The evolution of enzyme kinetic power

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Evolution of the kinetic potential of enzyme reactions is discussed. Quantitative assessment of the evolution of enzyme action has usually focused on optimization of the parametric ratio $k_{\text{cat.}}/K_m$, which is the apparent second-order rate constant for the reaction of free substrate with free enzyme to give product. We propose that the general form $k_{\text{cat.}}[E]_T/K_m$ (where $[E]_T$ is total enzyme concentration), which is designated the 'kinetic power', is the real measure of kinetic/catalytic potential *in situ*. The standard paradigm of 'perfection' dictates the evolutionary maximum of 'kinetic power' to be $k_{+s}[E]_T/2$, where k_{+s} is the diffusion-controlled rate constant for formation of the ES complex (and, hence, for the overall enzyme reaction). We discuss the role of protein conformational mobility in determining this state of 'perfection', via gating of substrate binding and determination of the catalytic configuration. Going beyond the level of the individual enzyme, we indicate the manner by which the organizational features of enzyme action *in vivo* may enhance the 'kinetic power'. Through evolutionary 'perfection' of the microenvironment, one finds that the 'kinetic power' of enzymes can be affected by alteration of $[E]_T$ as well as the unitary rate constants. At this level of complexity, we begin to realize that the 'kinetic' description of cell metabolism must be supplemented with thermodynamic concepts.

Rapid chemical dynamics is a hallmark of cell metabolism. This temporal quality has been tailored by the evolutionary emergence and development of enzymes, which are charged with the task of catalysing material transformations at rates and, equally importantly, under appropriate conditions commensurate with the vitality of the cell. Previously, we suggested (Keleti, 1975; Welch & Keleti, 1981) three biological stages with which enzyme evolution might be directly linked: (i) abiogenesis, with the appearance of 'eobionts' (transition forms) from coacervates, microspheres and the like; (ii) early biological evolution, wherein were forged bottlenecks that tended to unify organisms, as regards a narrowing of the possible modes of efficient execution of metabolic processes; (iii) higher evolution, with its attendant specialization, integration of diverse activities and designs for bioregulation.

As discussed for example by Koshland (1976), the early 'struggle for existence' probably placed keen importance on increase in both the power (rate) and the specificity of enzyme catalysis. A

usual working hypothesis is that proteins evolved in a gradual stepwise manner, by addition (or elimination) of amino acids in a given structure. Hence one might posit that evolutionary elaboration of enzyme structure around the active site led to greater catalytic capacity, while generating a geometry for increased specificity.

By following this paradigm, it has been maintained that, when protein structure became large enough to accommodate conformational flexibility, a major achievement in the evolution of enzyme function must have been realized (Koshland, 1976). The way was paved for co-operativity, as well as the basis for other molecular schemes of metabolic regulation (e.g. isomerization, oligomerization, dynamic compartmentation etc.). Also, means were established for integrating enzyme action into the 'spatial' structure of the cell, by virtue of the organization (e.g. aggregation, membrane association) of multienzyme systems (Welch & Keleti, 1981; Srere, 1984).

Quantitative assessment of the evolution of catalytic capacity and specificity for enzymes has

usually focused on optimization of the ratio $k_{\text{cat.}}/K_m$, where $k_{\text{cat.}}$ is the catalytic turnover constant and K_m the Michaelis constant (Fersht, 1977). Brocklehurst (1977) has given a detailed and cogent treatment concerning the evolution of 'catalytic power' for individual enzymes. In the present paper we extend further the significance of the parametric ratio $k_{\text{cat.}}/K_m$, relating it to the evolution of conformational flexibility and enzyme organization. We propose a general form, designated the 'kinetic power', according to which one might view evolution of the kinetic potential of enzyme reactions at various levels of biological complexity.

Theory

I. Existing kinetic scheme

Let us consider the following simple Michaelian enzyme system:

with the usual form for the forward velocity:

$$v_f = \frac{V_{\text{max.}}[S]}{K_m + [S]} \quad (2)$$

and the definitions:

$$V_{\text{max.}} = k_{\text{cat.}}[E]_T \quad (3)$$

and

$$K_m = \frac{k_{-s} + k_{\text{cat.}}}{k_{+s}} \quad (4)$$

where $[E]_T$ is the total enzyme concentration.

