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Role of Protein Motions in Catalysis by Formate Dehydrogenase

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Abstract

We have analyzed the reaction catalyzed by formate dehydrogenase using transition path sampling. This system has recently received experimental attention using infrared spectroscopy and heavy-enzyme studies. Some of the experimental results point to the possible importance of protein motions that are coupled to the chemical step. We found that the residue Val123 that lies behind the nicotinamide ring occasionally comes into van der Waals contact with the acceptor and that in all reactive trajectories, the barrier-crossing event is preceded by this contact, meaning that the motion of Val123 is part of the reaction coordinate. Experimental results have been interpreted with a two-dimensional formula for the chemical rate, which cannot capture effects such as the one we describe.

INTRODUCTION

There has been continuing interest in the coupling of protein motions to the chemical step of enzymatic reactions. These include theoretical studies that have examined both fast femtosecond motions^{1–3} and slow conformational motions^{4–7} and a significant number of experimental studies.^{8–12} Some authors believe that dynamics does not play a role in catalysis,^{13,14} while others think that this disagreement is a matter of semantics rather than the substance.^{15,16}

A system that has attracted attention is formate dehydrogenase (FDH). Recently, Cheatum and co-workers¹⁷ studied the dynamics of *Candida boidinii* FDH (CbFDH) complexed with azide and nicotinamide (NAD⁺), using infrared photon-echo spectroscopy. The transition-state analogue of FDH, azide, is a good infrared probe. Unlike other experimental techniques that only probe global protein motions such as conformational fluctuations, two-dimensional infrared spectroscopy probes the dynamics around the chromophore, which can include the dynamics of the local environment around it. It can probe fluctuations on the ps time scale

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by measuring the frequency autocorrelation function of the vibrational chromophore. In most cases, this function can be fitted with exponential decay forms. However, in ref 17, it was found that in CbFDH, this function exhibits oscillatory motions with frequencies 9 and 24 cm^{-1} , which were found to be related to motions of the NAD ring. In a recent work,¹⁸ Cheatum and co-workers mutated a Val that lies behind the NAD ring to the smaller Ala and Gly and found that the activity of the mutants was reduced, and the KIE, which is temperature-independent on the native enzyme, became temperature-dependent. These KIE results indicate that while in the native enzyme, the reaction proceeds through tunneling, and in the mutants, the smaller residue behind the NAD ring leads to a larger average donor-acceptor distance, therefore to classical transfer.

A recently developed technique^{19–24} for probing the effect of protein motions is heavy (isotopically substituted) enzymes. The idea is to affect the frequencies of fast atomic motions in the enzymatic system without affecting the electronic potential energy surface that controls chemistry. Theoretical studies of heavy enzymes can direct mutagenesis experiments that improve the catalytic power of the native enzyme.^{25,26} Recent experiments by Cheatum and Kohen^{27,28} on heavy FDH showed complicated behavior. Experiments were performed for both singly labeled ^{15}N FDH and doubly labeled ^{13}C – ^{15}N . The ^{15}N -labeled FDH was found to have larger KIE, and the temperature dependence of the KIEs showed a slope that increased more than would be expected from a simple protein mass effect. These results suggest that there are complex interactions and possibly coupled dynamics effects in the active site.

The study of quantum hydride transfer in condensed phases is a challenging theoretical problem. Quantum electron transfer is simpler because tunneling takes place from the ground state and if a simplified two-dimensional representation is appropriate, the rate is given by the Marcus formula. However, the transfer of the heavier hydride takes place from excited states and rate calculations are complex even for simple model systems.^{29,30} It should also be pointed out that because the barrier-crossing event for a hydride transfer is of the order 10 fs, the free-energy barrier (an ensemble average) is not the barrier that the hydride traverses.³¹ Experimental results on systems with hydride transfer have often been interpreted in the framework of a Marcus-like model for the reaction rate, which has two limitations. First, as mentioned, excited states contribute to hydrogen transfer. Second, the Marcus formula is a two-dimensional model which cannot capture possible motions (other than donor-acceptor distance modulations) that are coupled to the reactive event. We will show below that this is indeed what is happening in FDH.

