COMPUTER-AIDED MODELLING OF ENZYME - SUBSTRATE INTERACTION

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Experimental rating of enzyme efficiency towards different substrates is the standard procedure. However, modern computational methods may be fast and not expensive approach before extensive biochemical assay is started. The paper describes a special case of computer-aided molecular modelling when two very similar substrates are to be concerned.

1. Introduction

One of the important questions in enzymology is the efficiency of substrates coming into interaction with enzymes [1]. This paper concerns some computational aspects of this problem.

Assume a simple enzymatic reaction

$$E + S \rightarrow E + P \tag{1}$$

where E is the enzyme, S is the substrate, P is the product. The rate of the enzymatic reaction is defined by Eq.2

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt} \tag{2}$$

where [S] and [P] are the concentrations of S and P.

Our task is to estimate relative quality of two similar substrates, *S(L)* and *S(D)*, from the ratio of their rates [2]

$$\frac{v(L)}{v(D)} \tag{3}$$

by means of the computer-aided methods.

2. Michaelis–Menten equation

In 1913 Michaelis and Menten [3] found that many enzymatic reactions may be described by a formula

$$v = \frac{k_{cat}[E]_0[S]}{K_M + [S]} \tag{4}$$

where k_{cat} , *turnover number*, and K_M , *Michaelis constant*, can be derived from the plot of experimental data.

3. Theoretical interpretation of the Michaelis-Menten equation

Brown [4] in 1902 proposed the hypothesis that a specific *complex* is formed between any enzyme and substrate [5]. Michaelis and Menten applied this idea to two-step enzyme reactions, where only one intermediate is formed,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} E + P \tag{5}$$

Where E and S are the free enzyme and free substrate, ES is the enzyme-substrate complex, P is the product of the reaction , k_1 and k_2 are the rate constants of the forward reaction, k_{-1} is the rate constant of the reverse reaction.

In a special case, when k_{cat} is small, they derived for k_{cat} and K_M using the steady-state approximation

$$k_{cat} = k_2 \text{ and } K_M = \frac{k_{-1}}{k_1} \tag{6}$$

The Michaelis constant, K_M, in this case, is written as K_{s}. Briggs and Haldane [6] assume a more general situation : k_2 is not small. Then K_M is equal to

$$K_M = \frac{(k_{-1} + k_2)}{k_1} \tag{7}$$

4. Eyring theory of transition state

Eyring [7-8] formulated a theory based on the concept of *transition state* (or activated complex) which exists as an intermediate stage in any chemical reaction [9]. In the case of two-step enzyme reaction we write now

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (E-S)^{\#} \overset{k_2}{\rightleftarrows} ES \rightarrow ES^{\#} \rightarrow E + P \tag{8}$$

where the first transition state $(E\text{-}S)^{\#}$ is controlled by attractive forces (van der Waals and Coulomb forces) and repulsion forces (e.g., deformation of both enzyme and substrate). The ES state is identified as a non-covalent (Michaelis or van der Waals) complex. The second transition state, $ES^{\#}$, may be modelled by a covalent complex of intermediate tetrahedral geometry. This is valid,e.g.,for serine and cysteine proteases.

From the classical thermodynamics, as well as Planck and Eyring theories we can derive an expression relating the n-th rate constant to the Gibbs energy of activation [10-12], $\Delta G^{\#}_{\mathrm{n}}$,

$$k_n = C_n \exp(-\Delta G_n^{\#} / RT) \tag{9}$$

where C_n is the preexponential factor dependent on the reaction order, R is the gas constant, T is the absolute temperature in K.

5. Comparison of two substrates

Eyring theory enables us to calculate rate constants from known heights of energy barriers along the reaction pathway and *vice versa*. In this chapter, we will focus on the task of estimating efficiency of two similar substrates, when the energy profiles of their reactions are at least, partly, known. Assume two-step reaction (Fig. 1), as described by Eq.(8), and known concentrations of $[E]_o$ and [S]. A general comparison of two substrates is difficult. However, there are several situations where the Michaelis–Menten equation (4) can be simplified.

