THE THERMODYNAMIC DESCRIPTION OF ENZYME-CATALYZED REACTIONS

THE LINEAR RELATION BETWEEN THE REACTION RATE AND THE AFFINITY

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ABSTRACT The rate of enzyme-catalyzed reactions is a proportional function of the reaction affinity over a range of more than 2 kcal/mol, (i.e., 25-fold substrate concentration change). For kinetically irreversible reactions, proportionality is obeyed when the substrate concentration is of the same order of magnitude as the K_m of the reaction. Linearity can be obtained by proper choice of product concentration or alternatively by a linear transformation which allows the description of the system by slightly different parameters. For kinetically reversible reactions, the linear range could be obtained and extended to both sides of equilibrium provided the concentration of the substrate is fixed at a proper value and the affinity is varied by the product concentration. In oxidative phosphorylation, a coupled system of enzymatic reactions, proportional regions are found for both oxidation and phosphorylation. These findings justify the use of linear phenomenological equations in bioenergetics.

In nonequilibrium thermodynamics, the rate of a chemical reaction, the reaction flow (J_r), is a function of its thermodynamic driving force; the chemical affinity (A), as defined by De Donder (1), is $A = -\sum_i \nu_i \, d\mu_i$, where ν_i represents the stoichiometric coefficients and μ_i is the chemical potential of the reactants and the products. A useful form of this function is the linear function $J_r = L_r A$, which could be included in the linear phenomenological description of complex systems that involve interactions (i.e., coupling) between various flows. However, in general, the validity of the linear relation is very limited. Reaction rates obey the mass action law in which $J_r = f(C_a, \cdots, C_n)$, where C_a to C_n are the concentrations of both reactants and products. Thus, for the linear thermodynamic equation to be valid it is necessary that $[f(C_a, \cdots, C_n)]/A$ remain constant when A is changed, since $J_r = [f(C_a, \cdots, C_n)/A] \cdot A$. The term $f(C_a, \cdots, C_n)/A$ might be constant only in a limited region of A (and C's) and only when A is changed in a restricted manner (such as by keeping all C's constant except for changing C_i).[1] It was first demon-

[1] These restrictions are not peculiar to the thermodynamic description of reaction rates. Even in the classical linear system of diffusion, the diffusion coefficient is constant only over a small range of concentrations. In the coefficient of the reaction flow the dependence is on the concentration of more than one species and it is difficult to change the affinity without affecting this coefficient.

strated by Prigogine et al. (2) that for a class of simple reactions, where there is a linear dependence of the rate on the concentration, the thermodynamic description is valid in a very limited region, very close to equilibrium, where $RT \gg A$. It is important to realize that the linearity of these reactions near equilibrium is not the result of some fundamental property of the system but of the fact that in this domain $f(C_a, \cdots, C_n)/A$ is constant. Thus, it is not unlikely that for other kinetic systems there is another domain, not necessarily near equilibrium, in which the linear description is valid. Such domains, indeed, should exist on approaching saturation kinetics when the reaction order is changing from high-order to zero-order kinetics.

Enzyme-catalyzed reactions obey very special kinetic laws. The most common description of enzyme kinetics is in terms of the Michaelis-Menten equation (3) where V, the reaction rate, is given by

$$V = \frac{V_S \cdot S}{K_S + S}, \tag{1}$$

where S is the substrate concentration, V_S is the maximal velocity (at excess substrate concentration), and K_S is a constant (Michaelis constant) related to the association between the enzyme and the substrate.

This relation can be derived from a kinetic description of the steady state of the reversible system

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} SE \rightleftarrows PE \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E. \tag{2}$$

In a steady state the net rate of the reaction $S \rightarrow P$ is given by

$$V = \frac{(V_S S/K_S) - (V_P P/K_P)}{1 + (S/K_S) + (P/K_P)}; \tag{3}$$

Eq. 3 reduces to Eq. 1 when $P = 0$. However, in this condition the affinity A is not defined. Nevertheless, many enzyme systems can be described by Eq. 1 even though P is not negligible. This is a result of the conditions $V_S \gg V_P$ and $K_P \gg K_S$. This situation is associated with high standard free energy as seen from the Haldane relation (4)

$$K_{eq} = \frac{V_S}{V_P} \cdot \frac{K_P}{K_S}. \tag{4}$$

Thus when $V_S \gg V_P$ and $K_P \gg K_S$, P even at high concentration does not affect the rate of the reaction $S \rightarrow P$. For those systems which obey Eq. 1 we can vary the affinity (and the rate) by changing the substrate concentration while the product concentration is kept constant. The affinity of enzyme reactions is given by

$$A = RT \ln \left(K_{eq} \cdot \frac{S}{P} \right) = RT \ln \left(\frac{V_S}{V_P} \cdot \frac{K_P}{K_S} \cdot \frac{S}{P} \right). \tag{5}$$