For simple (i.e. non-regulatory) enzymes the major attention, in discussions concerning evolution, has been given to the maximization of the overall rate. The primary focus is the ratio $k_{\text{cat.}}/K_m$, which is an apparent second-order rate constant relating to the reaction of free enzyme with free substrate to give product (as seen when $[S] \ll K_m$ in eqn. 2). Rigorous reasoning leads to the conclusion that maximization of rate entails an evolutionary pressure to increase K_m (relative to an ambient substrate concentration), with a consequent increase in $k_{\text{cat.}}$ over k_{-s} (Fersht, 1977; Brocklehurst, 1977; Cornish-Bowden, 1976; Brocklehurst & Cornish-Bowden, 1976). In these evolutionary discussions k_{+s} (the second-order rate constant for collision/binding of enzyme and substrate) is usually regarded as something of a 'backdrop'. Since evolutionary alteration in protein structure cannot affect the rate at which substrate diffuses to the enzyme molecule, k_{+s} supposedly represents a limiting rate constant for product formation.

When $k_{\text{cat.}} \gg k_{-s}$ and $K_m \gg [S]$, such that $[E]_T \simeq [E]_0$ (i.e. the concentration of free enzyme), eqn. (2) attains the limiting form $v_f \simeq k_{+s}[E]_0[S]$, where k_{+s} is diffusion-controlled (e.g. according to the Smoluchowski theory). A so-called 'perfectly evolved enzyme reaction' is one for which $k_{\text{cat.}}/K_m$ is at (or close to) the diffusion limit, and also for which $K_m \lesssim [S]$ (Fersht, 1977; Brocklehurst, 1977). Furthermore, it has been suggested (Knowles, 1976; Brocklehurst, 1977) that a compromise in the evolution of simple enzyme function leads to the condition, $k_{-s} \simeq k_{\text{cat.}}$, so that no one transition-state barrier in a multistep enzyme reaction is rate-limiting. It should be noted also that $k_{\text{cat.}}/K_m$ is an important determinant in the specificity for competing substrates (Fersht, 1977).

II. Definition of enzyme 'kinetic power'

We suggest that the evolution of enzyme action would be manifested more appropriately in an increase of the quantity $V_{\text{max.}}/K_m$, the real measure of the kinetic-catalytic potential *in situ*. This is an apparent first-order rate constant relative to the conversion $S \rightarrow P$ (i.e. the rate of such conversion per unit concentration of substrate S , when $[S]$ is limiting). It should hold the status of a fundamental kinetic constant of enzyme action, alongside $V_{\text{max.}}$ [Although K_m relates (inversely) to the affinity of the enzyme for substrate under some conditions, it is not an independent variable (Cleland, 1977).] We propose to call $V_{\text{max.}}/K_m$ the 'kinetic power' of the metabolic conversion and symbolize it as ' k_Γ '. (Contrasting, $k_{\text{cat.}}$ may be regarded as the 'catalytic power', i.e. the rate per unit concentration of E , when $[E]$ is limiting.)

Looking at the reciprocal relation:

$$\frac{1}{k_\Gamma} = \frac{K_s}{V_{\text{max.}}} + \frac{1}{k_{+s}[E]_T} \quad (5)$$

we see that k_Γ depends on the equilibrium dissociation constant (K_s) for the ES complex, the maximal catalytic capacity ($V_{\text{max.}}$), and the diffusion-controlled rate constant for product formation (regarding $k_{+s}[E]_T$ as an apparent first-order constant in the limiting case $v_f \simeq k_{+s}[E]_0[S]$). The state of 'evolutionary perfection', as indicated in Section I above, implies that k_Γ attains the value $k_{+s}[E]_T$. Writing eqn. (5) in the form:

$$\frac{1}{k_\Gamma} = \frac{1}{k_{+s}[E]_T} \left(\frac{k_{-s}}{k_{\text{cat.}}} + 1 \right) \quad (6)$$

and applying the aforementioned evolutionary 'compromise' condition $k_{-s} \simeq k_{\text{cat.}}$, shows the evolutionary maximum of 'kinetic power' (k_Γ) to be $k_{+s}[E]_T/2$, i.e. half the diffusion-controlled rate constant.

