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Enzyme dynamics point to stepwise conformational selection in catalysis

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Abstract

Recent data increasingly reveal that conformational dynamics are indispensable to enzyme function throughout the catalytic cycle, in substrate recruiting, chemical transformation, and product release. Conformational transitions may involve conformational selection and induced fit, which can be viewed as a special case in the catalytic network. NMR, X-ray crystallography, single-molecule FRET, and simulations clearly demonstrate that the free enzyme dynamics already encompass all the conformations necessary for substrate binding, preorganization, transition-state stabilization, and product release. Conformational selection and substate population shift at each step of the catalytic turnover can accommodate enzyme specificity and efficiency. Within such a framework, entropy can have a larger role in conformational dynamics than in direct energy transfer in dynamically promoted catalysis.

Introduction

Evolution has optimized enzymes to selectively and efficiently catalyze biochemical reactions. It is generally accepted that the catalytic power of enzymes largely derives from their ability to stabilize transition states, lowering the barrier that reactants have to pass to reach productive states. However, the clear and attractive transition-state theory does not account for the reaction story in its entirety mainly because of the enzymes' conformational ensembles and dynamics. On the basis of theoretical arguments, we suggested that the entire transition-state region, rather than a single saddle point, contributes to reaction kinetic; thus instead of optimizing the binding to a well-defined transition-state structure, enzymes are optimized by evolution to bind efficiently with a transition-state ensemble [1]. Further, we proposed that preexisting substates at the bottom of folding funnels of the free enzyme and population shifts can usefully explain enzyme pathways [2], and that there is no basic difference between funnels depicting ensembles of conformers of single molecules with loop, or domain motions, as compared to allosteric movements of subunits in multimeric

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quaternary structures. All states pre-exist, and evolution makes use of these for enzyme function [2]. Experimental evidence has strongly supported the role of conformational dynamics in catalysis [3]. Yet, the question of whether protein dynamics contribute to enzyme catalysis and if so to what extent [3,4^{●●},5[●]] has been a debated issue. Since it is very difficult to establish a clear-cut linkage between protein flexibility and enzymatic function by experiment, it has largely been addressed by integrating experiments with computations.

Enzyme single-molecule studies have clearly shown fluctuations in conformations and in catalytic activity over a broad timescale ranging from 1 ms to 100 s [6]. Barrier crossing is over a much shorter, femtosecond to picosecond timescale. Before and following the barrier crossing leading to products, enzymes apparently spend most of their time assisting the reactants to reach the transition state, and escorting exiting products. Here, we show that at every step enzymes select their appropriate conformation optimized for substrate binding, chemical reaction, and product release, reviewing the literature published since 2007 in this light. We also note that conformational selection is general, and was observed for protein–protein, protein–DNA, protein–RNA, and protein–small molecule interactions [7[●],8].

Conformational selection and population shift constitute the catalytic network

The classical Michaelis–Menten equation (1) (Figure 1) is based on the assumption that an enzyme has only one (or ensemble-averaged) conformation, in which enzyme E quickly binds with substrate S to form Michaelis complex ES, followed by a reaction to EP and release of Product P.

The conformational fluctuations of enzymes [6,9] lead to ensembles of the Michaelis ES and Product EP complexes and their related transition states [1,6]. Therefore, a more complete description of the overall catalytic process has to consider the interconverting conformers at each step, as in Eqn (2) (Figure 1), via ‘catalytic networks’ [6,10[●]]. Catalytic networks can be defined as parallel reactions where each reaction step has multiple enzyme conformers in equilibrium. Even though each ‘single molecule’ does not necessarily exhibit Michaelis–Menten (MM) steady-state kinetics [11], in most cases, a single fluctuating enzyme can either follow or be reconciled with ensemble-averaged MM kinetics [6,11]. Eqn (2) indicates that many enzyme conformations pre-exist allowing the substrate to bind different conformers [12]. The populations of the Michaelis complex ES and Product complex EP will shift upon binding, depending on time and environment. Collectively, the conformational dynamics of the Michaelis ES and the Product EP complexes affect the overall turn-over rate, which corresponds to the most important transition-state barrier crossing (K2) and the product release steps (K3). Single-molecule studies indicate that the catalytic velocity of a single enzyme molecule fluctuates among the catalytic networks over a broad range of time-scales, from 10^{-3} to 10 s [6] among the catalytic networks (Eqn (2), Figure 1) indicating a strong dynamic influence; however, the question as to whether the dynamics of Michaelis complexes ES can contribute to barrier crossing is highly debated, and the dynamics of the EP complexes are usually less noticed.