5.1 Concentration [*S*] is much higher than K_M

In this case, Eq. (4) is reduced to the form of

$$v = k_2[E]_0 \tag{10}$$

and the relative efficiency of the enzyme reaction is given by

$$\frac{v(L)}{v(D)} = \frac{k_2(L)}{k_2(D)} = \exp\{[-\Delta G_2^{\#}(L) + \Delta G_2^{\#}(D)]/RT\} \tag{11}$$

5.2 Concentration [*S*] is equal to K_M

In this situation, which is close to the physiological conditions, the same expressions (10) and (11) are valid.

5.3 Concentration [*S*] is much lower than K_M

Under this assumption, the Michaelis-Menten equation (4) has the form

$$v = k[E]_0[S] \tag{12}$$

where $k = k_{cat}/K_M = k_1 k_2/(k_{-1} + k_2)$, known as *specificity constant*. Brot and Bender [13] realized that this constant is a proper measure for the substrate-enzyme efficiency. Laidler and Peterman [14] calculated the final change in Gibbs energy, ΔG_o,

$$\Delta G_0 = \frac{k_{-1}(\Delta G_1^{\#} + \Delta G_2^{\#} - \Delta G_{-1}^{\#}) + k_2 \Delta G_1^{\#}}{k_{-1} + k_2} \tag{13}$$

As can be seen, the resulting expression for Gibbs energy includes rate constants and activation energies, which makes the calculation complicated. However, the overall energy change, ΔG_o, is a weighted mean of two contributions which can be analyzed separately.

(a) Assume $k_2 >> k_{-1}$
Under the condition mentioned above, k is equal to k_1 and Eq.(3) can be written as

$$\frac{v(L)}{v(D)} = \frac{k_1(L)}{k_1(D)} = \exp\{[-\Delta G_1^{\#}(L) + \Delta G_1^{\#}(D)]/RT\} \tag{14}$$

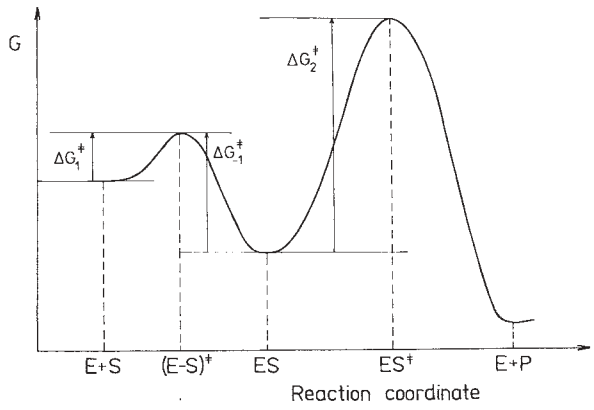

Fig.1 Energy profile along the pathway of the two-step enzyme - substrate reaction. Gibbs energy of following species : *E+S* free enzyme + free substrate; $(E\text{-}S)^{\#}$ first transition state; *ES* non-covalent complex; $ES^{\#}$ second transition state; *E+P* free enzyme + free product.

(b) Assume $k_2 << k_{-1}$
Then the final expression will be a fraction, where the numerator is

$$\exp[-(\Delta G_1^{\#} + \Delta G_2^{\#} - \Delta G_{-1}^{\#})/RT] \quad \text{for substrate L} \tag{15}$$

and the denominator will be a similar expression for substrate D.

6. Model for the first transition state

The first transition state, $(E\text{-}S)^{\#}$, should be calculated if k_1 and k_{-1} are to be utilized. General modelling of the $(E\text{-}S)^{\#}$ state is difficult. But there are several situations where the problem can be overcome. At least three conformations corresponding to the *E+S*, *ES* and $E^{def}+S^{def}$ states are feasible, where E^{def} is a symbol for isolated enzyme of the deformed (strained) conformation corresponding to the geometry of the *ES* complex. The same holds for the deformed substrate S^{def}. It is clear (Fig.2) that the unknown value of `d` can be eliminated from expression (16)

$$\exp[-(\Delta G_1^{\#} + d + \Delta G_2^{\#} - \Delta G_{-1}^{\#} - d)/RT] \tag{16}$$

Therefore, the point E on the strain curve (c) can be used instead of the point D on the Gibbs energy curve (b).

