COMMENTS

Comment on "Computational Studies of Enzyme-Catalyzed Reactions: Where Are We in Predicting Mechanisms and in Understanding the Nature of Enzyme Catalysis"

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Regarding the recent discussion between two theoretical groups on the entropic contribution to enzyme catalysis,[1−4] I would like to make some comments from an experimental viewpoint. At issue is how much entropy and free energy reduction is brought by preorganization, i.e., bringing reactants scattered in the solution into close proximity (restricting translational motions) and optimal alignment (restricting rotational motions) in the enzyme. Here, I will not get into the issue of motional freedom of the reactants in the enzyme−substrate complex. Rather, my comments concern the translational and rotational motions of a solute molecule in the solution, i.e., how much motional freedom is there to be reduced through preorganization?

In the gas phase, the standard translational/entropy entropy can be readily calculated using ideal gas statistics.[5] In solution, the calculations are much more complex and often have to rely on simplified models (for instance, see ref 6). For macromolecules such as proteins, the concept of solvent cage and cage volume runs into difficulty as the dimension of a macromolecule is larger than 1660 Å^3, the average volume for each solute molecule in a 1 M solution. In fact, for macromolecular aqueous solutions, the 1 M standard state is not thermodynamically self-consistent, its fictitious nature notwithstanding.[7] Experimental measurement offers an alternative to computation.

The results that my co-workers and I obtained for the enthalpy, entropy and free energy of each translational/rotational unit in aqueous solution at 1M standard state is $H^\circ_{tr} = 4.5 \pm 1.5RT$, $S^\circ_{tr} = 5 \pm 4R$, and $G^\circ_{tr} = 0 \pm 5RT$.[8] Thus, for the association of n molecules, the corresponding standard enthalpy, entropy and free energy *loss* are $(n-1)H^\circ_{tr}$, $(n-1)S^\circ_{tr}$, and $(n-1)G^\circ_{tr}$, respectively.

The result on H°_{tr} is a consequence of energy equi-partition of classical systems.[5] The result on S°_{tr} is based on experiments which compared the unfolding/dissociation reactions of homodimeric proteins with their disulfide cross-linked counterparts in aqueous solution.[9,10] Together, H°_{tr} and S°_{tr} leads to $G^\circ_{tr} \approx 0$ at 1 M. This empirical result provides an explanation as to why some computational approaches which ignored the translational/rotational term can still reproduce experimentally determined standard binding affinity.[11,12]

The result on S°_{tr}, 5 ± 4 R, is an order of magnitude smaller than the gas-phase value, which is $\sim 50R$ for molecules with molecular weights in the range of 5−25 kDa.[8] On the other hand, this result is very close to $-R$ ln 1/55, the value conventionally assigned to the cratic entropy of solutes in a 1 M aqueous solution. Cratic entropy was originally proposed by Gurney to denote ideal mixing entropy.[13] However, in a 1 M macromolecular aqueous solution, neither the solute nor the solvent behaves ideally. Further, the mole fraction of most macromolecular solutes in aqueous solution at 1 M standard state is a meaningless negative number rather than 1/55.[7] Thus, our result cannot be interpreted as the cratic entropy. The various meanings later bestowed to the cratic entropy are outside the scope of this comment.

Whether translational and rotational motions of polar solutes in aqueous solutions are amenable to computation in a quantitative fashion is outside my expertise. However, since vapor condensation is also caused by molecular association, a computational approach capable of treating molecular association in solution should be able to reproduce numerically Trouton's rule on vaporization entropy and deviations (e.g., vaporization entropy of water) from this rule.[14,15] Thus, data on vaporization entropy can calibrate and validate such computational approaches. In fact, the data show that, among isomers, the more spherically symmetric ones have lower entropy reduction upon condensation, providing strong evidence that rotational motions are indeed restricted in the liquid phase.

In summary, our results indicate that water already imposes severe restrictions on the translational and rotational motions of polar solutes and there is little benefit to be gained through enzyme preorganization in terms of S°_{tr}. Obviously, preorganization involves contributions other than translational and rotational motions. One such contribution is solvation.[16] Indeed, the relationship among vaporization entropy, solvation entropy and translational/rotational entropy is worth further investigation, as pointed out by Wertz over 2 decades ago.[17] The aforementioned results on the relative vaporization entropy values among isomers clearly indicate that solvation cannot be considered to be independent of the translational/rotational motions of the solute molecule.

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