In this work, we use transition path sampling to study the chemical event, identify the reaction coordinate, and identify possible protein motions that are coupled to the catalytic event in FDH. We will briefly review earlier experimental and theoretical studies on FDH. Then, we will describe the simulation protocol, the TPS study, and analysis of the results.

FDH is a homodimer that catalyzes the NAD^+ -dependent oxidation of formate to CO_2 . It plays a key role in the energy supply of bacteria and in the stress response in plants.³² It is used as a catalyst for NADH regeneration in the synthesis of chiral compounds with NAD^+ -dependent dehydrogenases. Formate dehydrogenase is interesting as a model system for

analyzing the catalytic mechanism of dehydrogenases because the chemical event consists of a single hydride transfer without an accompanying proton transfer, and this hydride transfer is partially rate-limiting. A lot of the experimental work has been performed on *Pseudomonas* sp. 101 FDH (PsFDH). Because the sequence of residues is different than the CbFDH we will study, for clarity, we will number the residue sequence according to CbFDH, with the numbering in PsFDH in parentheses.

Analysis of crystallographic structures^{32–34} of PsFDH suggested that the cofactor and substrate are involved in multiple interactions in the active site. In Figure 1, we show the active site, with hydrogen bonds that are formed by important surrounding residues.

In solution, there is a competing reaction to hydrogen transfer, the nucleophilic addition of formate to NAD⁺. The substrate must be oriented so that the competing reaction is prevented. This is accomplished by three residues. The guanidinium protons of Arg258 (284 in PsFDH) form hydrogen bonds with one oxygen of the formate. Arg258 (284) is involved in substrate binding and it may support the catalytic conformation of the nicotinamide ring through electrostatic interactions.^{35,36} Mutagenesis of Arg258 to Ala leads to loss of enzymatic ability. The other oxygen of the formate forms hydrogen bonds with Asn119 (146) and Val93 (Ile122). The residue Ile122 in PsFDH becomes Val93 in CbFDH. This position can be occupied by different residues because the hydrogen bond with the formate oxygen is contributed by the nitrogen of the backbone.

His311 (332) forms a H-bond with an oxygen of the carboxamide group of NAD⁺ but may also form a H-bond with the formate oxygen that is coupled to Arg258 (284).³⁵ Mutagenesis of His311 to Phe leads to loss of enzymatic ability, but the exact role of this residue is debated. It was suggested that His311 may bend the nicotinamide ring at the TS into a “boat” conformation^{32,36} assumed to lower the activation barrier; however, this was not consistent with later experiments.

Two residues that are not shown in Figure 1, Thr256 (282) and Asp282 (308), form H-bonds with the carboxamide group of the cofactor, ensuring proper positioning and polarization of the nicotinamide ring. Pro68 (97) (not shown) lies behind the formate forming a hydrophobic wall. Behind the nicotinamide ring, Val123 (150) and Ile175 (202) also form a hydrophobic wall.

Popov³³ performed a search for conserved amino acids in 125 enzymes of the superfamily of D-specific 2-hydroxy acid dehydrogenases (these catalyze the reaction (*R*)-lactate + acceptor $\rightleftharpoons$ pyruvate + reduced acceptor). Arg258 (284), His311 (332), and Asp282 (308) were present in 100% of the enzymes examined, 79% had Val or Ile in position 93 (122), and Val123 (150) was conserved similarly to the amino acid in position 93.

Computational studies of FDH have identified important interactions in the active site, first, in classical simulations by Bruice³⁶ and, later, in QM/MM studies by Popov.³⁵ Free-energy calculations by Moliner,³⁷ with the use of the AM1 semiempirical Hamiltonian, found a free-energy barrier equal to 12.1 kcal/mol.

