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Response to Comments on “Extreme electric fields power catalysis in the active site of ketosteroid isomerase”

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Abstract

Natarajan *et al.* and Chen and Savidge comment that comparing the electric field in ketosteroid isomerase's (KSI's) active site to zero overestimates the catalytic effect of KSI's electric field because the reference reaction occurs in water, which itself exerts a sizable electrostatic field. To compensate, Natarajan *et al.* argue that additional catalytic weight arises from positioning of the general base, whereas Chen and Savidge propose a separate contribution from desolvation of the general base. We note that the former claim is not well supported by published results, and the latter claim is intriguing but lacks experimental basis. We also take the opportunity to clarify some of the more conceptually subtle aspects of electrostatic catalysis.

Although the active site of ketosteroid isomerase (KSI) exerts a very large electric field (magnitude close to 150 MV/cm) onto its bound substrate, the substrate experiences a rather large electric field (magnitude 80 MV/cm, on average) when interacting with water in aqueous solution. A key point raised by Natarajan *et al.* (1) and Chen and Savidge (2) in their Comments is that bulk water would exert a catalytic effect on KSI's reaction proportional to its electric field. Chen and Savidge further imply that polar reactions are always accelerated by a polar medium. These suggestions reflect fundamental misunderstandings. Marcus theory teaches us that in reactions (such as KSI's) during which dipoles reorient and charges move, a polar solvent can actually have an inhibitory effect because there is an energetic cost (the reorganization energy) imposed by the requirement for the solvent sphere to forfeit the conformation that stabilizes the reactant's charge configuration to adopt a conformation that stabilizes the transition state's (TS's) charge configuration (3). An electric field (regardless of magnitude) can only have a catalytic effect by the model in figure 1B of our paper [Fried *et al.* (4)], if it adopts an orientation that specifically stabilizes the TS—that is, it is preorganized (5). The observation of narrow C=O bands in all the KSI active sites studied suggests that KSI's active-site electric field is fixed and preorganized [see also (6)] and therefore capable of producing a catalytic effect proportional to field magnitude. Solvent reaction fields (such as in bulk water) are not preorganized because they stabilize the reactant's (and not the TS's) charge configuration and have large fluctuations. The importance of this distinction is evidenced by the fact that several fundamental polar reactions are faster in the gas phase than in aqueous solution (7).

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Therefore, we do not think a priori that water itself will provide a catalytic effect due to its electric field relative to the gas phase, and for this reason, we counted the electric field of the KSI active site in full to estimate its contribution to catalysis.

Natarajan *et al.* are correct to emphasize that the electric field C=O experiences is the result of both the residues that create the field and those that position the steroid ligand within it; indeed, the “electric field picture” (8) illuminates why key hydrogen-bond–donating residues cooperate with positioning residues to confer maximal catalytic effect. To be clear, we reserve the term “chemical positioning” to refer to the positions of components that participate in chemistry (i.e., breaking and forming of bonds); this should be largely separable from electrostatic catalysis, which depends on the positions of atoms that define the environment in which the reaction occurs but do not necessarily participate in the reaction. Natarajan *et al.* suggest that chemical positioning provides the majority of KSI’s catalytic effect. This suggestion seems unlikely to us, since several experiments conducted by Herschlag and colleagues that directly examined the effect of positioning Asp⁴⁰ are consistent with our 10^{2.5}-fold assignment: The introduction of mutations that mis-position Asp⁴⁰ (9, 10) reduces activity by a factor of 10^{1.5} to 10³. It would be very interesting to conduct “chemical rescue” studies (11) in which activity is restored to an Asp⁴⁰Gly mutant of KSI with exogenous acetate. Our assignment for the contribution of chemical positioning [figure 3C in (4)] could be tested by measuring the effective acetate concentration that provides an equivalent rate as wild-type KSI.

Natarajan *et al.* emphasize experiments on a collection of mutants of KSI in which the oxyanion hole and its environs are removed and partially replaced with small residues, leaving a water-filled cavity in its wake (part of which gets displaced by the substrate). These mutants reduce KSI’s catalytic effect by 10³-fold, and the authors take this to imply that electrostatic stabilization of C=O by KSI’s active site provides a 10³-fold effect relative to water. We disagree with this claim because, first, the active-site electric field arises not only from the oxyanion hole but also from the enzyme scaffold as a whole (so removing the oxyanion hole side chains would not remove all contributions to the active-site electric field). Second, it is unlikely that the water molecules trapped in the active site are analogous to bulk water, because they will be (partially) pre-organized by the same enzyme scaffold that would otherwise organize Tyr¹⁶ and Asp¹⁰³. Enzyme design efforts have demonstrated that active-site waters (much like amino acid residues) can assist or impede catalysis depending on their positions, orientations, and dynamics (12, 13). Specifically in the case of KSI, water dynamics in the active-site cleft near a substrate analog were found to be substantially different from those of bulk water, even for a rather exposed region of the cleft (14).

Chen and Savidge suggest the existence of an important third catalytic contribution from the placement of Asp⁴⁰ in a nonpolar environment. Rephrasing their idea as we understand it, the concept is that just as C=O becomes more polar in the TS, and so would be stabilized by an active-site environment that exerts larger electric fields than water, the carboxyl group of Asp⁴⁰ becomes less polar in the TS, and so would be less destabilized by an environment that exerts smaller electric fields than water. Such proposals would have to be tested by measuring the electric field on the carboxyl group across several mutants and seeing if it

bears any relationship to rate. We would hesitate to assign this hypothetical effect a specific catalytic weight in the absence of experimental evidence, although in any event, it should still qualify under the heading of electrostatic catalysis.

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