VIBRATIONAL SPECTROSCOPY

Electric field maps in enzymes

How electric fields generated by enzyme active sites push and pull on substrates is important to their chemistry, but measuring them is difficult. Now, the electric field within an active site has been measured along two directions using a vibrational probe, revealing that the field effect in enzymes is different compared with that in bulk solvents.

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Do enzymes offer a special electrostatic environment for catalysing reactions? How is such an environment different from that offered by solvent molecules organized around a reactant? And is the electrostatic environment of enzymes the key to their exceptional catalytic power? These questions, which reside at the intersection of several fields, including catalysis, biochemistry, mechanistic organic chemistry and electrochemistry, have puzzled chemists of many stripes for decades, challenging and taunting those who seek to design enzyme mimics. And answering them is difficult, because the inner environment of an enzyme, which features internal fields generated through the combination of charged amino acids and their molecular dipoles, is inaccessible to many of the conventional tools used to measure electrostatic fields. One method by which to study the internal fields within enzymes is vibrational Stark-shift spectroscopy[1], which uses probe molecules that have vibrational signatures that are sensitive to electric fields. Thus, one may infer the electric field within a given enzyme by measuring the shift in the vibrational frequency of the probe molecule when compared with the frequency of the probe in a reference environment outside the enzyme.

Taking a step back, what if we forgo an enzyme and place a molecule in a solvent? Will the molecule feel an electric field? The simplest understanding of electrostatic solvation is that the solute molecule will organize the solvent molecules around itself, thereby creating a cavity. The field from the dipoles of the solvent do not cancel within this cavity owing to the organized nature of the solvent molecules, and if the solute happens to be a Stark-shift probe its frequency will shift proportional to the effective field in the cavity. In most solvents, such an effect is dominant and the resulting field will shift the frequency of the probe to lower values, that is, cause a redshift.

Now, writing in *Nature Chemistry*, Boxer, Markland and co-workers report[2] that the electric field within an enzyme is quite different from that within a solvent cavity. The Boxer lab has worked on the electrostatics of enzymes for many years using vibrational Stark spectroscopy, and now they have used a probe molecule with not one but two tractable vibrations, both with sensitivity to the electric field. In their molecule, a deuterated form of *N*-cyclohexylformamide (CXF-D), the C=O and C–D stretches are located next to each other at an angle of nearly 120° (Fig. 1), enabling them to measure the fields experienced by the probe along two directions.

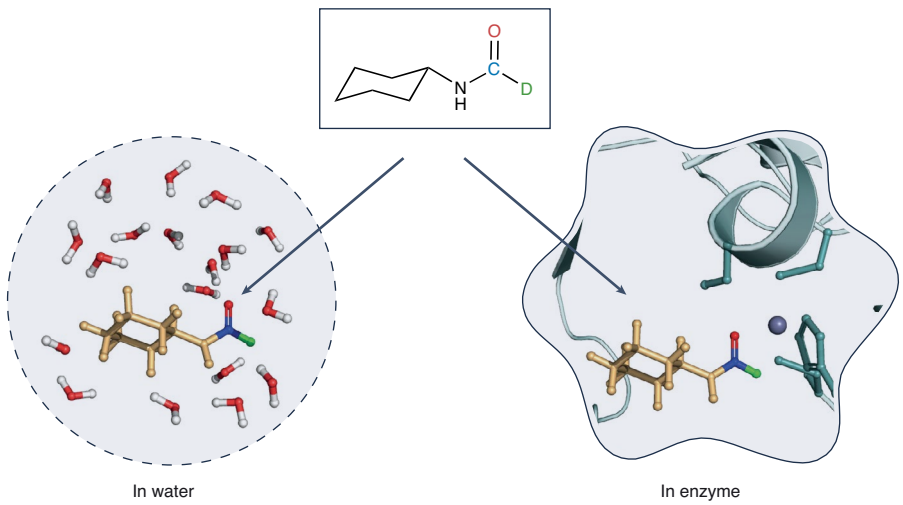

Fig. 1 | The molecule, CXF-D, studied using vibrational Stark-shift spectroscopy. The vibrational frequencies of C=O and C–D were measured in different solvents and the changes in frequencies were ascribed to different electrostatic environments around the C=O and C–D bonds. The molecule was then inserted into an enzyme and the frequency shifts were compared with those within the solvents. Figure reproduced with permission from ref. [2], Springer Nature Ltd.

First they established the sensitivity of both modes within CXF-D to an electric field through a series of experiments and molecular dynamics simulations. Measuring two vibrations at the same time and identifying correlations between their frequency shifts is key to this study. Then they measured the vibrational frequency shifts of the probe in several solvents. In addition to electrostatics, hydrogen bonding is also important to the frequency shift of the C=O and therefore their series of solvents included water as a hydrogen-bonding solvent.

Their first important finding was that the frequencies of the two vibrational modes changed in an anti-correlated way. That is, for solvents that cause the C=O frequency to redshift, the C–D frequency blueshifts — an effect observed over the entire series of solvents. They propose that this difference is because the C=O mode has a larger role in organizing the solvent molecules around itself owing to its larger dipole, and thus it redshifts as expected. However, the resulting field in the solvent cavity is such that the C–D stretch blueshifts.

After establishing the behaviour of the probe molecule in solvents, Boxer, Markland and co-workers put it in an enzyme — a liver alcohol dehydrogenase inhibitor. They saw that the frequencies of the C=O and C–D do not behave according to the correlation

found in the solvent series, showing that the electrostatic environment within the enzyme cavity is nothing like that of a bulk solvent. While a solvent cavity is formed by the combined interaction of solute and solvent — resulting in a field that induces correlated frequency shifts of C=O and C–D — in the enzyme, the electrostatics are mainly dictated by the protein residues, and the probe molecule complies with the enzyme field. The protein, in this case, exerts a heterogeneous field on the probe that is unlike any of the solvents that are studied.

The importance of the work from Boxer, Markland and colleagues is not only in the improved understanding of this enzymatic reaction but also in it serving as a guiding principle for the creation of better catalysts more generally and the creation of enzyme mimics. It highlights the importance of electrostatic engineering on the molecular scale. There have been sustained efforts to engineer the electrostatic environment created by molecular catalysts, such as appending ions near metal centres via crown ethers[3,4]. Similarly, promising efforts have been made in engineering the electrostatic environment near electrodes by using charged surfactants[5]. The work by Boxer, Markland and colleagues highlights that in all of these efforts, one must think beyond merely duplicating a solvent cavity of high dielectric constant. Molecular designs under these efforts need the appropriate mix of rigidity and flexibility in the scaffolds to create a heterogeneous field tailored for a given reaction. And what is next for Stark-shift spectroscopy? Perhaps even more detailed analyses of electrostatic correlations in solvents and enzymes using probes with several measureable vibrations. ❐

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Competing interests

The authors declare no competing interests.