METHODS

The starting point was the crystal structure 5DN9 from the Protein Data Bank, CbFDH complexed with NAD⁺ and azide. We replaced the azide with formate using Accelrys DiscoveryStudio. All simulations were performed using the CHARMM molecular dynamics package.³⁸ The QM region consisted of the substrate, part of the cofactor, and parts of the residues His311 and Arg258 that have been shown to be crucial, as discussed in the previous section. His311 was protonated at the NE2 position. The generalized hybrid orbital (GHO) method³⁹ was used for coupling the QM and MM regions. The boundary atoms were C5D for NAD⁺, CB for His311, and CD for Arg258. The QM/MM partition scheme is shown in Figure 1. The QM region was described by the semiempirical AM1 method. Each protein was solvated within a 15 Å sphere using TIP3 waters and neutralized using potassium ions. We minimized the system with 50 steps of the Steepest Descent method, followed by 2000 steps of the adopted Basis Newton-Raphson method. The system was heated slowly to 300 K for 30 ps, starting with harmonic constraints except on H atoms and TIP3 waters that were gradually reduced to force constant 1. We started the equilibration with 15 ps with harmonic constraints of force constant 1, which were then removed and the equilibration ran for 60 ps.

We used transition path sampling^{40–42} to generate and analyze the ensemble of reactive trajectories. As order parameters that define the reactant and product basins, we used the donor-hydride and acceptor-hydride distances. The reactant basin was defined as a donor-hydride distance smaller than 1.6 Å and the product basin as an acceptor-hydride distance smaller than 1.6 Å. To seed the generation of the path ensemble, we generated a biased trajectory by applying a harmonic force between the hydride and the acceptor and allowed the system to propagate for 250 fs. New trajectories were generated by the shooting algorithm: we perturbed the momenta of a randomly chosen slice of a reactive trajectory, and using the new momenta, we allowed the system to propagate forward and backward in time for 250 fs. A total of 120 reactive trajectories were generated.

To analyze this generated reactive path ensemble, we employed committor analysis. For a given trajectory, the committor probabilities are obtained by selecting slices along the trajectory and initiating dynamics by assigning random momenta to the atoms. After generating several trajectories from each slice, we obtained the probability that these trajectories end up in reactants or products. The transition state of a trajectory is defined as the structure in a trajectory with an equal probability to commit either to reactants or products. The collection of transition states from the path ensemble is called the separatrix. Next, one uses this separatrix surface to search for the reaction coordinate, which are the degrees of freedom that are orthogonal to the separatrix. One makes an initial guess of residues that may be part of the reaction coordinate, the system is propagated while these residues are constrained, and commitment probabilities are calculated for slices along this restrained walk. If the guess for residues to constrain was correct, the system would remain on the separatrix and the committor probabilities will be peaked at 0.5. If it is peaked elsewhere, we make a different choice of residues to restrain and repeat.

RESULTS AND DISCUSSION

Before we present the trajectory analysis, we briefly compare some geometrical aspects with earlier computational work. In agreement with earlier work,^{35,36,43} we find hydrogen bonding between one oxygen of the formate and Asn119 and Val93, between the other oxygen and Arg258, and also between NH₂ of NAD⁺ and Thr256 and Asp282. In agreement with ref 35, we found that His311 switches hydrogen bonding between O7 of NAD and a formate oxygen. Finally, we ran a long nonreactive trajectory and found the angle C4N–H–CO₂ to be 75% of the time in the range 130–180°, that is, the transfer hydride faces the acceptor, in agreement with the QM/MM calculations of Popov.

The committor distribution that best approached the expected distribution for a reaction coordinate was obtained when only the QM region was included in the group of residues that were constrained along walks that originate in the separatrix. Including other residues that are close to the active site did not improve the results. This is strong evidence that motions of these residues are not coupled to the chemical step, even though they play important roles in properly positioning the substrate and in TS stabilization. It should be noted that two active-site residues, His311 and Arg258, were part of the QM region along with the substrate and the cofactor. In addition, analysis of distances in the reactive trajectories did not show any coordination of motions with the chemical step.

