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Are there dynamical effects in enzyme catalysis? Some thoughts concerning the enzymatic chemical step

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ABSTRACT

We offer some thoughts on the much debated issue of dynamical effects in enzyme catalysis, and more specifically on their potential role in the acceleration of the chemical step. Since the term 'dynamics' has been used with different meanings, we find it useful to first return to the Transition State Theory rate constant, its assumptions and the choices it involves, and detail the various sources of deviations from it due to dynamics (or not). We suggest that much can be learned about the key current questions for enzyme catalysis from prior extensive studies of dynamical and other effects in the case of reactions in solution. We analyze dynamical effects both in the neighborhood of the transition state and far from it, together with the situation when quantum nuclear motion is central to the reaction, and we illustrate our discussion with various examples of enzymatic reactions.

Introduction

Enzyme catalysis is a complex process involving a series of kinetic steps. In order to complete a full catalytic cycle, these steps include at the very least substrate binding in the enzymatic active site, the chemical reaction *per se*, and product release into the solvent. These steps do not differ in a fundamental way from those for a bimolecular reaction in solution, where the overall reaction process involves the diffusion of the reacting pair, the chemical reaction *per se*, and then the dissociation of the newly formed products. But of course enzymatic reactions are so important and of such great interest because they involve catalysis in a biological context. Since the basic reaction classes involved are the same [1–6], the natural question is then just what, at the microscopic level, is key for the reaction acceleration in the enzyme compared to the solution reaction? This is of course a question of long standing with assorted proposed answers [2–11]. In this contribution, we will be concerned only with a small portion of the general question, namely: are there special "dynamical" effects that are key for enzymatic catalysis reactions? Further, we concern ourselves exclusively with the chemical step in the catalysis.

It seems fair to say that there is a degree of confusion about the answer to this question. The question is of course by no means a simple one to answer. But in our view, a significant contributor to the confusion concerning the importance of dynamical effects (or lack thereof) in enzyme catalysis is simply the ambiguity of the terms 'dynamical' and 'dynamics': these are frequently interpreted and/or employed by different authors in quite different fashions. Of course, even a rate constant itself could be labeled as evidence of the existence of dynamics, but this is certainly far from what anyone would currently intend. In the following, we will not necessarily insist on any procrustean definition of dynamics, but will instead give assorted interpretations, with commentary and as much clarity as we can manage.

We will find it quite useful in this effort to spend considerable time on key reaction rate features and concepts that have been elucidated over the years for chemical reactions in solution. We think that this helps both to focus the issues and to highlight what might be different for reactions in enzymatic and solution environments. We hope that this perspective for the chemical step in enzymatic catalysis will add something useful to the by now extensive literature discussion on the general issue [10–24]. The present article, which is limited to the scope that we have indicated, makes no pretense of completeness vis a vis the topics discussed or the references to the literature. We would like to point in particular to

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the very recent issue on "Protein Motion in Catalysis" [21] for recent contributions of relevance.

Certainly there are dynamics everywhere if one views with a molecular level eye chemical reactions in solution or in enzymes from beginning to end. And there are differences in the typical time scales that occur in these systems. For example, a characteristic feature of enzymatic environments is the presence of a very broad spectrum of protein conformational motions, which occur on timescales ranging from picoseconds to milliseconds (see e.g. [25]). Solvation dynamics in water typically takes place in the femto- to picosecond (10^{-15}–10^{-12} s) range [26], but much longer time scales can occur in e.g. aqueous ionic solutions (with long-lived ion atmospheres) or in viscous liquids [27]. But are these dynamics relevant for the reaction rates? Possibly, but not necessarily; *vide infra*.

It is true that major, large amplitude conformational changes in the protein may occur during substrate binding and product release, i.e. before and after the chemical transformation. These include for example loop motions or the opening of lids which gate the active site entrance. But our exclusive focus in this article is the chemical transformation itself. Here it is expected that smaller and faster conformational changes may occur in the active site. These can affect the interaction between the protein active site and the substrate. One impact of this would be to change the electrostatic properties and the hydrogen-bond network of the active site in order to favor the electronic rearrangement associated with the bond-breaking and -forming processes. In the solution reaction, such roles are served by the solvent itself. But as noted above, certain solvents and especially proteins possess particularly slow dynamics and it is conceivable that these slower motions have a role to play in the reaction rates.

The repeated reference to "reaction rates" just made emphasizes an important distinction that has already arisen and will recur at a number of junctures within. Slow motions can and undoubtedly often occur in the process of a reaction mechanism yet their dynamics have little or no impact on the reaction rate. For example, in a solution reaction, a particular vibration occurring along the reaction pathway might be critical for the reaction to occur and its activation can make a contribution to the effective barrier for the reaction. But if the rate of that activation is not sufficiently slow, the dynamics of that vibration will not explicitly enter the reaction rate constant. (We will see an example of this in the section 'Diffusion-influenced reactions'.)