III. Protein conformational flexibility and 'kinetic power'

With increasing size, various conformational degree of freedom in the protein molecule came to play roles in enzyme action. This flexibility placed additional constraints on the evolution of the 'kinetic power' k_{Γ} . For example, consider the binding of enzyme and substrate, as reflected in the constant k_{+s} . The binding may be dissected into two parts, as follows (North, 1964):

Hence, E and S must first diffuse into an encounter-pair configuration, with S in the 'recognition volume' of the enzyme active site (Welch, 1977a; Somogyi & Damjanovich, 1973). This diffusional step is defined by a constant k_D , which we may regard as the evolutionary upper limit on k_{+s} , according to the Smoluchowski theory (Fersht, 1977). Then follows the actual binding, characterized by an intrinsic bimolecular rate constant $\tilde{k}_{+s}$. The overall constant k_{+s} has the steady-state form:

$$k_{+s} = \frac{k_D \tilde{k}_{+s}}{k_D + \tilde{k}_{+s}} \quad (8)$$

Similarly, one finds that $k_{-s} = k_D \tilde{k}_{-s} / (\tilde{k}_{+s} + k_D)$, which becomes $k_{-s} = k_D \tilde{k}_{-s} / \tilde{k}_{+s}$ under diffusion-controlled conditions (North, 1964).

If the active-site region of the enzyme is motile, then the binding of substrate may be 'gated' by the local stochastic motions of the protein. Let us describe the dynamics of the 'gate' by two rate constants k_{+i} and k_{-i} , following the simple kinetic scheme:

Suppose that the opening/closing of the 'gate' is slow compared with substrate diffusion. Then theory (Szabo *et al.*, 1982) yields:

$$k_{+s} = \left(\frac{k_{+i}}{k_{+i} + k_{-i}} \right) \left(\frac{k_D \tilde{k}_{+s}}{k_D + \tilde{k}_{+s}} \right) \quad (10)$$

which becomes:

$$k_{+s} = \left(\frac{k_{+i}}{k_{+i} + k_{-i}} \right) k_D \quad (11)$$

when $\tilde{k}_{+s}$ is large. Also, when the 'gate' fluctuation is fast compared to diffusion, one finds similarly

(Szabo *et al.*, 1982) that:

$$k_{+s} = \frac{k_D \tilde{k}_{+s} k_{+i}}{(k_{+i} + k_{-i}) k_D + k_{+i} \tilde{k}_{+s}} \quad (12)$$

which becomes $k_{+s} = k_D$ when $\tilde{k}_{+s}$ is large.

Such conformational mobility may further influence the evolutionary maximum for k_{Γ} . For example, let $k_{+i} = k_{-i}$ in eqn. (11). Including the aforementioned 'compromise' condition $k_{\text{cat.}} \simeq k_{-s}$, eqn. (6) shows that $k_{\Gamma} = k_D [E]_T / 4$, as compared with the maximal value $k_D [E]_T / 2$ suggested in Section II above (with k_{+s} replaced by the upper limit of k_D).

Analogous reasoning and similar kinetic equations (e.g. eqn. 11) apply to the case of conformational isomerization, with differential catalytic activity attributable to the various conformers (Keleti, 1967, 1968). When the number of conformational substates is large, the number of unitary rate constants (and degrees of freedom) increases enormously (Keleti, 1968), with the potential for rather significant decrease in k_{Γ} .

These considerations imply some degree of caution in the usual quantitative assessment of enzyme 'perfection'. The standard practice is to determine the closeness of the value of $k_{\text{cat.}} / K_m$ to k_D . If slow conformational motions are involved (in regulatory enzymes or otherwise), then the reaction may be diffusion-limited (in the sense that $\tilde{k}_{+s} \gg k_D$), yet $k_{\text{cat.}} / K_m$ would be less than k_D by virtue of 'gating' or 'isomerization' (see eqn. 11).

Kinetic considerations alone suggest that the early evolution of enzymes (regulatory factors aside) would favour tight compact globular forms, whose conformational motions are relatively localized and fast, as opposed to loose, slow-moving ('floppy'), structures. As indicated in eqn. (12), k_{+s} would then approximate to k_D (when $\tilde{k}_{+s}$ is large), and k_{Γ} attains the upper limit of $k_D [E]_T / 2$.