One limitation of the current methods of extracting information about reaction coordinates from a TPS ensemble is that they search for information only near the transition state because they search for directions perpendicular to the separatrix. In some systems, there could conceivably exist motions that prepare the system into a configuration that is favorable for the reaction. We show below that this is indeed what is happening in FDH.

We turn our attention to Val123, whose mutation in the work reported in ref 18 led to changes in the enzyme activity. During a trajectory, the nicotinamide ring oscillates and as a result, the acceptor moves toward and away from the donor. We noticed that in all reactive trajectories, at 150–200 fs before the reactive event, Val123 approached the acceptor C4 at van der Waals distance, thus preventing the acceptor from moving away from the donor, forcing the system to remain prepared for the reaction. The moment of the closest approach is far enough from the TS that cannot be captured by the committor analysis that searches for directions perpendicular to the separatrix. We show the donor-acceptor, bond-forming, bond-breaking, and Val123-acceptor distances for a representative trajectory in Figure 2.

We will now examine in more detail the role of the motion of Val123. In Figure 3, we show a density plot of all trajectories in the transition path ensemble. The difference of bond-breaking minus bond-forming distances is plotted on the horizontal axis and the distance between C_γ of Val123 and the acceptor on the vertical axis. One tabulates into a histogram the values of this pair of variables for all slices along all reactive trajectories. The colors represent the percentage of slices in all trajectories having the values of a particular point on this plane. The color scheme is the same as a topographical map: green is low in density and yellow, brown, and white have increasingly higher number of slices. As can be seen in Figure 3, on the way to the TS, most trajectories pass through a slice, far away from the TS,

where the distance between C_{γ} of Val123 and the acceptor is equal to the sum of their van der Waals radii.

To understand whether this close approach between Val123 and the acceptor carbon always leads to the reaction, we ran a 20 ps trajectory which remained nonreactive and monitored the donor-acceptor distance and the Val123-acceptor distance. We found that even in this nonreactive trajectory, there are instances when the donor-acceptor and Val123-acceptor distances are both short, but the reaction does not happen, increasing the possibility in the reactive trajectories. Another event takes place concurrently with the Val123-acceptor compression. We have identified this to be the near-attack angle donor-hydride-acceptor.

We plot the donor-acceptor and Val123-acceptor distances and the donor-hydride-acceptor angle in Figure 4 (from 3 reactive trajectories) and Figure 5 (from nonreactive trajectories). We note that in the reactive trajectories, when Val123 pushes the NAD acceptor carbon toward the donor, the donor, hydride, and acceptor are almost collinear. On the other hand, in the nonreactive trajectories, when the donor-acceptor distance reaches the minimum value, the near-attack angle has veered away from the reaction-ready values.

These results strongly suggest that the motion of Val123 is part of the reaction coordinate. This has consequences for the interpretation of experimental results. As mentioned earlier, such a multidimensional reaction coordinate makes the use of Marcus-like models inappropriate. In addition, free-energy calculations should include both the Val123 and the donor-hydride-acceptor angle in their definition of the reaction coordinate.

In our earlier work on rate-promoting motions,¹⁻³ the residue motions that compressed the donor-acceptor distance were taking place immediately before the barrier-crossing event, which was effectively happening over a fluctuating barrier. The effect of dynamics on the current system is different, it is happening well before the bond-breaking, and the energetic barrier to the reaction is not affected by dynamics, rather dynamics affect the way the system is prepared for the reaction.

In Figure 6, we plot the Fourier transforms of the distances shown in Figure 4. We mentioned earlier that 2D-IR experiments found oscillatory patterns in the frequency autocorrelation function at frequencies 10 and 24 cm^{-1} that were attributed to motions of the NAD ring. In Figure 6, we note the presence of peaks in the distance time series at frequencies 10 and 30 cm^{-1} , similar to the experimental values seen in the 2D-IR experiments. The time series are noisy, and at low frequencies, the resolution of peaks in a Fourier transform series is not optimal, but nevertheless, Figure 6 provides support to the attribution of the peaks in the 2D-IR experiments to motions of the NAD ring.

