Supplementary Information

Structural and kinetic characterization of human deoxycytidine kinase variants able to phosphorylate 5-substituted deoxycytidine and thymidine analogs

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Content

Table S1: Steady state kinetic analysis of WT dCK and the R104M-D133A double mutant

	Nucleoside	dCK WT			dCK R104M-D133A		
		k_{cat} (s^{-1})	Km (μM)	k_{cat}/Km ($M^{-1}s^{-1}$)	k_{cat} (s^{-1})	Km (μM)	k_{cat}/Km ($M^{-1}s^{-1}$)
A	D-dC[c]	0.040±0.001[a]	< 3	> $13.3x10^3$	1.80±0.04	5.70±0.44	$315.8x10^3$
	D-dA[c]	2.13±0.35	115±4	$18.6x10^3$	4.51±0.33	1040±117	$4.3x10^3$
	D-dG[c]	2.60±0.10	231±20	$11.3x10^3$	1.73±0.12	1865±211	$0.9x10^3$
B	5-Me-dC	0.070±0.02	7.8±1.2	$9.0x10^3$	0.36±0.02	4.16±1.54	$87x10^3$
C	Gem[c]	0.39±0.03	16.1±3.5	$24.2x10^3$	2.68±0.07	56±17	$47.7x10^3$
	AraC[c]	0.34±0.01	13.1±1.1	$26.0x10^3$	1.43±0.03	137±10	$10.5x10^3$
D	D-dT[c]	nm[b]	nm[b]	nm[b]	1.74±0.01	144±10	$12.1x10^3$
	L-dT[c]	nm[b]	nm[b]	nm[b]	3.13±0.10	138±10	$22.7x10^3$
E	BVdU	nm[b]	nm[b]	nm[b]	1.21±0.08	108±17	$11.2x10^3$
	L-dU	nm[b]	nm[b]	nm[b]	10.60±1.44	1058±242	$10.0x10^3$

[a] standard deviation
[b] nm: not measured; rate is very low for these nucleosides with WT dCK.
[c] Data taken from Hazra et al, Biochemistry, 2009.
The concentration of ATP was kept constant at 1 mM.

Table S2: Efficiency change for dC-analog phosphorylation between WT and mutant dCK				
		dCK WT R1 = CE[a](dC) / CE(dC-analog)	**dCK R104M-D133A** R2 = CE(dC) / CE(dC-analog)	R2/R1[b]
A	D-dC	1	1	> 23.75[c]
B	5-Me-dC	> 1.47	3.63	> 2.47
C	Gem	> 0.55	6.62	> 12.04
	AraC	> 0.51	30.10	> 59.02

[a] CE = catalytic efficiency, k_{cat}/Km

[b] R2/R1 is a double ratio, i.e. the ratio of two ratios; this gives a measure of specificity change.

[c] This number the single ratio of k_{cat}/Km of dCK of the mutant versus WT dCK.