Since P is kept constant, we define C as $\ln (V_S/V_P)\cdot(K_P/P)$ and X as S/K_S and rewrite Eq. 5:

$$A = RTC + RT \ln X. \tag{6}$$

Assuming that there is another region of linearity in the function $J_r = f(A)$ that is not necessarily identical with the linear region close to equilibrium, we can write

$$J_r = K + L_rA = K + L_rRTC + L_rRT \ln X = K' + L_rRT \ln X. \tag{7}$$

The Michaelis-Menten equation (Eq. 1) gives

$$V = V_S \frac{X}{(X+1)}. \tag{8}$$

We now use the series

$$\ln X = 2\left[\frac{(X-1)}{(X+1)} + \frac{1(X-1)^3}{3(X+1)} + \cdots\right], \tag{9}$$

which is valid for positive X. When X is close to 1 (say between 0.3 and 3) the first term is a good approximation of the series. Thus

$$\ln X \simeq 2\frac{(X-1)}{(X+1)} = 2\left(-1 + \frac{2X}{X+1}\right), \tag{10}$$

and

$$\frac{X}{(X+1)} = \frac{1}{2} + \frac{\ln X}{4}. \tag{11}$$

Substituting Eq. 11 into Eq. 8 gives

$$V = \frac{1}{2}V_S + \frac{V_S}{4}\ln X. \tag{12}$$

It is observed that Eqs. 7 and 12 are of the same form; a comparison of the two gives

$$L_r = \frac{V_S}{4RT}; \qquad K = \frac{1}{2}V_S\left(1 - \frac{1}{2}C\right). \tag{13}$$

Thus when $C = 2$, $K = 0$ and $J_r = L_rA$.

Since P does not have an effect on the reaction rate it could be arbitrarily chosen,

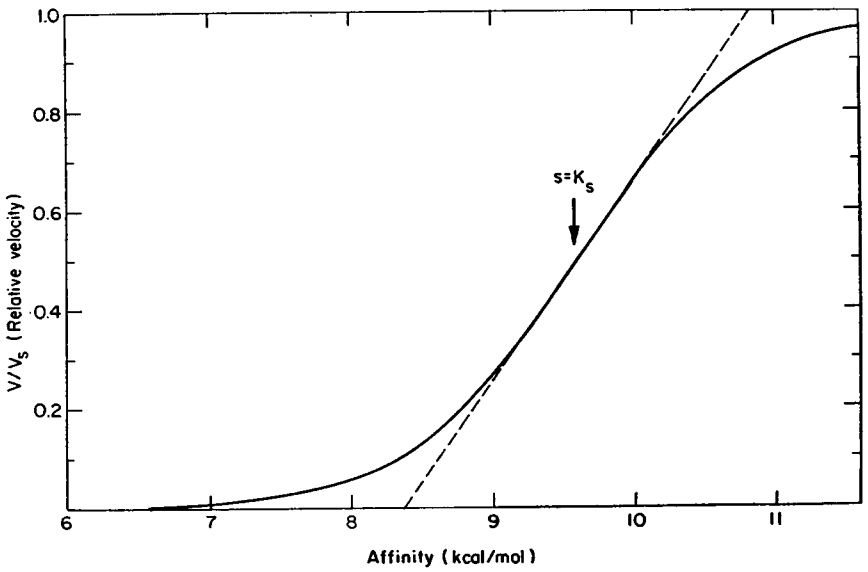

FIGURE 1 The rate of sucrose hydrolysis by invertase as a function of the reaction affinity. The rate was calculated from the Michaelis-Menten equation assuming K_S of 0.0167 M (3), and the affinity by assuming ΔG^0 of 6.57 kcal/mol (5). The affinity was varied by changing the sucrose concentration (from 1×10^{-4} to 1 M) while keeping the glucose and fructose concentrations equal and constant (at 1×10^{-2} M).

resulting in the condition

$$C = \ln \frac{V_S}{V_P} \cdot \frac{K_P}{P} = 2,$$

in which $J_r = L_r A$. This procedure might not always be possible, both because it could bring the system into a region in which Eq. 1 is not valid anymore, and because the concentration of P could be so high that it could not be realized in real systems. It is also possible to transform Eq. 7 into a linear equation by the following transformation of flows and forces: Let us define $\bar{J}_r$ as $J_r - \frac{1}{2}V_S$ and $\bar{A}$ as $A - RTC$. The dissipation function is given by $\phi = J_r A = \bar{J}_r \bar{A} + \frac{1}{2}V_S RTC$. In this new system when $\bar{A} = 0$, $\bar{J}_r = 0$ and $\bar{J}_r = L_r \bar{A}$ for $\bar{A}$ values up to ± 1 kcal/mol.

