Reply to Comment on the "Thermodynamic Trajectory of Enzyme Evolution"

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The comment made by Dunfold *et al.* concerns the interpretation of enzyme evolution.[1] In the comment, they use three-step model (eq 7) instead of two-step model (eq 1) and conclude that eq 7 does not lead to diffusion-controlled reactions when k_2 is much smaller than $k_{-\text{diff}}$. This is the nature of things, because the turning point and the diffusion-controlled reaction appear when k_2 becomes much larger. It is true that the subsequent steps after formation of an encounter complex determine the ultimate speed and specificity, if $k_{-\text{diff}} \gg k_2$.

However, there is no earthly reason why k_2 should be much smaller than $k_{-\text{diff}}$ in evolution of enzymes. It is more general to think that k_2 as well as k_3 increases with the evolution. Equations 8 and 9 do not hold any more, when k_2 becomes much larger. In our previous report,[1] k_{cat} is not limited to $k_{-1} \gg k_{\text{cat}}$. Namely, the turning point and the diffusion-controlled area appear when the value of k_{cat} becomes comparable to k_{-1} and larger than k_{-1}, respectively. In conclusion, when $\Delta G_{\text{ov}}^{\ddagger}$ decreases, there is a turning point, where specificity is the highest, and the diffusion-controlled area. However, as indicated by us in the previous paper,[1] *which goal enzymes will select depends upon which factor nature prefers.* In this respect, the model of Dunford *et al.* seems to be a special case of ours.

References and Notes

(1) Kodaka, M.; Hase, A. *J. Phys. Chem.* **1995,** *99,* 10686.