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 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 positions the TS—that is, it is preorganized (5). The observation of narrow C=O bands in all the KSI active site studies suggested 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). 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 of KSI’s charge configuration 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 102.5-fold assignment: The introduction of mutations that misposition Asp40 (9, 10) reduces activity by a factor of 101.5 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 104-fold, and the authors take this to imply that electrostatic stabilization of C=O by KSI’s active site provides a 104-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) preorganized by the same enzyme scaffold that would otherwise organize Tyr46 and Asp80. 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 (22, 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.

REFERENCES

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