Dynamic disorder driven substrate inhibition and bistability in simple enzymatic reaction

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***Short Abstract* — Conformations and catalytic rates of enzymes (biological catalysts) fluctuate over a wide range of timescales. Recent experimental and theoretical investigations demonstrated case studies where enzymatic rate still follows Michaelis-Menten(MM) rate law despite molecular fluctuations. In this letter we consider a simple kinetic scheme of enzyme catalysis where the product release step is treated explicitly and examine how conformational fluctuations affect the underlying rate law. Our results indicate that slow conformation fluctuations of the enzyme-substrate complex not only lead to a non MM behavior but also substrate inhibition and possible bistability of the reaction network.**

I. Introduction

Enzymes (protein catalysts) play important role in controlling the flux of biochemical reaction networks. Conformational flexibility and dynamics of enzymes play important roles in their functional properties. Fluctuations in the protein structure can bring changes in the substrate/product affinities and catalytic properties of enzymes [1-3]. The importance of conformational dynamics has been realized for quite some time and has been widely studied in the context of allosteric regulation – propagation of the ligand binding effect to a distant catalytic site[4-7]. In the recent years, a large number of different tools have been developed for probing the conformations of enzymes during catalysis. Single-molecule experiments[8-11] suggest that conformational fluctuations occur over vast distribution of timescales: from milliseconds to 100 s. Interestingly single molecule experiment on the enzyme β-galoctosidase show that the rate of the catalytic reaction fluctuate over the same range of time scales (10^{-4} to 10s) [11], a phenomena termed as dynamic disorder. These observations have inspired several theoretical studies [12-15] on the effects of conformational dynamics on the kinetics of single enzymatic reactions. The results of these studies indicate that in general the steady state kinetics of a fluctuating enzyme is non-MM[12,16], but depicts several limiting cases in which MM equation is obeyed even for single-molecule reactions. The limits considered include quasi-equilibrium limit when conformational dynamics is fast and equilibrates before chemical reactions occur. Another important limit motivated by experimental work is the quasi-static limit in which fluctuations in any one of the conformational states (E or ES) is very slow. In this work we consider a simple kinetic scheme of enzyme catalysis with more than two enzyme states and examine how conformational fluctuations affect the underlying rate law.

II. Results and Discussion

Our results indicate that for this extended reaction scheme, slow conformation fluctuations of the enzyme-substrate complex not only lead to a non- MM behavior, but manifest a decrease in the catalytic turnover rate at high substrate concentration - a phenomenon known as *excess substrate inhibition*.

Classically, substrate inhibition is assumed to take place when additional molecule of the substrate binds to allosteric site of the enzyme forming an enzyme substrate complex, which is catalytically inactive. Our results suggest an alternative way to achieve the same effect that does not require allosteric regulation but resultant from slow conformational dynamics of enzyme-substrate complex. To confirm the results of our numerical simulations we also construct an analytically tractable model demonstrating the substrate inhibition effect. Our model also provides a theoretical evidence of the fact that a simple reaction network can exhibit bistability when substrate inhibition is in action.

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