structure is a prerequisite for chemical activity, protein engineering is still caught up in this problem.

Although a general solution to the protein folding problem would greatly help design, solutions for simplified cases (*1*, *5*), such as for short, idealized, or repetitive polypeptide chains, have nonetheless allowed the field to move forward. Incorporation of sequence patterns conserved in evolution has also been very helpful, as has the use of naturally occurring protein fragments as building blocks. Successful design efforts have not only replicated some natural folds and enzymatic activities, but also generated new folds and catalytic activities (*1*, *2*). As in the well-known dictum by Richard Feynman, "What I cannot create, I do not understand," successful design is also a powerful way to show that a design principle has been understood.

One of the best model systems for protein design is the coiled coil, a fibrous fold formed by two or more helices in parallel or antiparallel orientation curved around a central axis (*6*). Coiled-coil helices pack along seams of residues that form regular, geometrically defined interactions; they thus have repetitive sequences of low complexity. They become structured at shorter lengths (often 25 to 30 residues) than do globular folds (typically more than 70 residues) and are more stable. Uniquely among proteins, their structure can be described by parametric equations (the Crick equations) (*7*), and can thus be computed (rather than simulated, a vastly more laborious process) (*4*, *5*, *8*–*10*). Recent studies, including that by Joh *et al.*, substantially extend the range of engineered coiled-coil forms (*4*, *9*, *10*).

"...Rocker can translocate three to four hydrogen ions in one direction for every Zn^{2+} ion in the other, even against a pH gradient..."

Huang *et al.* (*9*) designed antiparallel bundles of three and four helices that depart from the common coiled-coil sequence periodicity of seven residues by having 18- and 11-residue periodicities, respectively, and are unusually stable. The authors also built a parallel pentameric coiled coil, which marks a transition from bundles with tightly packed cores to barrels enclosing a central, solvent-accessible channel.

The interhelical interactions in coiled-coil barrels involve two seams of residues (rather than one, as in bundles) and are hence harder to design. Thomson *et al.* (*10*) classify these complex seams into three types according to their geometry and show that they have understood the principles governing one type by designing penta-, hexa-, and heptameric barrels. On the basis of experiments demonstrating differential chemical accessibility of the channels formed by such barrels (*11*), they highlight their potential as catalysts.

These channels also have a clear potential for transmembrane solute transport (*12*). Joh *et al.* now illustrate this by building a membrane-embedded coiled coil that contains two Zn^{2+} binding sites close to its ends. By design, the coiled coil (called Rocker) oscillates dynamically between two states, such that when Zn^{2+} is bound at one site, it is released at the other. By further designing the binding sites such that they can either coordinate zinc ions or hydrogen ions, but not both, Joh *et al.* ensure that these ions are transported in opposite directions. Indeed, they show that Rocker can translocate three to four hydrogen ions in one direction for every Zn^{2+} ion in the other, even against a pH gradient (see the figure). An extensive characterization of the structure and activity of this synthetic antiporter proves the accuracy with which all design goals have been met.

The study of Joh *et al.* convincingly breaks several barriers in protein engineering: taking protein design from the solvent into the membrane, aiming for dynamic properties rather than for stability, and achieving an advanced biomimetic function from first principles, without recourse to screening or directed evolution. The work opens up exciting new avenues for membrane protein engineering, given that most membrane proteins are helical bundles and that Grigoryan and Degrado (*13*) have provided a general extension of the Crick equations to these structures. It should not be overlooked, however, that extending these advances to other protein folds remains a challenge. ■

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BIOCHEMISTRY

More than fine tuning

Local electric fields accelerate an enzymatic reaction

By **Peter Hildebrandt**

How do electric fields affect enzymatic processes? Binding and crystallographic studies have shown that electrostatic interactions are important in the substrate-binding step that initiates enzyme catalysis. However, for the subsequent steps, experimental data have been limited. The study of electric field effects on the transition state was therefore largely restricted to theory (*1*). On page 1510 of this issue, Fried *et al.* (*2*) use a recently developed technique to quantify the electrostatic contribution to the rate-limiting step of an enzymatic reaction. They show that the local electric field accelerates the reaction, a finding that is likely to apply to other catalytic reactions.