Similar thinking applies to the optimization of k_{Γ} via increase in $k_{\text{cat.}}$ (see eqn. 6). The actual catalytic decomposition process of the ES complex can be modelled as a slow 'diffusion' (in configuration space) along a reaction co-ordinate (Karplus & McCammon, 1983; Welch *et al.*, 1982). Experimental evidence and molecular-dynamics studies have revealed a wide variety of internal protein motions (Karplus & McCammon, 1983; Careri *et al.*, 1975), most of which are much faster than typical values of enzyme catalytic turnover numbers ($k_{\text{cat.}}$). It is apparent though, that $k_{\text{cat.}}$ is dependent on the rate of conformer transitions in the enzyme molecule (Keleti, 1968; Welch *et al.*, 1982; Karplus & McCammon, 1983; Welch, 1985). Again, we recognize an evolutionary (kinetic) determinant towards compact globular construction of enzyme molecules, whose internal motions

are localized, modular and rapid. It has been suggested (Rupley *et al.*, 1983) that such kinetic factors relate to the internal 'domain' structure of globular proteins.

Of course, conformational flexibility provides the basis for such regulatory designs as allostery, co-operativity, hysteresis etc. (Koshland, 1976). In some enzymes, regulatory considerations take priority over the maximization of 'kinetic power'. For example, Ricard (1978) showed that kinetic co-operativity of a monomeric enzyme must be 'paid for' at the expense of catalytic efficiency, and that the monomeric enzyme cannot be simultaneously co-operative and catalytically 'perfect' (in the sense suggested in Section I above). Ricard (1978) suggested that the need to achieve regulatory power with optimal catalytic efficiency may have been an important selective pressure in the evolution of polymeric regulatory enzymes.

IV. Supramolecular influences on the 'kinetic power' of enzyme systems

For simple (i.e. non-regulatory) enzymes, one supposes that the evolutionary course dictates the optimization of the structure and dynamics of protein structure to a point as close as possible (as determined by the enzyme mechanism) to the 'kinetic power' $k_{\Gamma} = k_D[E]_{\Gamma}/2$. As discussed above, enzymes that exhibit this form *in vitro* are said to have attained a state of 'perfection'. This view would imply that evolution can mould such enzymes no further. Although this may be the case for certain types of enzyme, it falls short, in general, as a 'biological principle' of enzyme evolution.

The standard paradigm of 'perfection' presumes that the enzyme molecule operates in isolation in bulk solution. Accumulating evidence indicates that many enzymes of intermediary metabolism function in organized states *in vivo*, associated as protein-protein complexes and/or adsorbed on intracellular particulates (see Srere, 1984; Clegg, 1984; Wombacher, 1983; Masters, 1981; Welch & Keleti, 1981; Welch, 1977a; and other references cited therein). Both k_{+s} (i.e. k_D) and k_{cat} are subject to influence in these regimes, by extrinsic proximity and catalytic-enhancement effects. There are quite a number of supramolecular 'structural' factors that can be brought to bear on enzyme action within the confines of organized states (Welch & Keleti, 1981).

A major benefit of enzyme organization is metabolite compartmentation, in dynamically (Friedrich, 1974; Keleti, 1984) and statically (Welch, 1977a) interacting enzyme aggregates. An important concomitant is a decrease in (or elimination of) the steady-state transient time. For a particular enzymic step, this temporal element is

seen to be given by $\sim 1/k_{\Gamma}$ (Welch, 1977b). Thus, the greater is the 'kinetic power' (k_{Γ}) of the component enzymes in a sequence, the more efficient the metabolite compartmentation [and the lower the concentrations of intermediates required to sustain the steady state (Welch, 1977b)]. Referring to eqn. (6), it is realized that localization of enzyme systems in specific micro-environments *in vivo* can increase k_{Γ} , not only by extrinsic enhancement of k_{+s} (i.e. k_D) and k_{cat} , but also by increase in the effective (local) enzyme concentration. Multienzyme complexes, which 'channel' substrates, represent the highest level of efficiency.

