Published in final edited form as:

Chem Phys. 2014 October 16; 442(17): 132–136. doi:10.1016/j.chemphys.2014.02.018.

The enzymatic reaction catalyzed by lactate dehydrogenase exhibits one dominant reaction path

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Abstract

Enzymes are the most efficient chemical catalysts known, but the exact nature of chemical barrier crossing in enzymes is not fully understood. Application of transition state theory to enzymatic reactions indicates that the rates of all possible reaction paths, weighted by their relative probabilities, must be considered in order to achieve an accurate calculation of the overall rate. Previous studies in our group have shown a single mechanism for enzymatic barrier passage in human heart lactate dehydrogenase (LDH). To ensure that this result was not due to our methodology insufficiently sampling reactive phase space, we implement high-perturbation transition path sampling in both microcanonical and canonical regimes for the reaction catalyzed by human heart LDH. We find that, although multiple, distinct paths through reactive phase space are possible for this enzymatic reaction, one specific reaction path is dominant. Since the frequency of these paths in a canonical ensemble is inversely proportional to the free energy barriers separating them from other regions of phase space, we conclude that the rarer reaction paths are likely to have a negligible contribution. Furthermore, the non-dominant reaction paths correspond to altered reactive conformations and only occur after multiple steps of high perturbation, suggesting that these paths may be the result of non-biologically significant changes to the structure of the enzymatic active site.

Keywords

enzymatic catalysis; reaction coordinate; transition path sampling; lactate dehydrogenase

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Introduction

Enzymes accelerate chemical reactions by many orders of magnitude as compared to solution chemistry [1, 2]. As a result of this remarkable rate enhancement, the exact nature of chemical barrier crossing in enzymatic reactions is of great interest to the field of biophysical chemistry [3]. The catalytic power of enzymes has been traditionally attributed to the ability of the active site to preferentially bind the chemical transition state, a phenomenon known as transition state stabilization [4]. While efforts to create artificial catalysts based on this theory have achieved catalytic rate enhancements on the order of 10^8 [5], other effects must be considered in order to fully explain the rate enhancements routinely achieved by enzymes (in the range of 10^{17} to 10^{20}) [2]. While there is evidence that the preorganization of the enzymatic active site can account for this discrepancy [6], there is a growing body of work suggesting that dynamic effects in the enzymatic reaction coordinate contribute significantly to catalysis [1, 7-9].

In light of the current controversy surrounding the origin of the extraordinary rate enhancements demonstrated by enzymes, a complete understanding of the calculation of enzymatic reaction rates is essential. A popular approach to predicting rates in chemical reactions in general is transition state theory (TST) [10]. In gas-phase reactions, implementing TST while assuming a singular transition state is sufficient for the calculation of reaction rates, but condensed-phase reactions, including enzymatic reactions, have free energy surfaces that are too complex to validate this assumption. Adaptations of TST to condensed-phase reactions [11-13] have indicated the presence of multiple paths in reactive phase space, but the contribution of multiple reaction paths to enzymatic reaction rates has yet to be fully elucidated [14].

The study of chemical barrier crossing in enzymes presents with a unique set of challenges. Enzymatic systems are optimized to increase the probability of a specific reaction, but chemical transformation in an enzymatic reaction is still a rare event. The lifetime of the transition state (about 10 femtoseconds (fs)) is only $1/10^{11}$ a complete enzymatic reaction cycle (approximately 1 millisecond) [1, 2]. Furthermore, enzymes are large, complex molecules with a manifold of possible conformations and dynamic transitions [15, 16]. The rarity of chemical events combined with the complexity of enzymatic systems creates a substantial obstacle to the study of chemical reactions in enzymes [17] and demonstrates the difficulty of locating multiple reaction paths.

One way of surmounting this problem is to apply transition path sampling (TPS), a Monte Carlo technique developed by Chandler and colleagues for studying rare events in complex systems [18, 19], to simulations of chemical reactions in enzymes. In order to implement this method, an initial reactive trajectory that connects the reactant and product basins must be produced. Once this is accomplished, the next step of the TPS algorithm is to perturb velocities and/or coordinates of all atoms in a randomly selected timeslice along the initial reactive trajectory. Using the resultant set of coordinates and velocities, the final step of the algorithm is to run dynamics forwards and backwards in time to generate a new trajectory. This is done iteratively until an ensemble of reactive trajectories, called a transition path

ensemble (TPE), is generated. A transition state ensemble (TSE), also referred to as the stochastic separatrix [20], can then be obtained by analyzing the set of reactive trajectories.