Fig. 1 shows the relative rate of the reaction

$$\text{sucrose} \xrightarrow{\text{invertase}} \text{glucose} + \text{fructose}$$

as a function of the reaction affinity.

It is observed that in the region where the substrate concentration is of the same order of magnitude as the K_S there is a linear relationship between the reaction rate and the affinity. This relationship holds far away from equilibrium, particularly since the Michaelis-Menten treatment is valid only in cases where K_{eq} is large and P

is small, conditions which involve large A. Even though linearity is preserved only along a span of 1–2 kcal this is often the region of interest since in higher substrate concentrations the reaction becomes saturated (i.e., independent of the substrate concentration), whereas in lower substrate concentrations it becomes very slow. In the example which is shown in Fig. 1 the standard free energy is unusually large; therefore, the linear region in the vicinity of the K_S is quite far from the linear region found near equilibrium. When the standard free energy of the reaction is low the two linear regions could be united into one. For these reactions, which are kinetically reversible, the Michaelis-Menten equation is no longer valid. The accurate kinetic description of the mechanism described by Eq. 2 is given by Eq. 3. In a restricted system it is possible to transform Eq. 3 into one linear function of the affinity extending on both sides of equilibrium over most of the range in which the reaction is not completely saturated. This is demonstrated in the following treatment.

We will denote as substrate the substance with the higher maximal velocity ($V_S > V_P$). Let us multiply all terms by K_P/P:

$$V = \frac{(V_S K_P/K_S P) - V_P}{(K_P/P) + (K_P S/K_S P) + 1}. \tag{14}$$

Written in a slightly different form Eq. 14 becomes

$$V = \frac{[(V_S K_P S/V_P K_S P) - 1]V_P}{(K_S K_P/K_S P) + (K_P S/K_S P) + 1}. \tag{15}$$

Let us restrict our system to constant substrate concentration such that

$$S = K_S/[(V_S/V_P) - 1]$$

or

$$K_S = [(V_S/V_P) - 1]S. \tag{16}$$

Substitute this value of K_S into Eq. 15:

$$V = \frac{[(V_S K_P S/V_P K_S P) - 1]V_P}{[(V_S/V_P) - 1](K_P S/K_S P) + (K_P S/K_S P) + 1}$$

$$= \frac{[(V_S K_P S/V_P K_S P) - 1]V_P}{(V_S K_P S/V_P K_S P) + 1}, \tag{17}$$

and because of the Haldane relationship (Eq. 4)

$$V = \frac{\left(K_{eq}\frac{S}{P} - 1\right)V_P}{K_{eq}\frac{S}{P} + 1}. \tag{18}$$

Using again the first term of the series in Eq. 9 for values of $K_{eq}(S/P)$ close to 1,

$$V = \frac{\ln\left(K_{eq}\frac{S}{P}\right)V_P}{2}, \tag{19}$$

and from Eqs. 5 and 19,

$$V = \frac{V_P}{2RT}A. \tag{20}$$

Thus, under the prescribed conditions the reaction rate is linear with the affinity having the same coefficient on both sides of the equilibrium up to values of ± 1 kcal/mol.[2] It should be pointed out that the choice of the substance kept constant is dictated by the kinetic parameters, and it must be the substance with the higher V_{max}. This is due to the fact that the symmetry of the kinetic curve about equilibrium is achieved by lowering the maximal possible velocity of one reaction to that of the V_{max} of the slower reaction by keeping the concentration of the substrate of the faster reaction constant and low.

The fact that the linear region of Eq. 20 is centered at $A = 0$ brings the linear region of the enzyme to overlap the linear region of near equilibrium which exists for all reactions irrespective of mechanism (2). However, the range of the enzyme kinetics linearity is orders of magnitude larger than the near equilibrium region that is only limited for values of $A \ll \pm RT$.

