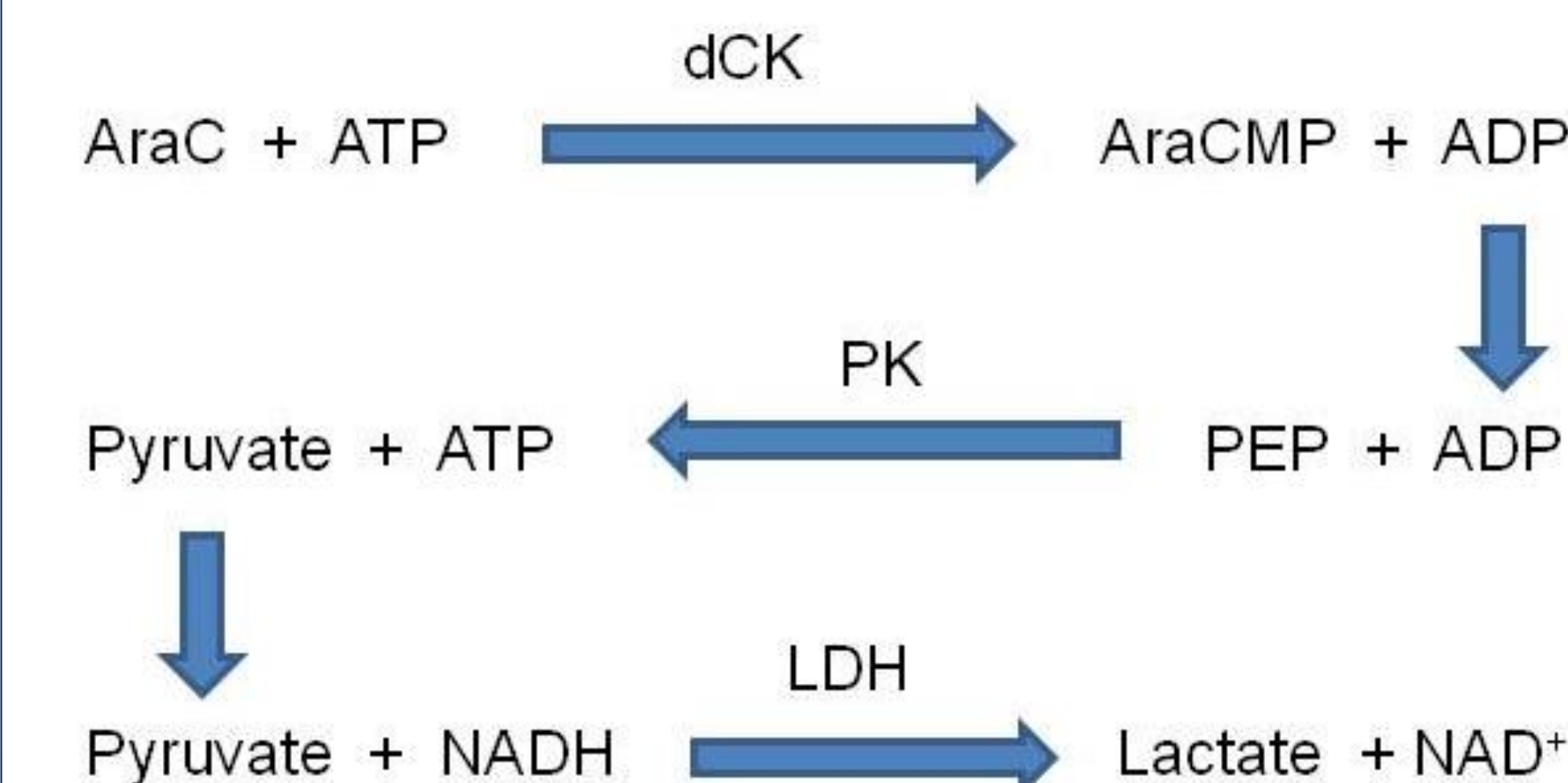
k_{cat} are in sec⁻¹, K_M are in μM, V_{max} are in μM/sec, and k_{cat}/K_M are in μM⁻¹sec⁻¹. V_{max} and K_M values are determined by curve fitting to the M-M equation. K_M is defined as the substrate concentration required to reach ½ V_{max} . Lower K_M indicates less substrate is required to approach maximum velocity. A higher k_{cat}/K_M value indicates a more efficient enzyme.

Conclusions

Our results showed that mutating the dCK residues will open up the active donor site to make it more accessible to bind ATP and UTP resulting in more phosphate donor flexibility. Significantly we found an increased rate for mutants D241N, D241N/F242L, and F242L. This research has the potential to progress anti-cancer therapy as a more efficient form of dCK can act as an inhibitor of both viral and cellular DNA replication. Kinetic assays with other nucleoside analogues will be performed to extend the results of our research.

Results

Overall Kinetic Assay Equation:



NADH absorbs light at 340nm, while NAD⁺ does not. Based on the above reactions, the ratio between NADH oxidation and phosphorylation by dCK is 1:1. Pyruvate kinase and Lactate dehydrogenase are orders of magnitude faster than dCK. Therefore, by measuring NADH oxidation we can measure phosphorylation by dCK. (Agarwal) [NADH] is determined by absorption: $A_{340} = \ell \times \epsilon \times [\text{NADH}]$, where A is the absorbance at 340nm, ℓ is the path length (1cm) and ϵ is the absorption coefficient of NADH=6200M⁻¹cm⁻¹.

References

- Agarwal, K. C. *et al.* (1978). *Methods in Enzymology*, 51, 483–490.
- Godsey, M.H. *et al.* (2006). Structural basis for the preference of UTP over ATP in human deoxycytidine kinase: Illuminating the role of main-chain reorganization. *Biochemistry*, 45 (2), 452-461.
- Kesler, G. *et al.* (2004). Stimulation of deoxycytidine kinase results in prolonged maintenance of the enzyme activity. *Nucleosides, Nucleotides & Nucleic Acids*, 23 (8 & 9), 1357–1361.
- Sabini, E. *et al.* (2003). Structure of human dCK suggests strategies to improve anticancer and antiviral therapy. *Nature Structural Biology*, 10 (7), 513-519.

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