Our group has previously applied TPS to the reaction catalyzed by human heart lactate dehydrogenase (LDH) [21-23]. LDH catalyzes the interconversion of the substrate pyruvate and the cofactor NADH to the substrate lactate and the cofactor NAD⁺. This chemical transition consists of a hydride transfer from a donor carbon of the cofactor nicotinamide ring to a substrate carbon acceptor, in addition to a proton transfer from a donor nitrogen of an active site histidine to a substrate oxygen acceptor (Fig. 1). Prior work has shown that the hydride transfer always precedes the proton transfer in human heart LDH, and the time lag between these two transfers is usually less than 50 fs [21-23]. We have also demonstrated that a sub-picosecond protein motion, termed a promoting vibration, facilitates this chemical reaction and favors a short time lag by simultaneously decreasing the donor—acceptor distances for both the hydride and the proton. Furthermore, earlier results indicate that this reaction can be characterized by a highly condensed TSE.

These previous TPS studies of the reaction catalyzed by human heart LDH have revealed that a markedly narrow reaction path is sampled by this enzyme. To ensure that this finding is not the result of an incomplete sampling of reactive phase space, we used a higher perturbation TPS algorithm in this study. While our previous TPS algorithm produced reactive trajectories with a frequency of approximately 26%, the algorithm used in this study generated an acceptance ratio of ~ 10%. We also employed both canonical and microcanonical path sampling algorithms to create two different types of TPEs. Perturbation was applied to the velocities in the microcanonical TPEs (mTPEs) and to the coordinates in the canonical TPEs (cTPEs). In this study, we created 10 independent TPEs (4 mTPEs and 6 cTPEs) of 50 reactive trajectories each. We found that, although alternate reaction paths are possible, the reaction catalyzed by human heart LDH is in fact dominated by one specific path through phase space.

Methods

Quantum Mechanics/Molecular Mechanics Simulation

The set-up for the enzymatic system outlined in this section is similar to those detailed in previous studies [21-23]. We used the CHARMM [24, 25]/MOPAC [26] interface and the CHARMM27 force field to conduct all molecular dynamics and quantum mechanics/molecular mechanics (QM/MM) simulation. As in previous studies, we used the 2.1 Å-resolution crystal structure of the human heart LDH homotetramer in a ternary complex with the cofactor NADH and the isosteric and isoelectronic substrate mimic oxamate (PDB ID: 1I0Z) [27] to generate the initial atomic coordinates for simulation. In order to achieve a reactive enzyme-substrate complex, we protonated the active site histidine (His193) and substituted the oxamate nitrogen with a carbon to create the pyruvate ligand. We generated the parameters for the pyruvate ligand previously [28]. We solvated each system with TIP3P explicit water models [29] and neutralized the charged ternary complex with potassium ions to ensure physiological relevance.

Since LDH exhibits no cooperativity, only the active site of one monomer was treated quantum mechanically using the AM1 semiempirical method [30]. This partition consisted of 39 atoms: 17 atoms of NADH, 13 atoms of protonated His193, and 9 atoms of the substrate pyruvate, or 16 atoms of NAD⁺, 12 atoms of neutral His193, and 11 atoms of the substrate lactate. We linked the quantum mechanical and classical mechanical regions via two boundary atoms (the alpha-carbon of the active site histidine and the NC1' carbon of the cofactor) using the generalized hybrid orbital (GHO) method [31]. For all dynamics performed, we used 1 fs as the timestep for integration. To minimize the potential energy of the system, we ran 300 successive steps of steepest descent (SD) followed by 700 steps of adopted basis Newton-Raphson (ABNR). We then heated the system from 0 K to 300 K over the course of 300 picoseconds and equilibrated using QM/MM dynamics at 300 K for 500 picoseconds.

Transition Path Sampling

We generated 10 transition path ensembles (TPEs) of 50 reactive trajectories utilizing high perturbation transition path sampling (TPS). This method is similar to standard TPS [18, 19] with slight alterations. As the first step of our TPS algorithm, we designated the reactant (pyruvate) and product (lactate) basins using the reactive bond lengths as the order parameters. We considered a bond formed when the distance between two reactive atoms was ≤ 1.3 Å. To initially connect the pyruvate and lactate basins, we used constrained QM/MM dynamics to generate a 250 fs reactive trajectory.

