

# COMMENTS

## Comment on “Thermodynamic Trajectory of Enzyme Evolution”

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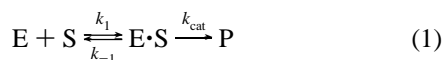
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Two papers in the past four years in *The Journal of Physical Chemistry* have addressed the question of whether the ultimate target of enzyme evolution is a diffusion-controlled reaction. In the earlier paper,<sup>1</sup> the temperature dependencies of rates of reactions of a series of heme proteins with hydrogen peroxide were analyzed in terms of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$ . Globins had  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  values much greater than that for an aqueous diffusion-controlled reaction, and peroxidases and catalases had smaller values. The globins have similar structures to the peroxidases and catalases, and if they are not evolutionary precursors, there would at least appear to be a common evolutionary ancestor.<sup>2</sup> In the sequel paper,<sup>3</sup> the same kinetic data were reanalyzed on the basis of a series of assumptions that led the authors to conclude that a corner could be turned and the diffusion-controlled target could be reached, despite being initially bypassed.

The assumptions made by Kodaka and Hase<sup>3</sup> are the following.

1. Equation 1 is an adequate model.



The rate constant  $k_1$  is assumed to be for a diffusion-controlled association reaction, yet  $k_{-1}$  is not for diffusion-controlled dissociation.

2. A linear free energy relation exists between  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  for the overall reactions:

$$\Delta H_{ov}^\ddagger = \beta \Delta S_{ov}^\ddagger + \gamma \quad (2)$$

3. A linear relation is also assumed to exist between  $x = (\Delta G_{-1}^\ddagger - \Delta G_{cat}^\ddagger)/RT$  and  $y = (\Delta H_{-1}^\ddagger - \Delta H_{cat}^\ddagger)/RT$ .

4. It is assumed that  $k_{-1} \gg k_{cat}$ . Then, on the basis of their eqs 10–15, they derive their eq 16 which can be put into the form of their eq 17,  $y = ax + b$ , where

$$a = \beta/(\beta - T) \quad (3)$$

$$b = -\{1/R(\beta - T)\}(\Delta H_1^\ddagger - \beta \Delta S_1^\ddagger - \gamma) \quad (4)$$

From a linear least-squares analysis of eq 2, based on Figure 1 of ref 1,  $\beta = 567$  K and  $\gamma = 5.85$ . The parameters<sup>1</sup> for an aqueous diffusion-controlled bimolecular reaction at 25 °C are estimated to be  $\Delta H_1^\ddagger = 18$  kJ/mol and  $\Delta S_1^\ddagger = -15$  J/(K mol). Therefore, the values of  $a$  and  $b$  can be calculated using their assumptions. Kodaka and Hase obtained the values  $a = 2.62$  and  $b = +16.9$ . They showed that there can be no turning point if  $b$  is negative.

5. Having derived their equations based on the assumption that  $k_{-1} \gg k_{cat}$ , they then assume that the equations are valid for  $k_{-1} \ll k_{cat}$ .

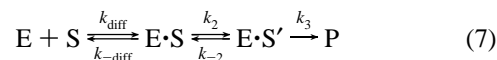
6. Their equations for the solid line showing a turning point in their Figure 4 are

$$\Delta H_{ov}^\ddagger = \Delta G_1^\ddagger + T \Delta S_1^\ddagger - RTy \exp(-x)/\{1 + \exp(-x)\} \quad (5)$$

$$\Delta S_{ov}^\ddagger = \Delta S_1^\ddagger - R \ln\{1 + \exp(-x)\} - Ry \exp(-x)/\{1 + \exp(-x)\} \quad (6)$$

The subscripts “ov” refers to the overall reaction and “1” to a bimolecular diffusion-controlled association reaction. Since conditions are chosen to force the last term in eq 5 to zero, and the last two terms in eq 6 to zero,  $\Delta H_{ov}^\ddagger$  is forced to equal  $\Delta H_1^\ddagger$ ,  $\Delta S_{ov}^\ddagger$  is forced to equal  $\Delta S_1^\ddagger$ , and the alleged target is reached.

Equation 1 is a satisfactory model to discuss the enzyme-catalyzed reaction in terms of an overall binding equilibrium followed by reaction, provided it is understood that  $k_1$  and  $k_{-1}$  both have physical and chemical components. We agree with assumption 2 that a fair linear correlation exists. Otherwise, we find the assertions of Kodaka and Hase to be without merit. A more realistic model to discuss diffusion control is the following:



Equation 7 leads to entirely different conclusions. For example, if one makes the simplifying assumptions about eq 7,  $k_{-diff} \gg k_2, k_3$ , similar to an assumption of Kodaka and Hase, and  $k_3 \gg k_{-2}$ , one obtains the equations

$$k_{ov} = k_2 K_{diff} \quad (8)$$

$$\Delta G_{ov}^\ddagger = \Delta G_2^\ddagger - RT \ln K_{diff} \quad (9)$$

where  $K_{diff}$  is the equilibrium constant for formation of an encounter complex, and  $k_2$  is the rate constant for conversion of the encounter complex  $E \cdot S$  to an enzyme–substrate complex which is properly oriented for reaction,  $E \cdot S'$ . Equation 7 does not lead to predictions of an ultimate evolutionary target of bimolecular diffusion-controlled reactions.

Diffusion control has existed since the beginning of time, for both the formation of an encounter complex<sup>4</sup> and its dissociation.<sup>5</sup> It is what happens in the subsequent steps that determines the ultimate speed and specificity of a reaction. Large

overall negative  $\Delta S^\ddagger$  values, observed experimentally for enzyme-catalyzed reactions (of the order of  $-100$  eu for the catalases), are a measure of the emphasis placed on specificity, and nature has better sense than to throw the specificity away.

### References and Notes

(1) Job, D.; Jones, P.; Dunford, H. B. *J. Phys. Chem.* **1993**, 97, 9259. The units for the ordinate of Figure 1 of this paper should be  $\text{kJ mol}^{-1}$ . In

eq 13,  $[E]$  should be replaced by  $[E]_0$ , the total enzyme concentration. The equation is valid for small values of  $[S]$ .

(2) Welinder, K. G. *Curr. Opin. Struct. Biol.* **1992**, 2, 388. Welinder, K. G.; Mauro, J. M.; Nørskov-Lauritsen, L. *Biochem. Soc. Trans.* **1992**, 20, 337. Takio, K.; Titani, K.; Ericsson, L. H.; Yonetani, T. *Arch. Biochem. Biophys.* **1980**, 203, 615.

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

(4) von Smoluchowski, M. W. *Z. Phys. Chem.* **1917**, 92, 129.

(5) Eigen, M. *Z. Phys. Chem. (Munich)* **1954**, 1, 176.