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Engineering nucleotide specificity of succinyl-CoA synthetase (SCS) in Blastocystis: The emerging role of gatekeeper residues

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SCS was discovered in 1958, there are extensive studies and crystal structures available, but mechanism of nucleotide specificity is still not clear. Hallmark concepts like "Lock and Key" and "Induced Fit" hypothesis were present for enzyme specificity. Here, we have proposed a novel strategy, employed by SCS in Blastocystis for discriminating between cognate and non-cognate ligands (ATP & GTP). Charged, solvent exposed residues at the entrance to substrate binding site (Gatekeeper residues), produce electrostatic dipole interactions with approaching substrates, and control their access by a novel mechanism called "Electrostatic Gatekeeper Effect". In this proof-of-concept study, we have demonstrated that nucleotide specificity of wild ATP-specific (Km=145±47 μM) SCS with positive gatekeepers (KK), can be engineered by altering electrostatic properties of its gatekeeper residues. Our enzyme kinetics results showed dual specificity [ATP (Km=230±34 μM) & GTP (Km=143±17 μM)] for gatekeeper mutant (ED) with negative gatekeepers (ED), which favored GTP access to binding site. However, nucleotide binding site mutant (LF) showed no GTP-specificity despite previously reported GTP-supporting residues (LF), and remained ATP-specific (Km=265±50 μM), because positive gatekeepers (KK) still precluded GTP access to binding site. Interestingly, combining gatekeeper mutant with nucleotide binding site mutant (ED+LF), resulted in exclusive GTP-specificity (Km=82±12 μM) and no detectable ATP-activity. This striking result was entirely due to; negatively charged gatekeeper residues (ED) favored GTP access, while nucleotide binding site residues (LF) altered ATP binding. These results were further supported by molecular modeling and simulation studies. Hence it is imperative to explore this strategy in different range of crucial enzymes (synthetases, kinases and transferases) to engineer substrate specificity for various industrial applications and substrate based drug design.

Biography

Kapil Vashisht is a Senior Research Fellow at National Institute of Malaria Research, New Delhi, India and registered for PhD program at Goa University, Goa, India. He is working under the supervision of Dr. Kailash C Pandey, currently at National Institute for Research in Environmental Health, Bhopal, India. Recently, he has submitted this interesting research in *Biochemistry* (Revised) as a first author and this study has also been filed for PCT at Indian Council of Medical Research, India (PCT/IN2015/000451). He is currently working to prove this concept in SCS of *P. falciparum* (another human parasite).

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