For microcanonical TPEs, we performed both shooting and shifting moves [32, 33] to attain an acceptance ratio of approximately 10%. For the shooting moves, we selected a random timeslice along the reactive trajectory and perturbed the momenta of all atoms in the system to generate a new set of velocities. This perturbation was taken from a zero mean Gaussian distribution, multiplied by a scaling factor, and then rescaled to ensure there was no net angular or linear momentum and that total energy was conserved. To conduct shifting moves, we used the resultant set of coordinates and velocities to run 20 fs of molecular dynamics while maintaining a zero net angular or linear momentum and conserving kinetic energy. Using the velocities generated during the last step of this dynamics run and the coordinates of the original timeslice, we propagated QM/MM dynamics forward and backward in time to create a new trajectory of 500 fs, conserving total energy. If this new trajectory was reactive, it was accepted and used as the seed to generate another 500 fs trajectory.

To generate canonical TPEs, performing only shooting moves was sufficient to achieve the desired acceptance ratio of $\sim 10\%$. To implement these moves, we selected a random timeslice along the reactive trajectory and perturbed the coordinates of all the atoms in a similar manner as the perturbation of velocities for microcanonical TPS. Using these new coordinates, we propagated QM/MM dynamics forward and backward in time to create a new trajectory of 500 fs while maintaining a constant temperature using a Nose-Hoover thermostat [34, 35]. Since we assume microscopic reversibility for the transition between the old and new trajectories, we were able to use the following protocol to accept reactive trajectories in a way that satisfied detailed balance: The new trajectory was accepted if it

connected the reactant and product basins and A) the ratio of the probability of the new slice and the probability of the old slice was greater than 1 or B) the ratio of the probability of the new slice and the probability of the old slice was less than 1 and smaller than a random number drawn from a uniform distribution in the interval of 0 to 1 [32, 33].

Committer Analysis

We employed committer analysis [19, 21, 22] to determine the probability of product formation for a specific timeslice of a trajectory. To implement this method, we initiated QM/MM dynamics from a specific timeslice using random velocities to generate 50 trajectories of 250 fs each while maintaining the appropriate ensemble for the given TPE (microcanonical or canonical). We considered 50 randomly initiated trajectories to be sufficient for the analysis of a single timeslice [21, 22]. We discarded trajectories that did not reach either basin in 250 fs. After detecting at least two timeslices with non-integer committer values for a given trajectory, we fit this data to a cumulative Gaussian distribution and considered the time of barrier crossing of an individual trajectory to be the time required for the probability of lactate formation to go from 0.1 to 0.9.

Principal component analysis

To determine the similarity of reactive conformations, we performed principal component analysis on enzymatic structures near the transition state of individual trajectories. This has been shown to be a valid method of elucidating conformational sampling when the first and second principal components account for the majority of the variance in a data set [36, 37]. We used the coordinates of the alpha-carbon of each side chain to generate a matrix with dimensions of the number of structures by the number of residues (332 for one monomer of human heart lactate dehydrogenase). We projected each enzymatic conformation onto the plane of the two largest principle components.

Results and Discussion

Possible mechanisms for the reaction catalyzed by LDH

We observed three distinct reaction mechanisms for the human heart LDH reaction. These reaction mechanisms are listed and described as follows: Type 1) hydride and proton transfers occur within the timescale of 5 bond vibrations (~ 50 fs); Type 2) hydride transfers first and proton transfers after an extended time lag; and Type 3) proton transfers first and hydride transfers after an extended time lag. Not all TPEs explored every possible reaction mechanism, and the different types of reaction mechanisms occurred at varying frequencies in each TPE (Table 1). In earlier work, we showed that the reaction of human heart LDH mainly proceeds via a Type 1 mechanism but can also exhibit a Type 2 mechanism in certain instances [21-23, 38]. We have seen the Type 3 mechanism in a mutant of human heart LDH [21] and the bacillus version of this enzyme [38], but never before in the wild type human heart isoform.