We mentioned earlier that it had been suggested that Val123 bends the nicotinamide ring in the TS into a “boat” conformation, which was inconsistent with later experiments. Our proposed role for Val123 leads to a different outcome, as can be seen in the snapshot of the TS of one of the generated reactive trajectories shown in Figure 7, where it is clearly seen that the nicotinamide ring is planar.

Finally, a Val at this position behind the nicotinamide ring plays a crucial role in the catalytic mechanism of other dehydrogenases. L-specific lactate dehydrogenases catalyze the same reaction as FDH, but in the opposite direction, with conversion of pyruvate to lactate with concomitant conversion of NADH to NAD⁺. They are evolutionarily unrelated to the D-specific lactate dehydrogenases that have been found to have sequence similarity with FDH. The active site of L-specific LDH has an Arg and His at exactly the same locations with respect to the substrate and the nicotinamide ring, as FDH has Arg258 and His311 (in human LDH, these are Arg109 and His195). The C4 atom of the NAD ring (the acceptor atom in FDH) is the donor atom in LDH and a Val that lies behind it was found^{44–48} to play an important role in catalysis: we have shown that it pushes the nicotinamide ring toward the lactate, shortening the donor-acceptor distance. Also, Klinman's work⁴⁹ in horse liver alcohol dehydrogenase has shown that mutating Val203 (which lies behind the nicotinamide ring) to a residue of smaller size leads to a reduction in both catalytic efficiency and tunneling.

In conclusion, we have shown that in the reaction catalyzed by FDH, the stochastic motions of Val123 are part of the reaction coordinate and they initiate the reaction-ready donor-acceptor compression. The interpretation we have suggested is consistent with experimental results¹⁸ where a mutagenesis of Val123 to a smaller residue such as Ala led to higher activity. Finally, this system highlights a lacuna in methods of extracting information from TPS trajectories because there is not yet a systematic way to identify events important for the reaction that take place far from the transition state. The only work we are aware that analyzed TPS trajectories and could identify events far from the transition state is ref 50, where machine learning methods were used. However, over one million trajectories were available for that analysis (transition interface sampling, a variant of TPS appropriate for systems with diffusive motion over the barrier, was used), making it impractical for typical enzymatic systems.

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Figure 1.

Active site of formate dehydrogenase. Arg258, Val93, and Asn119 form hydrogen bonds with the substrate. His311 during a reactive trajectory forms a hydrogen bond sometimes with the carboxamide group of NAD⁺ and sometimes with the substrate. We also show Val123, which lies behind the nicotinamide ring, that plays an important role, as we will show.

Figure 2. Bond-breaking, bond-forming, donor-acceptor, and Val123-acceptor distances for a typical reactive trajectory. The dashed vertical lines frame the segment of the trajectory that is analyzed in Figure 3.

Figure 3.

Projections of histogrammed densities of the structures along all reaction trajectories on the plane of the distance between C_γ of Val123 and the acceptor versus bond-breaking (D–H) minus bond-forming (H–A) distances. This map corresponds, roughly, to the area framed by vertical dashed lines in Figure 2. The color map (green for “plains”, i.e., zero density, up to brown for “mountains” and white for “snow peaks”, i.e., maximum density of structures) represents the allocation of a pair of the abovementioned geometric properties among structures along the reaction path.

Figure 4. Plots of the donor-acceptor and Val123-acceptor distances and the donor-hydride-acceptor angle for reactive trajectories. The units for the angle are shown in the right-hand side vertical axis. The location of the transition states is marked on the time axis.

Figure 5.
Similar as Figure 4 for nonreactive trajectories.

Figure 6. Fourier transforms of the distances shown in Figure 4. There are peaks near the frequencies of oscillations identified in 2D-IR experiments.

Figure 7. Transition state for a typical reactive trajectory. As can be seen, the nicotinamide ring has a planar conformation.