7. Model for the second transition state

The reaction path from the non-covalent complex *ES* to the second transition state $ES^{\#}$ requires formation of a covalent bond between enzyme (e.g., α-chymotrypsin or cathepsin B) and the substrate. Potentials available in the standard force field programs cannot describe properly formation and/or breaking of covalent bonds. Therefore, calculation of $\Delta G^{\#}_2$ and, consequently, k_2 is not a straightforward task. However, there are some situations where this obstacle can be avoided. As an example, we will show the case mentioned in section 5.3(b).

Assume that an increase in the Gibbs energy Δ (Fig.2) of valence bond formation between *E* and *S* is the same for

the *L*-type substrate as for the *D*-type substrate. Then the feasible rest of the $(\Delta G^{\#}_2 - \Delta)$ is given by a change in energy contribution which is different for the *L*-type and *D*-type substrates. Rating of the two substrates is possible because the unknown value of Δ in the exponential function of the final expression for *v(L) / v(D)*, is eliminated.

8. Master equation

In the previous sections, we have assumed the possibility of dividing Δ*G* into two independent contributions: a known part and unknown part. Ajay and Murcko [15] used a more general model adopted in the force field and wrote the Gibbs energy as an additive interaction of different parts. Such an expansion, called *master equation*, can simplify our calculations. Terms representing, e.g., the influence of water are, within a small error, the same for both similar substrates and, therefore, can be subtracted.

Furthermore, in the general thermodynamic formula

$$\Delta G = \Delta H - \mathrm{T}\Delta S \qquad (17)$$

similar entropic terms can be subtracted from many contributions to the master equation for the same reason. Instead of Δ*G*, the value of Δ*H* can only be calculated. Even this step can be simplified if we consider the most common assumptions:

(a) ensemble averages can be replaced by values corresponding to a single stable structure,
(b) a single conformation predominates in the free enzyme and free substrate.

Then the methods of molecular (force-field) mechanics can be utilized to estimate Δ*H* directly.

Certainly, we must not forget that this is a very rough approximation which may be applicable in congeneric series of compounds only [16].

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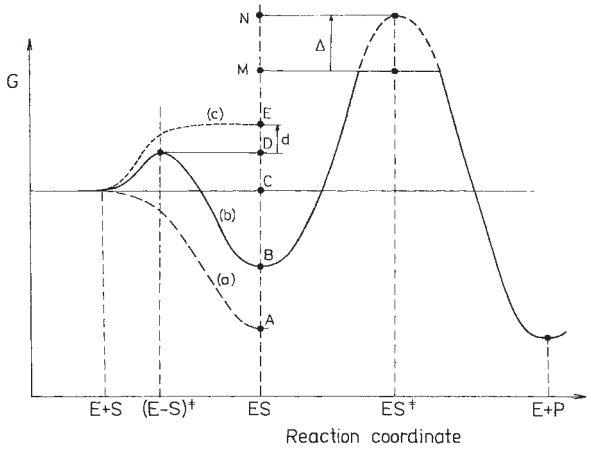

Fig.2 Definition of energies of attraction and repulsion : d and Δ, unknown contributions to Gibbs energy; (a) course of attracion, (b) = (a)+ (c) course of final Gibbs energy; (c) course of repulsion due to the distortion; CD = $\Delta G^{\#}_1$; BD = $\Delta G^{\#}_{-1}$; DE = *d* ; BN = $\Delta G^{\#}_2$; MN =Δ. Relative energy at A, B, C, E, M levels can be modelled with molecular mechanics methods. For

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