In agreement with previous studies, the Type 1 mechanism was the most common for all trajectories generated in this study (80.2% of trajectories generated), whereas the Type 2 mechanism was more rare (18.2%) and the Type 3 mechanism was the most rare (1.2%). In

all but one TPE, mTPE1, the Type 1 mechanism was the most dominant. The prevalence of the Type 2 mechanism in mTPE1 may have been the result of this TPE becoming trapped in a region of phase space surrounded by a high free energy barrier. Out of the TPEs that sampled the rarer mechanisms, we found that 3 out of 4 of the mTPEs and 1 out of 3 of the cTPEs returned to Type 1 trajectories after exploring Type 2 or Type 3 mechanisms. This result also suggests that, while the TPEs generated generally converge to a low-energy ensemble, it is possible for an individual TPE, such as mTPE1, to become stuck in a high-energy state. The rarer mechanism (Type 2 and Type 3) also only occurred well into the generation of each TPE, indicating these mechanisms are only accessible after many steps of high perturbation of the LDH system. Figure 2 illustrates this point by showing the progression of a specific TPE (cTPE1) through reactive phase space.

Characteristic barrier crossings for each LDH mechanism

In addition to differences in the timing of hydride and proton transfers, we discovered that each mechanism could be characterized by a specific type of barrier crossing. We identified the time of barrier crossing (the period where the probability of product formation rises from 0.1 to 0.9) for individual reactive trajectories by performing committor analysis [19, 21, 22] on a subset of trajectories of each type. The barrier crossing for Type 1 trajectories was swift (average of 3 fs for 15 trajectories), while Type 2 trajectories presented with a more gradual barrier crossing (average of 60 fs for 5 trajectories). In the Type 3 trajectories, the barrier crossing was fast (average of 3 fs for 3 trajectories), and coincided with the moment of hydride transfer even though the proton transfer occurs first. Figure 3 shows the difference in the timing of barrier crossings for each mechanism. In Figure 3C, a large vibrational amplitude of the carbon-hydride bond after formation is also apparent. This increased amplitude occurs in the majority of the Type 3 trajectories we produced (4 out of 6), indicating that Type 3 trajectories are high-energy reaction paths.

Differences in transition state formation for each LDH mechanisms

For each mechanism, we observed the formation of distinct transition states. Here, we define the transition states as the points along the analyzed trajectories corresponding to a 0.5 probability of product formation as determined by committor analysis [19, 21, 22]. These differing transition states occurred concomitantly with variations in donor-acceptor dynamics for both the hydride and the proton (Fig. 4). In the Type 1 mechanism, the donor-acceptor distances for each particle were at their minimums along the reactive trajectory during transition state formation. The transition state also corresponded to partially formed bonds between both particles and their acceptors. Transition states of Type 2 trajectories exhibited a partially formed bond between only the proton and its acceptor and coincided with short donor-acceptor distances for both particles. The decreases in these distances were also more gradual as compared to Type 1 trajectories. In the Type 3 mechanism, transition state formation did not correspond to a short hydride or proton donor-acceptor distance, and the transition state was characterized by a partially formed bond between the hydride and hydride acceptor.

Rarer LDH reaction mechanisms have altered reactive conformations

We found that each mechanism also corresponded to specific reactive conformations of human heart LDH (Fig. 5). We implemented principal component analysis to compare enzymatic structures near the transition state regions of individual trajectories in a TPE generated previously via standard TPS [21] and in a TPE created using high-perturbation TPS. This analysis has been shown to be a valid method of detecting conformational sampling if the two largest principal components account for the majority of the variance in the data set [36, 37]. For the standard TPE and the high-perturbation TPE, the amount of variance that was explained by the first and second principal components was 84.5% and 64.6%, respectively. After projecting the reactive conformations of these two TPEs in the plane of the first and second principal components, we found that each mechanism localized to specific regions of reactive conformational space (Fig. 5). The conformations representing the rarer Type 2 and Type 3 mechanisms also clustered in regions of conformational space that were not explored in earlier work.

Conclusions

Although this study demonstrates that several different mechanisms are possible for the reaction catalyzed by human heart LDH, the Type 1 mechanism dominates the enzymatic reaction coordinate. Furthermore, we see that the majority of TPEs generated converge back to Type 1 trajectories after exploring rarer trajectory types. These results confirm the validity of previous TPS studies of human heart LDH which were able to atomistically describe this dominant mechanism. Since the different mechanisms observed in this work can be characterized by distinct barrier crossings, unique transition state formation, and specific reactive conformations of the enzyme, it is reasonable to assume that they also correspond to disparate paths through reactive phase space. Describing these reaction paths in full as opposed to relying solely on order parameters to delineate different reaction mechanisms also allows us to more comprehensively define the free energy landscape of the reactive phase space of LDH.

