On the Origin of the Temperature Dependence of Isotope Effects in Enzymatic Reactions: the Case of Dihydrofolate Reductase

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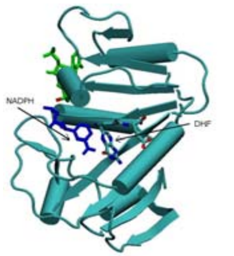
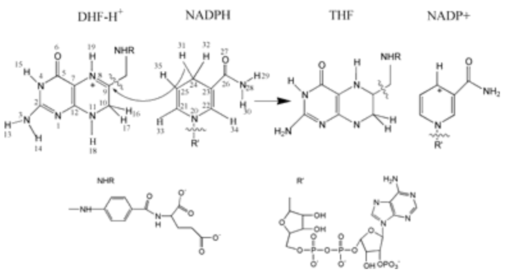
DHFR

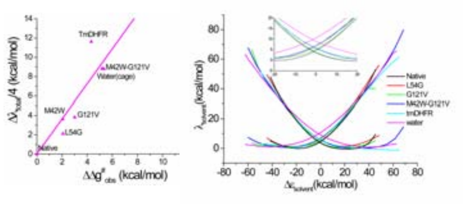


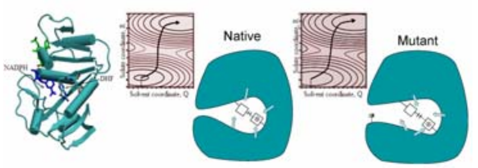
Very flexible protein;
Catalyzes the reduction of dihydrofolate to tetrahydrofolate;
The rate determining step of the reduction is a hydride transfer step from cofactor NADPH to protonated dihydrofolate.

Chemical Reaction



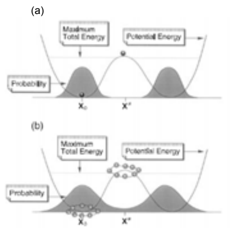
Our previous results about mutation effect[1]





Quantum classical path (QCP)

centroid path integral approach[2,3] provides a very effective way of simulating NQM effect



Schematic diagrams of the behavior of classical (a) and quantized (b) particles on a double-well potential surface. In order to make the discussion simple we only consider the contribution to the probability distribution from energies that are smaller than the barrier height, $p(x)$=with $\int_0^{E_{max}} P(x,E)\exp(-\beta E)dE$ with $E_{max} < U(x^{\neq})$, where $P(x,E)$ is the probability to be at the given x with the designated energy (i.e., the probability obtained by running a trajectory with the given E). This probability distribution is represented by a shaded area. The upper figure (a) represents the classical particle by a single sphere. The lower figure (b) describes the quantum mechanical particle by a ring of beads. When the particle is at the transition state region, the beads can be at points with energy lower than $U(x^{\neq})$, and this allows the particle to be at $x^{\neq}$ and to tunnel through the barrier (in this case we have nonzero probability to be at $x^{\neq}$). When the particle is at the bottom of the potential well the beads can see points with potential higher than $U(x_0)$. This dispersion is reflected by the zero point energy. The figure is adapted from ref [2].

EVB surfaces:

$$H_{ii} = \varepsilon_i = \alpha^i_{gas} + U^i_{\text{intra}}(\mathbf{R},\mathbf{Q}) + U^i_{Ss}(\mathbf{R},\mathbf{Q},\mathbf{r},\mathbf{q}) + U_{ss}(\mathbf{r},\mathbf{q})$$

Mapping potential:

$$\varepsilon_m = (1-\theta_m)\,\varepsilon_1 + \theta_m\varepsilon_2 \quad (0 \le \theta_m \le 1)$$

In the QCP approach:

$$k_{qm} = F_{qm}k_BT/h\,\exp(-\beta\Delta g^{\ddagger}_{qm})$$

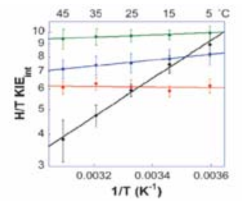
Quantum particles are sampling using Feynman's path integral formulation:

$$U_{qm} = \sum_{k=1}^{p}\frac{1}{2p}M\,\Omega^2\Delta x_k^2 + \frac{1}{p}U(x_k)$$

$\Delta x_k = x_{k+1} - x_k$ (where $x_{p+1}=x_1$), $\Omega=p/h_\beta$, M is its mass

$$Z_{\mathbf{q}}(\bar{x}) = Z_{\mathbf{cl}}(\bar{x})\langle\langle\exp\{-(\beta/p)\sum_k U(x_k) - U(\bar{x})\}\rangle_{\mathbf{fp}}\rangle_U$$

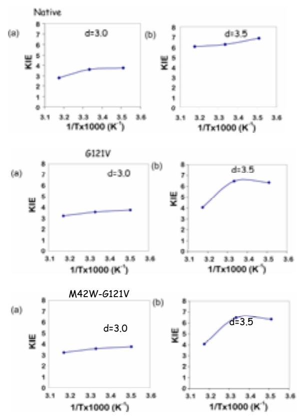
Experimental Isotope Results

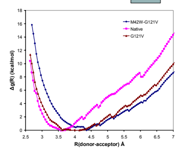


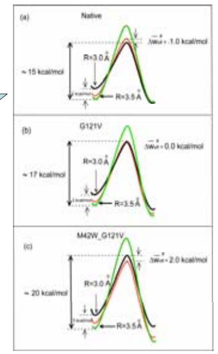
Comparison of the Arrhenius plots of intrinsic HT KIEs of WT(red), G121V (green), M42W (blue), and G121V–M42W (black) ecDHFRs[4]

Results

Calculated results at fixed distance



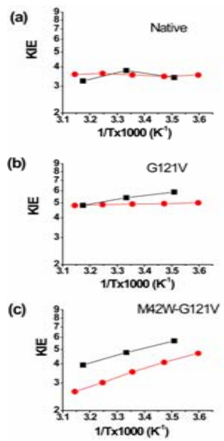


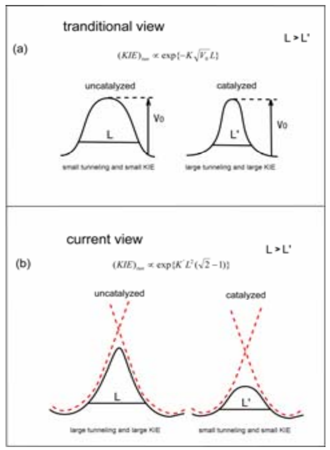


$$\bar{k}_{qm} = \int P(R)^{\neq}_{cl}(k_BT/h)e^{-\Delta\Delta g^{\neq}_{qm}(R)\beta}$$

$$P(R) = e^{-(\Delta g^{\neq}_{cl}(R)\beta + w(R)\beta)}\int e^{-w(R)\beta}dR = e^{-\Delta w^{\neq}(R)\beta}\int e^{-w(R)\beta}dR$$

What do we learn?





[1] Liu, H.; Warshel, A. *Biochemistry* **ASAP**. [2] Hwang, J.-K.; Warshel, A. *J. Am. Chem. Soc.* **1996**, *118*, 11745. [3] Hwang, J.-K.; Warshel, A. *J. Phys. Chem.* **1993**, *97*, 10053. [4] Wang, L.; Goodey, N. M.; Benkovic, S. J.; Kohen, A. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15753.