ELECTRIC FIELDS AND ENZYME CATALYSIS

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Status of the physical basis for enzymatic catalysis

This field, which is relatively new to my lab, has a long history with numerous textbook chapters and reviews. Despite textbook descriptions of “how enzymes work,” the descriptions do not agree and so this continues to be vigorously debated. In particular, the contribution of electrostatic interactions to catalysis, strongly advocated by Warshel [1] and other computational biochemists, has been difficult to evaluate experimentally due to a lack of local probes for measuring intermolecular interactions and electric fields with a consistent physical framework. While it is simple to measure rates and binding constants, both are complicated convolutions of contributions and therefore difficult to interpret within a consistent physical framework, even given X-ray crystal structures and multiple mutants. Much current work in this field is focused on directed evolution for improved or novel function [2], and primarily uses selection and screening strategies. De novo design [3], where it has been successful, tends to make extensive use of empirical correlations and heuristic models.

Recent Work from Our Lab on Enzymatic Catalysis

My group has been using Stark spectroscopy to characterize the displacement of charge (change in dipole moment or $\Delta \vec{\mu}$) for electronic and vibrational transitions for many applications [4]. $\Delta \vec{\mu}$ for electronic transitions in dye molecules leads to shifts in the absorption and emission maxima of dyes in solvents of different polarities, typically blue for non-polar solvents and red for polar solvents. A similar solvatochromic effect is observed for vibrational transitions, e.g. for the carbonyl group (–C=O). Vibrational Stark spectroscopy (VSS), which probes the effect of a known applied electric field (units MV/cm) on a transition, provides a measure for $|\Delta \vec{\mu}|$ (units of cm$^{-1}$/MV/cm) as illustrated in Fig. 1a. Note that $\Delta \vec{\mu}$ is a vector and for relatively localized high frequency vibrations, like –C=O and –C= N, the direction of this vector is expected and has been shown to lie along the diatomic bond axis (thus its orientation is known by X-ray crystallography when used as a probe in a protein). VSS is, in effect, a calibration step that
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establishes the sensitivity of the vibrational frequency (measured by IR or Raman spectroscopy) to an electric field. Once calibrated, the probe can be inserted into different environments and observed frequency shifts (Fig. 1b) interpreted in terms of changes in the electric field sensed by the probe going from solution into a particular site (e.g., in a protein), or the effect of titrating nearby charged groups, making a mutation, folding/unfolding, etc.

A key step towards obtaining experimental information on the absolute electric fields — the field “felt” by a substrate inside an enzyme active site and potentially lowering the activation barrier for catalysis — was to connect the VSS data to vibrational solvatochromism, illustrated schematically in Fig. 1c. As discussed
above, such frequency shifts in solvents of different polarity are well known, but by itself “polarity” is not a useful concept for precisely quantifying intermolecular interactions. This required the use of MD simulations with various force fields, all of which found a linear correlation between the average simulated value of the electric field in the solvent and the observed peak frequency in the IR, as shown for the carbonyl group of acetophenone in Fig. 1d (note that the field fluctuates in a simple solvent and this is reflected in the simulations; plotted in Fig. 1d are the time-averaged values) [5]. Both hydrogen bonded and non-hydrogen bonded carbonyl frequency shifts are found on the same correlation, and the slope of the correlation is found to be $|\Delta \mu|$ (see below). This experimental check gives confidence that the force fields used to calculate the electric fields are reasonable. Furthermore, the standard deviations of the simulated electric field distributions correlate well with the observed IR linewidths: going from non-polar to polar solvents the linewidth increases substantially (largely inhomogeneous broadening). Assuming that $|\Delta \mu|$ does not depend on field (a key but potentially testable assumption), a simple conversion is possible between the observed frequency (the vertical axis in Fig. 1d) and the average calculated absolute electric field (the horizontal axis), i.e. we can read off the field from observed frequencies (dashed lines in Fig. 1d).

This concept was applied to evaluate the electrostatic contribution to catalysis in the enzyme ketosteroid isomerase (KSI), introduced to our lab in collaboration with D. Herschlag where we studied changes in electric fields (Fig. 1b) around the active site using –SCN probes [7]. Figure 2a shows KSI’s mechanism established by work from many labs. Close inspection shows charge is displaced at the carbonyl group of the steroid substrate going from the $E\cdot S$ complex to the first intermediate, and we reasoned that a substrate-like inhibitor’s carbonyl group could be used to probe the electric field sensed by the substrate during this rate-limiting step [8]. The inhibitor 19-nortestosterone was bound to the active site of KSI and a very large shift to the red was observed, even compared with water (Fig. 2c). Following the conversion in Fig. 1d, this implies the electric field sensed by 19-NT’s carbonyl group is very large. Furthermore, the vibrational transition is sharp, suggesting a narrow distribution of electric fields in this equilibrium measurement, i.e. the enzyme creates and holds this very large field projected onto the bond involved in charge displacement during catalysis.