Though we show that the Type 2 and Type 3 reactive paths are allowed, their contribution to the enzymatic reaction rate is likely negligible. This is best exemplified by the frequency of occurrence of these mechanisms in the canonical TPEs (3.67% for Type 2 and 1.33% for Type 3). In a canonical ensemble, these frequencies are inversely proportional to the free energy barriers separating these specific reaction paths from other regions of phase space, so the probability human heart LDH reacting via a Type 2 or Type 3 mechanism is low. However, it is possible that a reaction path with a low probability of occurrence could still contribute to the overall reaction rate if that particular path also presents with a low barrier to reaction. Since we did not see a change in the acceptance ratio during the generation of Type 2 or Type 3 trajectories as compared to Type 1 trajectories, we can conclude that Type 2 and Type 3 reaction paths are not indicative of a significantly lower barrier.

The reaction paths corresponding to the Type 2 and Type 3 mechanisms were also only observed after many steps of high perturbation, suggesting that these rarer mechanisms could be the result of changes to the structure of the enzymatic active site. This conclusion is supported by the unique reactive conformations detected for each mechanism. Since the

changes in barrier crossing and transition state formation between mechanisms also coincided with differences in the dynamics of the hydride and proton donor-acceptor distances, it is also possible that each enzymatic reactive conformation might be distinguished by changes in the dynamics of the promoting vibration.

Acknowledgments

The authors acknowledge the support of the National Institutes of Health Grant GM068036.

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Highlights

- High-perturbation transition path sampling on the chemistry of lactate dehydrogenase.
- We find that multiple reaction paths are possible in this enzymatic reaction.
- In agreement with previous studies, one reaction path is dominant.
- The rarer paths are high energy and due to changes in enzymatic structure.

Figure 1.

Schematic of the reaction catalyzed by human heart lactate dehydrogenase shown from the side of pyruvate. The hydride and proton transfers are depicted in red and blue, respectively. Arginine 169 coordinates the substrate in the active site, and arginine 106 acts to polarize the bond of the substrate keto group.

Figure 2.

Progression of a TPE through reactive phase space. This graph shows all 50 reactive trajectories of an example TPE (cTPE1). The y-dimension is the time length of each trajectory (500 timeslices of 1 fs each), and the x-dimension is the trajectory number within the ensemble. The solid and dashed black lines follow along the points of hydride and proton transfer (respectively) for each trajectory. The blue region represents Type 1 trajectories, the orange region contains Type 2 trajectories, and the red region corresponds to Type 3 trajectories.

Figure 3.

Example trajectories for each LDH mechanism. Dynamic distances between the particles and the particle acceptors are red lines for the hydride and blue lines for the proton. Cyan regions correspond to the pyruvate basins, and the lavender regions represent the lactate basins. (A) Type 1 mechanism with a barrier crossing of 3 fs, (B) Type 2 mechanism with a barrier crossing of 78 fs, and (C) Type 3 mechanism with a barrier crossing of 3 fs.

Figure 4.

Transition state formation for each LDH mechanism. Example trajectories of (A) a Type 1 mechanism, (B) a Type 2 mechanism, and (C) a Type 3 mechanism are shown as graphs. The gray lines represent the transition state as determined by committor analysis, red dashed lines are the hydride donor—acceptor distances, and blue dashed lines are the proton donor—acceptor distances. Schematics of the transition states for each mechanism are explicitly shown with the hydride—acceptor bond in red and the proton—acceptor bond in blue.

Figure 5.

Reactive conformations of LDH. The black squares represent the first 50 reactive trajectories of a TPE previously generated using standard sampling methods, all of which display a Type 1 mechanism. The circles correspond to 50 reactive trajectories comprising a TPE generated via high-perturbation TPS (cTPE1). Blue circles represent trajectories which exhibited a Type 1 mechanism, whereas orange circles are trajectories that display a Type 2 mechanism and red circles are trajectories that react via a Type 3 mechanism.

Table 1

Relative frequencies of the different types of reaction mechanisms in each TPE.

Type	cTPE1	cTPE2	cTPE3	cTPE4	cTPE5	cTPE6	mTPE1	mTPE2	mTPE3	mTPE4
1 o	38	48	50	50	49	50	15	25	36	42
3	4	-	-	-	-	-	-	1	1	-