Further theoretical refinement on the internal thermodynamics of perfect enzymes

Gaochao TIAN

Biochemistry Program, Ohio State University, Columbus, OH 43210, U.S.A.

By solving simultaneously the equation for ‘uniform binding’ [Albery & Knowles (1976) *Biochemistry* **15**, 5631–5640] and the equation for ‘differential binding’ [Chin (1983) *J. Am. Chem. Soc.* **105**, 6502–6503], I derived the following simple equation for perfect enzymes (with single substrate and single product) under irreversible conditions:

$$K_2 = \frac{\beta(1 + R_s)}{1 - \beta(1 + R_s)}$$

where K_2 is the internal equilibrium constant and β is the Brönsted coefficient of the elementary catalytic step, and R_s is defined as $[S]_0/K_s$, with $[S]_0$ being the physiological substrate concentration and K_s being the substrate dissociation constant. The equation suggests that the perfect enzyme can have different internal thermodynamic properties depending on physiological conditions.

INTRODUCTION

Despite the development of a powerful ‘perfect enzyme theory’ by Albery & Knowles [1] a decade ago, a major issue concerning the internal thermodynamics of perfect enzymes, namely whether it is ‘matched’ [1] or a ‘descending staircase’ [2], remains controversial.

According to Albery & Knowles [1], enzymes evolve to perfection via three mechanisms in the order of increased difficulty: uniform binding, differential binding and catalysis of elementary steps. The system used in the theory of Albery & Knowles [1] is:

The equilibrium constants of the individual steps and the overall reaction are expressed by K_1 , K_2 , K_3 and K_e respectively. For an irreversible reaction, the uniform binding leads to:

$$\frac{1}{K_1[S]_0 k_{+2}} = \frac{1 + 1/K_2}{k_{+3}} \quad (2)$$

where $[S]_0$ is the physiological substrate concentration. By a detailed treatment, they suggest that differential binding leads to:

$$K_2 = \frac{\beta}{1 - \beta} \quad (3)$$

in which β is the Brönsted coefficient of the elementary catalytic step. Since typical β values for an elementary step lie between 0.3 and 0.7, Albery & Knowles [1] predicted that the internal equilibrium constant K_2 of perfect enzymes is close to unity. This prediction appears to be satisfied by triose-phosphate isomerase [1] and several other glycolytic enzymes [3]. The hypothesis of ‘matched internal thermodynamics’ has also been observed in many other enzymes [4–9].

However, experimental deviations from the hypothesis of ‘matched internal thermodynamics’ have been observed in some enzymes, mainly those that catalyse irreversible reactions. For example, the internal equilibrium constant is about 10–15 for pyruvate kinase [2]

and large for fructose biphosphatase [10]. More recently, the mathematical basis of eqn. (3) has been questioned, and an improved relationship has been derived [11] [eqn. (4) in the next section]. On the basis of these facts, Benner and co-workers have proposed a ‘descending staircase’ model to accommodate the experimental deviations observed in enzymes catalysing irreversible reactions. Although the ‘descending staircase’ model appears to be more general, the improved relationship for differential binding [eqn. (4) in the next section] is a complex one and has not been applied to Benner’s theory. Therefore it is still not clear theoretically whether the internal thermodynamics of perfect enzymes is ‘matched’ or a ‘descending staircase’. In the present paper a piece of theoretical evidence is presented to suggest that the internal thermodynamics of perfect enzymes that catalyse irreversible reactions could be either ‘matched’ or a ‘descending staircase’, depending on the physiological conditions.

RELATIONSHIP FOR DIFFERENTIAL BINDING

As pointed out in the Introduction, the relationship derived by Albery & Knowles [1] (eqn. 3) has been found incorrect and an improved version has been provided [11], which is given as below:

$$\left(\frac{K_2^\beta}{K_2 + 1}\right)^{\frac{1}{2}} \cdot \left(\frac{K_2}{\beta} - K_2 - 1\right) = \left(\frac{k_d[S]_0 K_e}{C}\right)^{\frac{1}{2}} \quad (4)$$

where C is the constant in the linear free-energy relationship of the elementary catalytic step:

$$k_{+2} = CK_2^\beta \quad (5)$$

and k_d is the diffusion constant for the collision between the substrate and enzyme molecules. As pointed out in the Introduction, eqn. (4) is a complex one, by which it is not apparent whether the internal thermodynamics is ‘matched’ or a ‘descending staircase’.