Eqn (2) (Figure 1) can suggest two simplified scenarios for the relative contribution of 'induced fit' and 'conformational selection' to the overall catalytic mechanism. If one neglects the conformational fluctuations of the Michaelis ES and the Product EP complexes, conformational selection can be 'narrowly' defined by Eqn (3) (Figure 1). At the other extreme, if one ignores the conformational substates of the apo enzyme and only considers the conformational changes of Michaelis complexes ES, an induced fit mechanism can be described by Eqn (4) (Figure 1). The relative contributions of induced fit or conformational selection may depend on on-rate K_1 and off-rate K_{-1} [13[●]] and can be examined using the flux through conformational selection (Eqn (3)) and induced fit (Eqn (4), Figure 1) pathways [14[●]]. In the case of NADPH binding to dihydrofolate reductase (DHFR), at a very low ligand concentration about 80% of the flux goes through the conformational selection pathway, but induced fit rapidly dominates as ligand concentration increases. For the *Desulfovibrio desulfuricans* flavodoxin folding and cofactor binding, which is an example of an apparently exclusive induced fit mechanism, the flux goes through each path as a function of the holoprotein concentration [14[●]]. This work indicates that both conformational selection and induced fit pathways are often used, essentially supporting the complete description in Eqn (2) (Figure 1).

Sullivan and Holyoak [15] used phosphoenolpyruvate carboxykinases (PEPCK) as a representative enzyme in which transition between the open and closed conformations occludes the active site from the solvent. The crystal structures of the PEPCK revealed that the active site is lid-gated. Since a closed form does not allow substrate binding, it was argued that induced fit rather than conformational selection must operate [15]. However, crystal structure snapshots do not provide the entire conformational dynamic picture, particularly of loop movements. If loop dynamics are fast, its structure cannot be solved by X-ray crystallography. This suggests that snapshots often cannot directly obtain adequate conformational sampling. As conformational sampling indicated for bacterial phosphotriesterase [16[●]], maltose-binding protein [17], and choline oxidase [18], many lid-gated enzymes have both closed and open conformations in equilibrium before ligand binding, suggesting that the binding mechanism of lid-gated enzymes could also follow a conformational selection pathway [14[●]]. The opening–closing binding site dynamics may couple with large domain motions, as in the case of the cytochrome *bcl* complex (Figure 2) [19]. In summary, the catalytic network in Eqn (2) (Figure 1) with conformational selection and population shift could provide a more comprehensive view of enzyme action. The range of binding constants (K_1) and conformational flexibility of ES/EP complexes can be tuned to enzymatic function. One example is the versatile cytochrome p450, which recognizes a broad range of substrates. Tight binding ligands can restrict the conformational substates, while weak binders can broaden the distribution [20].

Enzyme specificity can be controlled through multiple, consecutive conformational selection steps

To allow active site substrate selectivity, transition-state stabilization, and product release, enzymes have to balance specificity and efficacy. A 'lock-and-key' mechanism could provide 'perfect' selectivity. However, enzymes classified as following a lock-and-key

mechanism also present a dynamic conformer selection process. Serine protease is conventionally regarded as fitting the rigid lock-and-key model. However, nanosecond timescale binding loop movement was observed to select an inhibitor conformation [21]. The flaviviral nonstructural 3 protease (NS3pro), a chymotrypsin-like serine protease also presents conformational selection to facilitate substrate binding and product release, promoting the formation of the catalytically competent oxyanion hole [22].

Evolution appears to have optimized the enzyme's conformational substates toward its successive tasks, substrate binding, transition-state stabilization, and product release. As shown in Scheme 1 and Eqn (2) (Figure 1), conformational fluctuations take place at all catalytic stages. Thus, compared with the lock-and-key rigid specificity for substrate binding or transition-state stabilization, the multistep conformational selection mechanism could similarly provide a rigorous selection. For example, even though each stage yields only a 50% correct selection ratio, over all three stages it can reach 87.5%.

A first step toward validation of such a mechanism would require a demonstration that the enzyme can have all prior conformations with conversion speed comparable to enzyme turnover rate. Such evidence has emerged recently [23,24,25^{●●},26^{●●},27]. In a study combining experimental and computational approaches, Henzler-Wildman *et al.* identified conformational substates along the reaction trajectory of adenylate kinase, and determined the timescales for the transitions among them [23]. They observed that the hierarchy of timescales in protein dynamics is linked to enzyme catalysis, with picosecond to nanosecond timescale fluctuation in the hinge regions of the adenylate kinase facilitating the large-scale, slower lid motions that produce a catalytically competent state. Using NMR relaxation experiments, the dynamics of the prolyl *cis-trans* isomerase cyclophilin A (CypA) were studied in the enzyme's substrate-free state and during catalysis. Characteristic enzyme motions detected during catalysis were observed in the free enzyme state with frequencies corresponding to the catalytic turnover rates. This correlation could suggest that protein motions necessary for catalysis are an intrinsic property of the enzyme and might even limit the overall turnover rate [24]. The direct link between the intrinsic motions and the catalytic turnover rate was revealed using ambient-temperature X-ray crystallographic data collection and automated electron-density sampling of interconverting substates of the human pro-line isomerase CypA [25^{●●}].