Several mutants of key active site residues were studied for which information had already been obtained on $k_{cat}$ by other labs. As $k_{cat}$ decreased, the observed 19-NT carbonyl group shifts to the blue; this data was recently supplemented by subtler, structure-preserving changes where Cl-tyrosine was introduced at each of the active site tyrosines [9]. As seen in Fig. 2d, a robust linear correlation between the activation free energy (obtained from $k_{cat}$ using transition state theory) and the observed electric field, derived from the measured frequencies using a conversion like Fig. 1d, was observed. The slope of this line gives information on the displacement of charge in the transition state relative to the $E\cdot S$ complex ($|\Delta \mu_{rxn}|$, Fig. 2b), and the
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Fig. 2. Mechanism, concept of electrostatic catalysis, and observed electric field catalysis of KSI. (a) 5-Androsten-3,17-dione, the steroid substrate, is converted to the conjugated isomer, 4-androsten-3,17-dione, via the enolization (first step) and reketonization (second step) of the carbonyl. (b) Schematic illustration of the possible effect of the enzyme electric field, $\vec{F}_{enz}$, to differentially stabilize the transition state relative to the bound substrate by interacting favorably with $\Delta \vec{\mu}_{rxn}$. (c) KSI exerts a very large and homogeneous electric field on 19-nortestosterone's carbonyl bond relative to water's reaction field, based on the significant red-shift and band narrowing detected in the IR spectrum [8]. (d) Linear correlation ($R^2 = 0.98$) between KSI's catalytic power (expressed as $\Delta G^\ddagger$ and the electric field its active site projects onto the carbonyl of the inhibitor 19-nortestosterone. Black points represent mutations of the oxyanion hole residues to other canonical amino acids [8]; colored points represent subtle mutations to non-canonical amino acids [9]. Figure adapted from Ref. [6].

intercept, extrapolated to zero electric field (i.e. no net electrostatic stabilization) suggests that more than 70% of the catalytic power of this enzyme is due to the lowering of the transition state free energy by the electric field (the remainder is largely entropic). We believe that this approach is general, and it has now been used to re-interpret data from other groups already in the literature [10].

Outlook to future developments of research on enzymatic catalysis

Technical issues: The frequency-field conversion shown in Fig. 1d should have a slope equal to the independently measured Stark tuning rate (by VSS). However, we have consistently found that they differ by a factor of 2. We believe that the largest source of discrepancy is that the external electric field in the VSS measurement is not the field felt by the probe because of the “local field correction,” a longstanding source of uncertainty in a variety of spectroscopies. While a consensus value around 2 appears appropriate, as discussed in depth in [11], some variation has been observed [10]. The discrepancy may also reflect systematic errors in the
force fields used to calculate the field in solvents, and more sophisticated methods may be required. This affects the value extracted for $|\Delta \vec{\mu}_{\text{rxn}}|$, but not the value of the activation free energy extrapolated to zero field (Fig. 2d). A second technical issue concerns \textit{in situ} measurements of the Stark tuning rate to be certain that $|\Delta \vec{\mu}|$ is unaffected by the environment. While we have good evidence that this is the case in fields spanned by simple solvents [12], and \textit{ab initio} calculations suggest that $|\Delta \vec{\mu}|$ is mostly unaffected for the fields of the magnitude created by environments observed to date [8], this should be confirmed \textit{in situ}, with the probe in enzyme active sites. The problem is S/N, a limitation that might be overcome by use of high intensity tunable IR sources instead of a conventional FTIR.

\textbf{Conceptual issues:} A subtle but important issue concerns the direction of the dipole of a substrate undergoing catalysis in its ground state relative to an altered geometry in the transition state (Fig. 2b). For KSI, this direction likely does not change substantially, but for cases where the transition state geometry is very different from that of the bound inhibitor, one only obtains the projection of the field probed by the inhibitor’s geometry [6, 10]. Thus, probe/inhibitor design is critical. Ideally, what is needed is the ability to probe the electric fields as experienced by both ground and transition state analogs. Catalysis by electrostatic preorganization would imply that an enzyme would maximize the field projected onto the TS geometry compared to that projected onto the GS geometry. In conjunction with binding measurements and crystal structures, this has the potential to directly report on the functional fields relevant for catalysis from the perspective of the substrate, providing design principles for how to engineer active sites for electric field catalysis. It would be very interesting to use the vibrational Stark effect as a quantitative metric during computational enzyme design or as an observable during screening. It would also be very interesting to investigate the relationship between the evolutionary history of an enzyme and the electric field it creates. Finally, while we have shown that electric fields are correlated with increased rates, a large electric field in and of itself is not diagnostic of a good catalyst. This is clearly exemplified by water, where the average electric field and field distributions are large, though water, in general, is a poor catalyst [1]. The electric fields measured in aqueous solution and in enzyme active sites represent different manifestations of the electric field with respect to catalysis by transition state stabilization, a point that is often misunderstood [6]. One might explain these observations by pointing out that the electric field generated by a preorganized active site specifically stabilizes the transition state, while the field generated by solvent reorganization generally stabilizes the ground state, though this point needs to be investigated further.

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References