As described in the original paper by Albery & Knowles [1], the uniform binding mechanism for im-

Fig. 1. Plots of K_2 versus R_s at different values of β , according to eqn. (7)

proving the enzyme catalytic efficiency will stop once eqn. (2) is satisfied. For further refinement of the enzyme catalytic efficiency, the differential binding is then required. However, this refinement process, due to the differential binding, will make the relationship defined by eqn. (2) for uniform binding unsatisfied, and the uniform binding will resume until eqn. (2) is satisfied again. These two mechanisms of refining the enzyme catalytic efficiency operate over and over until the enzyme becomes perfect, when eqn. (2) for the uniform binding and eqn. (4) for the differential binding are both satisfied. Simultaneous solution of eqn. (2) and eqn. (4) then leads to a simplified relationship for perfect enzymes.

Substitution of eqn. (5) into eqn. (2) and solving for K_2^β gives:

$$K_2^\beta = \frac{k_{+3}K_2}{C[S]_0K_1(1+K_2)} \quad (6)$$

Bringing eqn. (6) into eqn. (4) yields the simplified relationship between K_2 and R_s (defined as $[S]_0/K_s$, where K_s is the substrate dissociation constant):

$$K_2 = \frac{\beta(1+R_s)}{1-\beta(1+R_s)} \quad (7)$$

Plots of K_2 versus R_s at different β values are shown in Fig. 1.

DISCUSSION

Unlike eqn. (4), eqn. (7) shares simplicity with eqn. (3). It is also very similar in format to eqn. (3). However, it has different implications from those that eqn. (3) has, owing to the extra term $(1+R_s)$. Because of this term, eqn. (7) clearly indicates that the internal thermodynamics of perfect enzymes could be either close to or deviate from unity, depending on the magnitude of R_s . For example, if $K_s \gg [S]_0$, eqn. (7) reduces to eqn. (3), and the internal thermodynamics is 'matched'. On the other hand, as R_s increases, K_2 becomes large and the internal thermodynamics becomes a 'descending staircase'. This dependence of K_2 on R_s is also shown in Fig. 1.

Although R_s can take on different values for perfect enzymes, restrictions on its magnitude deserve some considerations. First of all, the Michaelis constant K_m will be of the order of (but probably appreciably higher than) substrate concentration, such that changes in metabolic flux will not bring about catastrophic results, as discussed by Atkinson [12]. Such a 'safety factor' will require reasonably low R_s values for the perfect enzymes. In addition, since the numerator of eqn. (7) must be positive, R_s has to be smaller than $(1-\beta)/\beta$, and an upper limit can be set on R_s . Principally, β can have values between 0 and 1. As suggested by Albery & Knowles [1], however, most reactions have values for β between 0.3 and 0.7; as a result the upper limit will be expected between 0.4 and 2.3. Thus, for most perfect enzymes, K_s will be expected to be close to or higher than the substrate concentration. This conclusion, assuming $K_s = K_m$, is generally consistent with the ones to which other authors [13,14] have arrived.

Concerning enzyme evolution, one question is whether it is an advantage for perfect enzymes to be stable enough that not much adjustment will be required even when a significant change in $[S]_0$ takes place during the course of the enzyme evolution. Although this question may not yet have been answered, eqn. (7) does suggest a way by which an enzyme can evolve to a stable perfection. As shown in Fig. 1, if R_s is small, the sensitivity of K_2 to the change in $[S]_0$ will also be small. Therefore, by evolving to have K_s higher than $[S]_0$, the perfection of the perfect enzymes will be less affected by changes in physiological conditions that result in a change of $[S]_0$.

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