The outline of the remainder of this contribution is as follows. The section 'Transition State Theory' is devoted to a discussion of Transition State Theory, its assumptions and some of its principal ingredients. Deviations from this theory – which serves as our reference throughout – which are due to events occurring in the neighborhood of the transition state are discussed in the section 'Dynamical effects in the transition state neighborhood', in a general theoretical context and then in terms of applications to solution and enzymatic reactions. The special case of quantum nuclear particle transfer reactions is dealt with in the section 'Dynamics for reactions involving quantum nuclear motion', where the issue of "promoting modes" – which has garnered considerable attention in an enzymatic catalytic context – is considered. The section 'Dynamical effects away from the transition state neighborhood' deals with deviations from Transition State Theory due to events occurring away from the transition state region with applications to solution and enzymatic reactions. The section Concluding Remarks summarizes our key points.

Transition State Theory

One quite useful and commonly employed reference – and the one given pride of place in our discussions – is *Transition State Theory* (TST), also known in former times as "Activated Complex Theory". One definition of 'dynamical effects' for reactions is the departure of a reaction rate constant k from its TST value k_{TST}. The standard measure of this departure is the *transmission coefficient* κ, defined by the ratio k/k_{TST}. TST has been described in many different ways (not all of which are very compelling), especially in the older literature; but nowadays most would accept the 'no recrossing rule' version enunciated by E. Wigner in the 1930s [28], which we now present.

The TST rate constant for the forward reaction is given by the equilibrium average, normalized with respect to the reactants R, of the one-way flux across the transition state surface

$$k_{TST} = \langle J_+^{\ddagger} \rangle_R \tag{2.1}$$

Fig. 1 for a collinear (gas phase!) atom transfer reaction provides a useful illustrative perspective for the terms to be defined. Here the brackets $\langle(\ldots)\rangle_R$ denote the above-mentioned equilibrium average and $J_+^{\ddagger}$ is the one way flux

$$J_+^{\ddagger} = \frac{p}{m}\theta_+(p)\delta(x - x^{\ddagger}) \tag{2.2}$$

Here p and m are the momentum and mass associated with the reaction coordinate x at the transition state (TS), $\theta_+(p)$ is the step function assuring that only positive p values are included – corresponding to trajectories crossing the TS surface $x = x^{\ddagger}$ in the direction from reactants R to products P – and the delta function $\delta(x–x^{\ddagger})$ restricts the reaction coordinate x to its TS surface value. The basic assumptions here are a description by classical mechanics for the nuclei, the idea that the rate constant for a system in chemical equilibrium is the same as in a non-equilibrium kinetics experiment, and that (to repeat) there is no recrossing of the TS surface $x = x^{\ddagger}$, i.e. all trajectories crossing from the side of reactants to the side of the products continue on to become (stable) products (Figs. 1 and 2). The latter fundamental assumption of TST could legitimately be – and often is – termed a dynamical assumption, since it is an edict about the trajectories, but we will not insist on this in the present discussion. Note that, in the simple model illustration, both panels of Fig. 1 emphasize that the TS is really a surface (and not a point), and that there must be a *distribution* of trajectories that cross this surface, an aspect not always recalled.

Equation (2.1) can readily be shown (e.g. [29]) to yield the familiar formulas associated with TST, such as

$$k_{TST} = \frac{k_B T}{h}\frac{Q^{\ddagger}}{Q_R}\exp\left[-\Delta V^{\ddagger}/k_B T\right] = \frac{k_B T}{h}(C^0)^{1-n}\exp\left[-\Delta G^{\ddagger}/k_B T\right] \tag{2.3}$$

involving TS and reactant partition functions $Q^{\ddagger}$ and Q^R, the TS activation potential energy $\Delta V^{\ddagger}$ and free energy $\Delta G^{\ddagger}$, a reference concentration factor C^0 to guarantee correct dimensions, and the famous Eyring prefactor involving the ratio of the thermal energy and Planck's constant h. Despite assorted statements in the literature about the meaning of the latter factor, this factor does not represent any physical speed in the problem; indeed, the quantum factor h is canceled by an inverse h factor in the partition function ratio [29]. The activation free energy aspect in the simple model illustration Fig. 1 arises (primarily) from the differing distributions in the reactant and transition state transverse coordinates. In solution and enzymatic reactions to be discussed, the same basic structure of k_{TST} applies, but the coordinates differ.

An alternate form of Eq. (2.3) more convenient for our purposes is

$$k_{TST} = \frac{\omega_R}{2\pi}\exp\left[-\Delta G^{\ddagger}/k_B T\right] \tag{2.4}$$

Here ω_R is a collision frequency of the reactants for a bimolecular reaction (with appropriate units) and is a vibrational frequency in a reactant free energy well for a unimolecular reaction. In this way, the activation free energy $\Delta G^{\ddagger}$ refers to the same number of degrees of freedom in the TS and in the reactants [30].