DNA polymerases may have the highest specificity pressure to ensure fidelity. Conformational transitions which are involved in the series of steps could assist in safeguarding against errors. Before substrate binding, DNA polymerase I samples the open and closed conformations in millisecond timescale [26^{●●}]. DNA polymerase μ (pol μ) has a rate-limiting 'precatalytic translocation step' to ensure accuracy and retain efficiency [27]. The conformational dynamics of the Y-family DNA polymerase Dpo4 was also shown to control its selectivity [28,29]. Of particular note is the flexible region surrounding the H-helix of the thumb domain, which selects the correct Watson-Crick base pair [28]. In the DNA polymerase Klentaq1, there is a fast equilibration between the fingers subdomain in the open and closed states. Even though the difference in the ground-state binding affinity to each of the dNTPs is very small, the enzyme's selectivity between Watson-Crick and non-Watson-

Crick base pairs amplifies small differences [30]. A similar control exists for ribonucleases [31,32].

Why can product release be a rate-limiting step and conformational dynamics of the EP complex control the enzymatic reaction? Intuitively, if the product cannot be stabilized, it could go back to become a reactant and the reaction is reversed. In ribonuclease A (RNase A), the product release-related conformational change limits the overall catalytic rate [33]. Formamidopyrimidine-DNA *N*-glycosylase provides an example of control by product release [34]. In addition, nonequilibrium dynamics may also be involved in retaining a favorable enzyme configuration if product release follows a slow conformational relaxation, resulting in dynamic acceleration of enzyme turnover at high substrate concentration [35●].

Conformational substates may help transition-state barrier crossing

With enzyme conformations changing constantly over varied timescales and catalytic steps, one can wonder if the energy transfer accompanying these changes can be used to help decrease the transition-state barrier. Kamerlin and Warshel asked ‘At the dawn of the 21st century: Is dynamics the missing link for understanding enzyme catalysis?’ [4●●]. Pisiakov *et al.* have shown that enzyme millisecond conformational dynamics do not couple with fast motions, since the specific memory of fast motions dissipates during the slow conformational fluctuations [36].

Coupled motions spanning femtoseconds to milliseconds in DHFR catalysis were proposed to promote catalysis already years ago [37]. These coupled motions explain nonadditivity effects in the hydride transfer rates for mutations distal to the active site [38]. NMR studies found that each intermediate in the catalytic cycle of DHFR samples low-lying excited states whose conformations resemble the ground-state structures preceding and following the intermediates. The maximum hydride transfer and steady-state turnover rates are governed by the dynamics of the transitions between the ground and excited states of the intermediates [39]. Computational analysis revealed that protein dynamics can be changed by ligand binding via network-bridging effects which modify the residue interaction network in the protein [40]. Such effect was recently confirmed by NMR relaxation dispersion measurement, showing that the dynamics of the substrate and product binary complexes are governed by quite different kinetic and thermodynamic parameters [41]. However, even though these works revealed that structural fluctuations in DHFR are exquisitely optimized for every intermediate in the catalytic cycle [41], the exact motion that helps the hydride transfer remains to be unveiled. Evidence in support of promoting vibration modes was reported for a similar system (pentaerythritol tetranitrate reductase) with a hydrogen tunneling mechanism [42●].

The transition-state region is often energetically flat, with a range of structures which are very close in energy [1,43]. Substates with a large population may lead to transitionstate ensemble [1,44,45]. Thermodynamically, a barrier could decrease due to an entropy compensation effect [1,46]. Therefore, entropy can have a larger role in the conformational change dynamics than in direct energy transfer [47●]. The entropy contribution is also directly related to the complex scenarios described in Eqn (2) (Figure 1).

Conformational dynamics may greatly facilitate the positioning of the substrate toward barrier crossing or product release through a preorganization mechanism [4^{●●}]. RNA polymerase has a trigger loop (TL) which can fold into α -helices (TH) during catalysis. TH formation helps in the geometrical alignment of reactants in the enzyme's active site, allowing rapid nucleotidyl transfer [48]. Conformational dynamics-controlled substrate alignments which in turn control catalysis are also observed in other systems [49,50]. The bacterial phosphotriesterase has both closed and open conformations in the apo state. The closed conformation is ideally preorganized to lower the reaction barrier, but it is not compatible with product release. In contrast, the open conformation is better organized for product release but not for chemical reaction [16[●]].

