Post-translational phosphorylation of serine 74 of human deoxycytidine kinase favors the enzyme adopting the open conformation making it competent for nucleoside binding and release

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Supplementary Information

Supplementary data include Table S1 and Figures S1, S2.

Table S1: Kinetic analysis of WT dCK and mutants DM and DM.S74E.

	Nucleoside	WT dCK			R104M/D133A dCK			R104M/D133A + S74EdCK		
		k_{cat}[a]	Km[a]	k_{cat}/ Km[b]	k_{cat}[a]	Km[a]	k_{cat}/ Km[b]	k_{cat}[a]	Km[a]	k_{cat}/ Km[b]
A	D-dC	0.040±0.001[c]	<3	>13.3	1.80±0.04	5.70±0.44	315.8	4.53±0.1	19.5±1.60	232
	D-dA	2.13±0.35	115±4	18.6	4.51±0.33	1040±117	4.3	3.56±0.2	1415±114	2.5
	D-dG	2.60±0.10	231±20	11.3	1.73±0.12	1865±211	0.9	0.37±0.02	699±63	0.50
C	Gem	0.39±0.03	16.1±3.5	24.2	2.68±0.07	56±17	47.7	9.44±1.50	386±83	24.4
	AraC	0.34±0.01	13.1±1.1	26.0	1.43±0.03	137±10	10.5	4.24±0.78	616±182	6.9
D	D-dT	-	-	-	1.74±0.01	144±10	12.1	5.60±0.27	315±34	17.7
	L-dT	-	-	-	3.13±0.10	138±10	22.7	8.60±0.66	562±80	15.3
E	BVdU	-	-	-	1.21±0.08	108±17	11.2	3.34±0.19	728±76	4.60
	L-dU	-	-	-	10.60±1.44	1058±242	10.0	6.78±0.60	1103±159	6.15
F	5-Met dC	0.070±0.002[a]	7.8±1.2	9.0	0.36±0.02	4.16±1.54	87	3.62±0.42	38.38±11.08	94
	5-Pro-dC	-	-	-	0.59±0.02	22.67±3.80	26	3.33±0.12	40.36±5.54	82
	5-Br-dC	0.045±0.002	22.0±4.0	2.0	0.30±0.01	5.87±0.72	51	2.05±0.05	8.21±1.28	250
	5-I-dC	0.032±0.001	58.9±6.0	0.5	0.16±0.01	7.10±1.52	23	1.10±0.04	10.32±1.90	107

[a] k_{cat} units are sec^{-1}, Km units are in µM.
[b] $\times 10^3$ sec^{-1}/M
[c] standard deviation
- activity undetectable or extremely weak

Gem: gemcitabine; AraC: cytarabine; BVdU: bromovinyl-deoxyuridine; 5-Met-dC: 5-methyl-deoxycytidine; 5-Pro-dC: 5-propinyl-deoxycytidine; 5-Br-dC: 5-bromo-deoxycytidine; 5-I-dC: 5-iodo-deoxycytidine

Figure Legends

Figure S1: Conformational plasticity of dCK's nucleotide and nucleoside binding sites. Shown is an overlay of the dCK structure solved in the presence of L-deoxythymidine (L-dT) and ADP (PDB ID 3HP1, blue), and that of the S74E variant that also contained L-dT but UDP instead of ADP (green). In both cases the enzyme also contained the double mutations (DM) of R104M+D133A – these mutations confer thymidine binding ability to dCK. Right zoom inset: The base-sensing loop adopts a different main-chain conformation that is determined by the nature of the base of the nucleotide. However, the phosphates of UDP and ADP overlay nearly perfectly. Left zoom inset: The structure with ADP is the closed state in which residues 63 to 77 of the insert region cannot be modeled. The structure of the S74E containing mutation adopted the open state that reveals extra residues of the insert. Notably, the insert in the open state has adopted a different angle of helix a2, and a longer helix a3 that is followed by a loop. The side chain of E74 is shown. The position of L-dT is different between the open and closed conformations. Only in the closed conformation can the 5'-hydroxyl of L-dT (marked by black arrows) become activated by the carboxylic group of Glu53.

