A Metal Dependent Conformational Change Provides a Structural Basis For The Inhibition Of *E. Coli* CTP Synthase By Gemcitabine-5'- Triphosphate

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CTP synthases (CTPS) catalyze the de novo production of CTP using UTP, ATP, and l- glutamine with the anticancer drug metabolite gemcitabine-5¢-triphosphate (dF-dCTP) being one of its most potent nucleotide inhibitors. To delineate the structural origins of this inhibition, we solved the structures of *Escherichia coli* CTPS (ecCTPS) in complex with CTP (2.0 Å), 2'-ribo-F-dCTP (2.0 Å), 2'-arabino-F-CTP (2.3 Å), dF-dCTP (2.2 Å), dF-dCTP and ADP (2.1 Å), and dF-dCTP and ATP (2.6 Å). These structures revealed that the increased binding affinities observed for inhibitors bearing the 2'-F-arabino group (dF-dCTP and F-araCTP), relative to CTP and F-dCTP, arise from interactions between the inhibitor's fluorine atom exploiting a conserved hydrophobic pocket formed by F227 and an interdigitating loop from an adjacent subunit (Q114-V115-I116). Intriguingly, crystal structures of ecCTPS•dF-dCTP complexes in the presence of select monovalent and divalent cations demonstrated that the in crystallo tetrameric assembly of wild-type ecCTPS was induced into a conformation similar to inhibitory ecCTPS filaments solely through the binding of Na^{+}-, Mg^{2+}-, or Mn^{2+}•dF- dCTP. However, in the presence of potassium, the dF-dCTP-bound structure is demetalated and in the low-affinity, non-filamentous conformation, like the conformation seen when bound to CTP and the other nucleotide analogues. Additionally, we demonstrated that the product, CTP can also induce the filament-competent conformation linked to high-affinity dF-dCTP binding in the presence of high concentrations of Mg^{2+}. This metal-dependent, compacted CTP pocket conformation therefore furnishes the binding environment responsible for the tight binding of dF-dCTP and provides insights for further inhibitor design.