Enzyme conformational dynamics raise an interesting question related to the 'internal friction' [51] incurred during the barrier crossing, and the movements of the substrate or product in the protein. Around the barrier crossing area, the coupling between the protein conformational kinetics and the reaction kinetics can increase the transmission coefficient by a small ratio and Kramers' model may not be a good choice [52]. However, Kramers' model is very effective in measuring how viscosity will affect substrate or product diffusion and thus catalysis [51]. The generalized Kramers' model can explain the conformational dynamics of enzymatic reaction [53], predicting that intermediates along the reaction pathways can also promote barrier crossing [54]. Therefore, collectively, conformational transitions among the steps (as illustrated in Eqn (2)) will promote enzyme reaction.

Conclusions

Even though it is still highly debated whether the enzyme dynamic motions contribute to decrease the chemical reaction barrier, the consensus picture emerging from experimental and computational studies indicates that enzyme conformational transitions are highly organized increasing enzyme specificity and efficiency. It is striking that the enzyme structure has evolved to have all functionally relevant conformational substates [2], and that these are optimized for substrate binding, chemical reaction, and product release. Even enzymes catalyzing multiple chemical steps can still accommodate the conformational reorganization required to stabilize the transition states [55[●]] via pre-existing substates. We further note that the ensemble continuum between extreme conformations (open and closed) can also be controlled allosterically [56,57], since allosteric substates similarly pre-exist and undergo population shift [2]. Broader substrate specificities as in the multiply promiscuous hydrolase [58] or via allosteric mutations as in the directed evolution of an enantioselective Baeyer–Villiger monooxygenase [59] further highlight the conformational ensemble heterogeneity and the ruggedness of the free energy landscape. Such conformational dynamics suggest a description of enzyme turnover by a 'catalytic network', which initiates with conformational selection of substrate binding and proceeds via conformational substates population shifts which selectively stabilize transition states and facilitate product release. Induced fit can be viewed as a specific pathway within this broad spectrum. Multiple conformational steps can boost enzyme specificity and reaction rates regardless of whether the rate-limiting step is substrate binding, pre-organization, chemical reaction, or product release.

Ideally, substrate-binding energy can be used to promote both ground-state association and transition-state stabilization, or be translated into barrier crossing promoting motion. However, even though experimental evidence implicates vibration assistance in hydrogen tunneling, additional experimental data are needed to reveal the complex energetic benefit of the promoting motions. Thermodynamically, entropy can have a larger role in the conformational change dynamics than in direct energy transfer in catalysis. Enzymes can also use multimers to perform complex tasks. In the case of the homing endo-nuclease I-AniI, the two monomers in the dimer have different specificities: one for substrate binding, the other for transition-state stabilization [60]. Since the dimer is symmetric, conformational changes must exist to accommodate the two different tasks. A possible mutual conformational selection between DNA and a dimeric protein can be best illustrated by the glucocorticoid receptor (GR), in which DNA tailors the activity of the receptor toward specific target genes via a sequence-specific allosteric mechanism [8,61].

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Figure 1.

Conformational fluctuations of enzymes lead to 'catalytic networks'. Eqn (1) is the classical Michaelis–Menten equation. Eqn (2) considers that the conformational ensemble of enzymes allows multiple catalytic reactions to occur in parallel, forming catalytic networks. Eqn (3) illustrates a narrowly defined conformational selection pathway, where only unbound enzyme has multiple conformers while the Michaelis ES and the Product EP complexes are not affected by conformational fluctuations. Eqn (4) is an induced fit mechanism where substrate binding leads to an ES complex with different enzyme conformation.

Figure 2.

Conformational dynamics of the binding site for the extrinsic domain of the iron-sulfur protein (ISP-ED) subunit in the cytochrome *b* (cyt *b*) subunit of the cyt *bc*₁ complex is coupled with large motion in ISP-ED (Ref. [18]). MD simulations of the *bc*₁ complex from the *R. sphaeroides* (*Rsbcl*) with the bound inhibitor stigmatellin reveal that if no inhibitor exists, the binding pocket opens (B Ma, L Esser, R Nussinov, D Xia, unpublished). The *Rsbcl* catalyzes the electron transfer from ubiquinol to cytochrome *c* and simultaneously pumps protons across the membrane. In the right panel, the simulated nonsymmetrical dimer complex shows that the inhibitor (blue balls) is bound to only to one cyt *b* subunit (depicted by a red ribbon), while the other cyt *b* subunit (green ribbon) has an apo binding site. The yellow ribbons are cyt *c*₁ subunits. The ISP subunit with the 2Fe–2S clusters are shown in pink ribbons. The ISP-ED can have a large motion accompanying the electron transfer reaction. In the left panel, two *b* subunits are superimposed to compare the binding site changes. While the bound subunit (red ribbon) keeps the closed form, the apo subunit (green ribbon) opened up during simulation.

Scheme 1.

Multiple conformational selection steps can boost enzyme specificity. This hypothetical scheme illustrates that if there is only 50% specificity for each of the three steps, only 12.5% lead to product release, assuming that the rates of other pathways are slow.