The authors exploit the vibrational Stark effect (VSE), which results from the perturbation of molecular vibrational energy levels by the electric field. This effect is particularly strong for the stretching modes of carbonyls and nitriles, which exhibit dipole moments along their bond axes. When such probes are incorporated into proteins through binding of additional ligands or chemical modification of amino acid side chains, the frequency shifts induced by local electric fields can be determined by infrared (IR) spectroscopy (*3*–*7*).

Using this technique, Fried *et al.* study the enzyme ketosteroid isomerase (KSI), which catalyzes the isomerization of a group of ketosteroids to their conjugated isomers (*8*, *9*). The reaction cycle involves an enolate intermediate and is associated with substantial changes of the dipole moment of the keto group (see the figure). KSI has one of the highest known unimolecular rate constants in biochemistry. Despite extensive study (*9*), the mechanistic basis for this high rate and specifically the role of electrostatics remain matters of debate.

The KSI enzyme also binds 19-nortestosterone (19-NT), but this ketosteroid cannot react owing to the position of the C=C double bond. Nevertheless, the C=O group of 19-NT adopts essentially the same position in the active site and experiences the same

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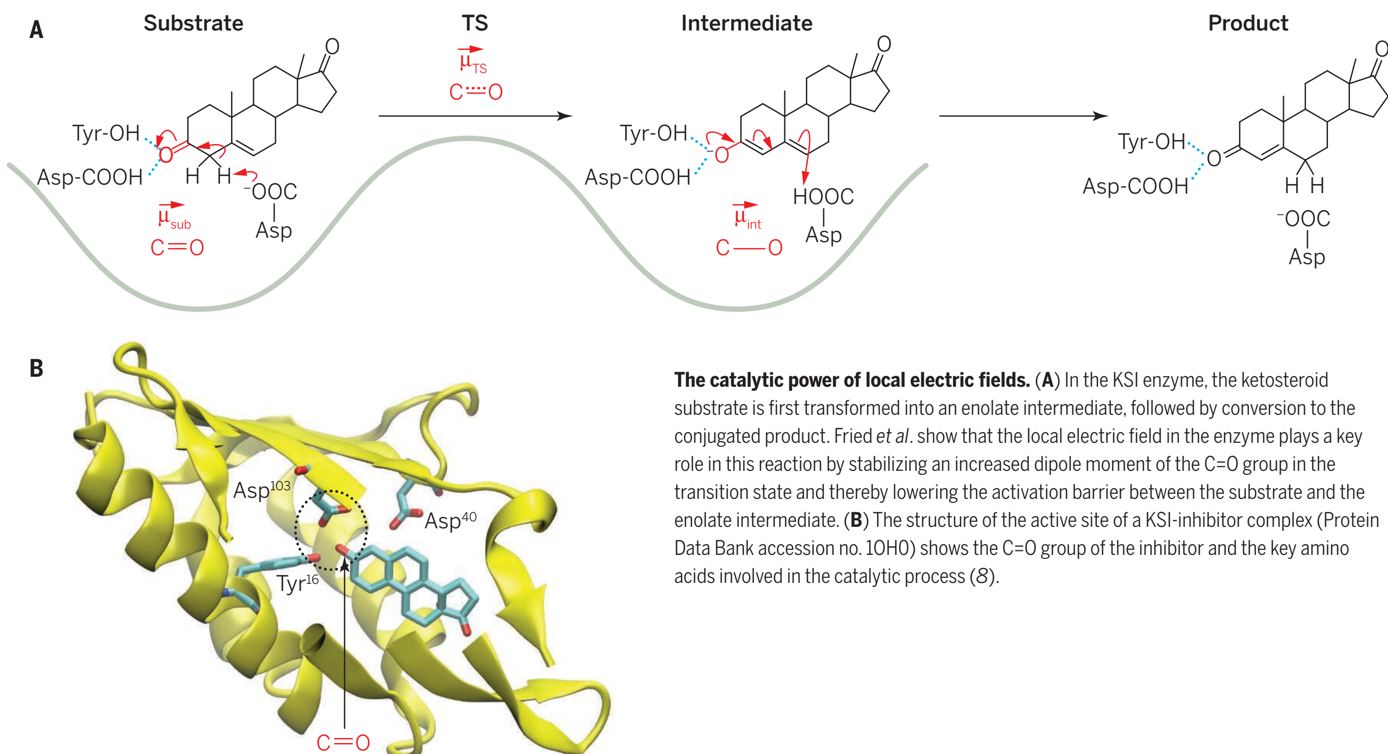