A complete multienzyme sequence, seen as a unit, is the most likely focus in the evolutionary optimization of cell metabolism (Keleti, 1975; Welch, 1977a). Accordingly, assertions of 'perfection' in the evolution of enzyme 'kinetic power' will be of limited value, unless something of the micro-environment *in situ* of the given enzyme is known (see also Brocklehurst, 1977). The notion of 'perfection' currently in usage maintains, basically, that the enzyme molecule evolves to the point where its kinetic behaviour is controlled by its environment (through k_{+s} and $[E]_{\Gamma}$). We wish simply to extend this perspective, through the realization that the microenvironment itself is evolving, and that this microenvironment can modify any or all of the parameters on the right-hand side of eqn. (6). There are indications that the very globular structure of many enzymes has been 'perfected' evolutionarily just to integrate into supramolecular regimes *in vivo* (McConkey, 1982; Srere, 1984).

V. Beyond the kinetic description

The 'kinetic' description of chemical reactions expresses the rate in terms of concentrations and unitary rate constants. The reaction rate is determined by the probability that a molecule has a critical 'activation' energy per population, multiplied by the actual population size (i.e. the concentration). This approach has proven extremely valuable, particularly in the analysis of multistep chemical processes. Rendering the rate constants according to the Smoluchowski theory, collision theory, transition-state theory etc., we gain insight into the molecular mechanisms of such processes. And, in principle, the kinetic approach can even be used to quantify steady-state concentrations of metabolic intermediates (Welch, 1977b).

Yet, the kinetic description does not give an adequate representation of the driving force in chemical processes. One must turn to thermodynamics here. In fact, the equilibrium character of the reaction $S \rightleftharpoons P$ tempers the maximization of

'kinetic power', k_{Γ} . A more general expression for k_{Γ} is as follows:

$$\frac{1}{k_{\Gamma}} = \frac{1}{k_{+s}[E]_{\Gamma}} \left(\frac{k_{-s}}{k_{cat.}} + \frac{1}{K_{eq.}} + 1 \right) \quad (13)$$

where $K_{eq.}$ is the equilibrium constant (Cleland, 1975; Brocklehurst, 1977). It is only for an irreversible reaction (i.e. $K_{eq.} \gg 1$) that eqn. (13) reduces to eqn. (6). (We note that the Haldane relation specifies the condition $K_{eq.} = k^f_{\Gamma}/k^r_{\Gamma}$, where indices 'f' and 'r' denote forward and reverse reactions respectively.)

Thermodynamically, the rate of the reaction will be some function of the chemical-potential difference ($\mu_s - \mu_p$) between reactants and products. Kinetic rate constants are determined by the free-energy levels of saddle-point transition states ('hills'). {For example, k_{Γ} depends on the free-energy difference between $E+S$ and the $[ES]^{\ddagger}$ transition state (Fersht, 1977).} In contrast, the quantity $\mu_s - \mu_p$ relates to the free-energy difference between stable reactant and product states ('valleys'). In the evolutionary 'optimization' of cell metabolism, this differential element is itself subject to alteration, as seen for example in the ubiquity of activated intermediates (Atkinson, 1969). Also, within the molecular environment of organized multienzyme systems that 'channel' substrates, the free-energy level (chemical potential) of the intermediates may well differ from that in bulk solution (Welch, 1977a; Welch & Keleti, 1981; Welch & Kell, 1985). Although, at present, there is little empirical information on the physico-chemical nature of such compartmentalized metabolites, it is evident that the chemical potentials must depend intimately on their interaction with the surrounding matrix (Hill, 1977).

In conclusion, we saw in Sections I–III how far in evolution the individual enzyme molecule can go, in optimizing the 'kinetic power' of cell metabolism. Studies performed *in vitro* suggest an (approximate) upper limit of $k_{\Gamma} = k_D[E]_{\Gamma}/2$. But, in Section IV, we found that evolution can go much further in tailoring the dynamical character of enzyme action, when the enzyme molecule is imbedded in its natural setting. At this higher level of complexity, the quantitative assessment of enzyme 'kinetic power' will require a marriage of conventional kinetics with newly emerging thermodynamic formalisms (Hill, 1977; Westerhoff, 1982; Stucki, 1982; Welch, 1984).

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