Figure S2: Sequence alignment of the human nucleoside kinases deoxycytidine kinase (hdCK), deoxyguanosine kinase (hdGK), thymidine kinase 2 (hTK2) and the *Drosophila melanogaster* deoxynucleoside kinase (dNK). The secondary structure of dCK is shown above the sequences, arrows depicting beta strands (numbered 1 through 5), and corkscrews depicting alpha helices (1 through 10). The Insert region that connects helix 2 to helix 3 is present in hdCK and hdGK, but not in hTK2 and dNK. In dNK, a short turn replaces the 12-15 residue long Insert region.

Figure S1

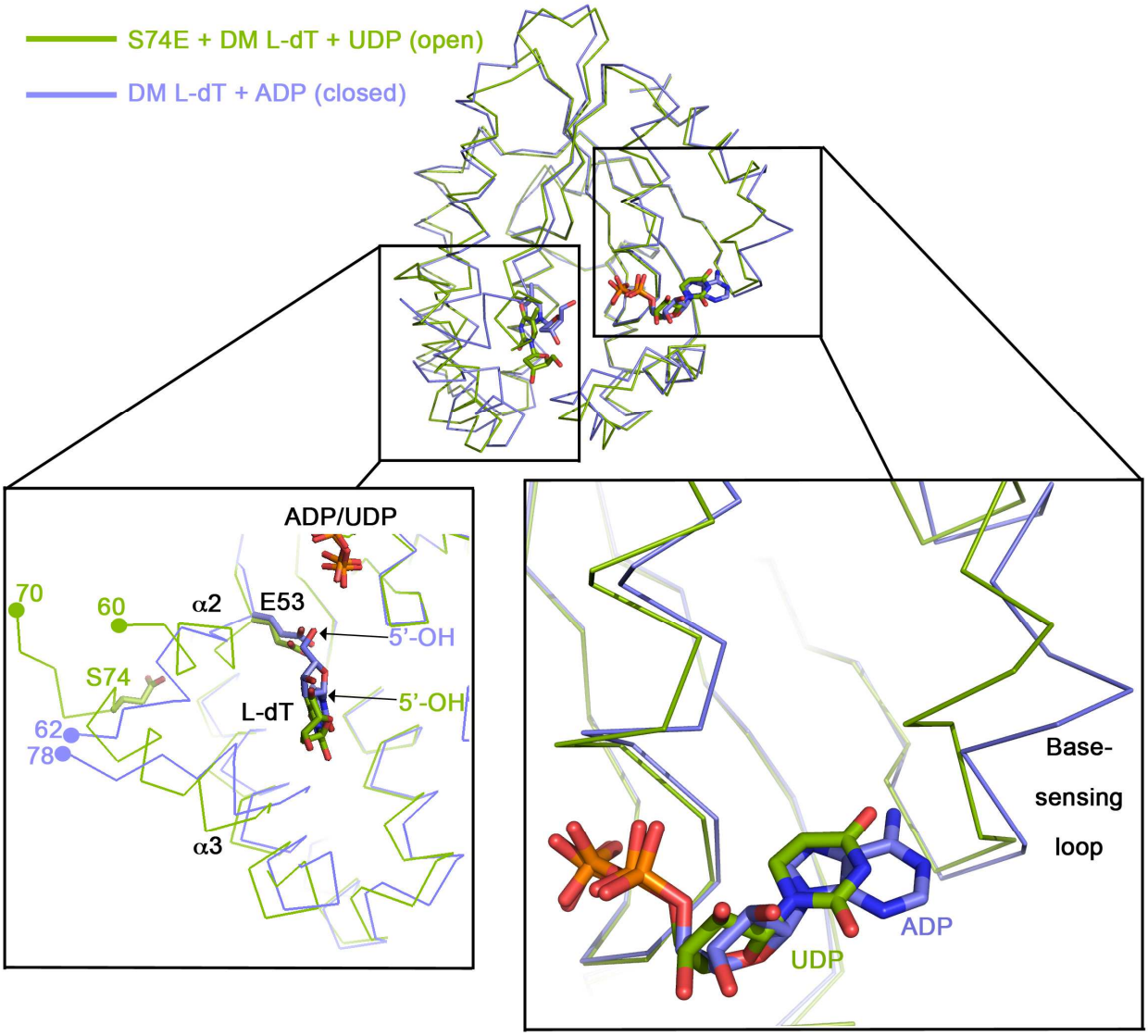
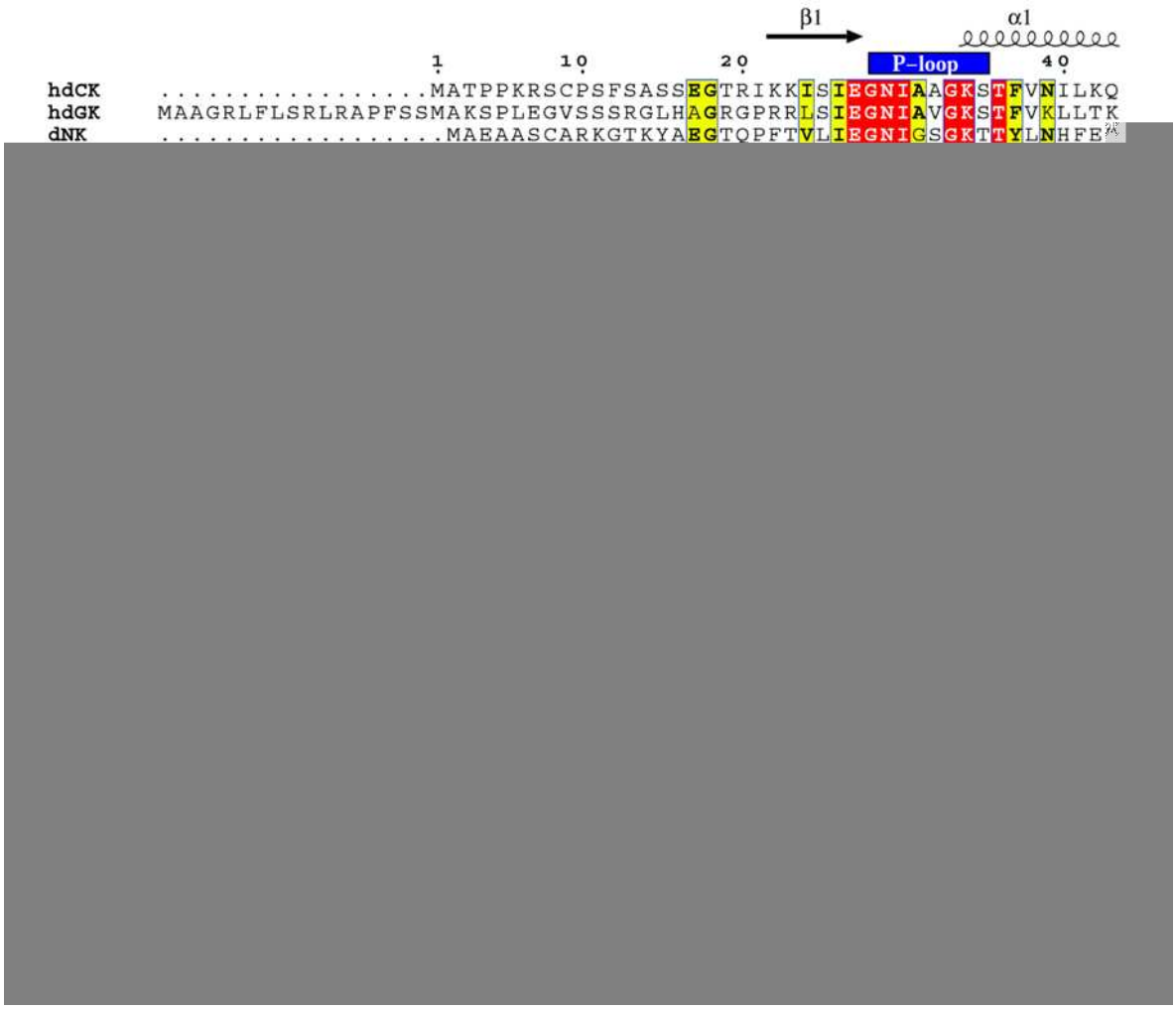

S74E + DM L-dT + UDP (open)
DM L-dT + ADP (closed)
ADP/UDP
70
60
α2
E53
5'-OH
S74
L-dT
5'-OH
62
78
α3
Base-
sensing
loop
ADP
UDP

Figure S2


β1
α1
1
10
20
P-loop
40
hdCK .....MATPPKRSCPSFSASSEGTRIKKISIEGNIAAGKSTFVNILKQ
hdGK MAAGRLFLSRLRAPFSSMAKSPLEGVSSSRGLHAGRGPRRLSIEGNIAVGKSTFVKLLTK
dNK .....MAEAASCARKGTKYAEGTQPFTVLIEGNIGSGKTTYLNHFE