The catalytic power of local electric fields. (**A**) In the KSI enzyme, the ketosteroid substrate is first transformed into an enolate intermediate, followed by conversion to the conjugated product. Fried *et al*. show that the local electric field in the enzyme plays a key role in this reaction by stabilizing an increased dipole moment of the C=O group in the transition state and thereby lowering the activation barrier between the substrate and the enolate intermediate. (**B**) The structure of the active site of a KSI-inhibitor complex (Protein Data Bank accession no. 1OH0) shows the C=O group of the inhibitor and the key amino acids involved in the catalytic process (*8*).

hydrogen bond interactions as a reactive substrate. Hence, as for reactive substrates, the C=O bond of the bound 19-NT may be elongated, resembling a putative transition-state structure (see the figure). In this sense, the C=O group of 19-NT is an ideal probe for the electric field environment in the first step of the catalytic reaction.

Fried *et al.* used IR difference spectroscopy to identify the C=O stretching mode of the bound 19-NT on the background of protein signals. When 19-NT is bound to the wild-type (WT) enzyme, the C=O stretching frequency is drastically downshifted compared to the value measured for 19-NT in water. To translate this frequency shift into electric field strength, the authors carried out calibration experiments on 19-NT in different solvents. The analysis provided a linear correlation between the C=O stretching frequency of 19-NT and the electrostatic field.

The authors next examined the frequency shifts of the 19-NT keto function in different mutants of the enzyme. Substitutions for amino acids in the active site led to smaller downshifts with respect to water as compared to the WT protein. These findings confirm that the formation of the enolate is the rate-limiting step of the catalytic cycle. They also show that the local electric field stabilizes an increased dipole moment

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> ***"It is very likely that the electric field–dependent acceleration of elementary reactions is a general concept in biological catalysis and perhaps also in chemical catalysis..."***

of the C=O group in the transition state, thereby reducing the energy barrier for the formation of the enolate intermediate (see the figure). Assuming that similar electric fields act on the C=O function in the ground and transition state, the authors determine a ~70% electrostatic contribution to the lowering of the activation barrier in the WT enzyme compared to the uncatalyzed reaction in solution.

By reporting quantitative experimental data on the electrostatics of the rate-limiting step—including the contribution of the crucial hydrogen bond interactions—the study is important not only for understanding the enzymatic mechanism of KSI (*9*). It may also help to elucidate other enzymatic processes involving the attack at polar functional groups. It is very likely that the electric field–dependent acceleration of elementary reactions is a general concept in biological catalysis and perhaps also in chemical catalysis, as suggested, for instance, for zeolite-based catalytic reactions (*10*).

Fried *et al.*'s methodology for determining the local electric field is not restricted to enzymatic reactions. VSE spectroscopy may also be applied to other processes involving proteins, particularly those that take place at or in membranes and thus under the influence of additional external electric fields. Here, the key question is how changes of the transmembrane potential can modulate local electric fields to steer or initiate specific processes. Use of model membranes assembled on gold electrodes may allow VSE spectroscopy to be extended to transmembrane proteins; this setup enables control of the transmembrane potential and ensures increased sensitivity of surface-enhanced infrared absorption for signal detection (*11*). ■

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More than fine tuning

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