Fig. 2 shows plots of the rate of the reaction

$$\text{fructose-6-P} \xrightarrow{\text{phosphoglucose isomerase}} \text{glucose-6-P}$$

as a function of the reaction affinity. Each plot is obtained from Eq. 3 by keeping fructose concentration constant and varying the glucose concentration over a wide range. In Fig. 2 A, in which fructose concentration is extremely low, the curve could be approximately described by Eq. 1 (with the glucose V_S and K_S), and the linear region by Eq. 12.

As the fructose concentration is increased, the linear region is shifted toward equilibrium and its slope changed (Fig. 2 B). In the condition specified by Eq. 16 the curve becomes symmetric about equilibrium and its linear region is described by Eq. 20 (Fig. 2 B, $f = 2 \times 10^{-4}$ M). As the fructose concentration is further increased the curve shifts farther away from equilibrium until the linear region starts from its origin; this situation is obtained when $C = 2$.

It is noted that the two conditions for linearity in the reversible and (kinetically) irreversible system are different. Inspection of Fig. 2 indicates that while in the

[2] The deviation from linearity at $A = \pm 1$ kcal is 18%.

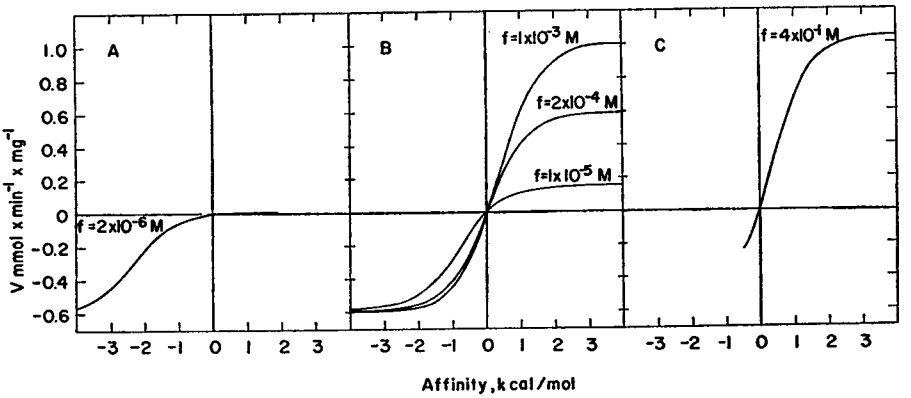

FIGURE 2 The rate of the reaction fructose-6-P → glucose-6-P as catalyzed by phosphoglucose isomerase. The kinetic constants are: V_f = 1.037 mmol × min^{-1} × mg^{-1} V_g = 0.586 mmol × min^{-1} × mg^{-1}; K_f = 0.17 mM; K_g = 0.37 mM (6). Rates were calculated and plotted by a computer from Eq. 3. For each curve the fructose concentration was kept constant and the affinity varied by changing the glucose concentration. The fructose concentration of each curve is indicated on the curve. A, low fructose concentration; B, intermediate fructose concentration; C, high fructose concentration.

reversible treatment we fix the center of the linear region at equilibrium, in the irreversible treatment we bring the beginning of this region close to equilibrium (since we are only considering the reaction $S \rightarrow P$). Thus, it is quite clear that, under various restricted conditions, enzymatic reaction rates show linear dependence on the affinity over most of the range in which the reaction is not saturated. Moreover, for studies of complex coupled systems it is quite possible to control the conditions experimentally so as to bring about linear behavior.

In a study of oxidative phosphorylation in rat liver mitochondria,[3] which is based on thermodynamic analysis (7), it was found that there is a wide range in which the oxidation reaction is linear both with the oxidation and the phosphorylation affinity, and that similarly the phosphorylation rate is linear with both affinities. This range is shown in Fig. 3; in higher or lower affinities linearity does not hold. It is seen that the phosphorylation rate (J_p) is linear with the phosphorylation affinity (A_p) and the coefficient of linearity (L_p) could be calculated from the slope. Likewise a different coefficient (L_o) relate the oxidation rate (J_o) to the oxidation affinity (A_o). On the other hand, the dependence of phosphorylation on the oxidation affinity is the same as the dependence of the oxidation on the phosphorylation affinity; in other words, $L_{po} = L_{op}$, which verifies Onsager's relation.

Thus the following equations could describe the system:

$$J_p = K + L_p A_p + L_{po} A_o , \tag{21}$$

$$J_o = K' + L_{op} A_p + L_o A_o . \tag{22}$$

[3] Rottenberg, H. Manuscript in preparation.

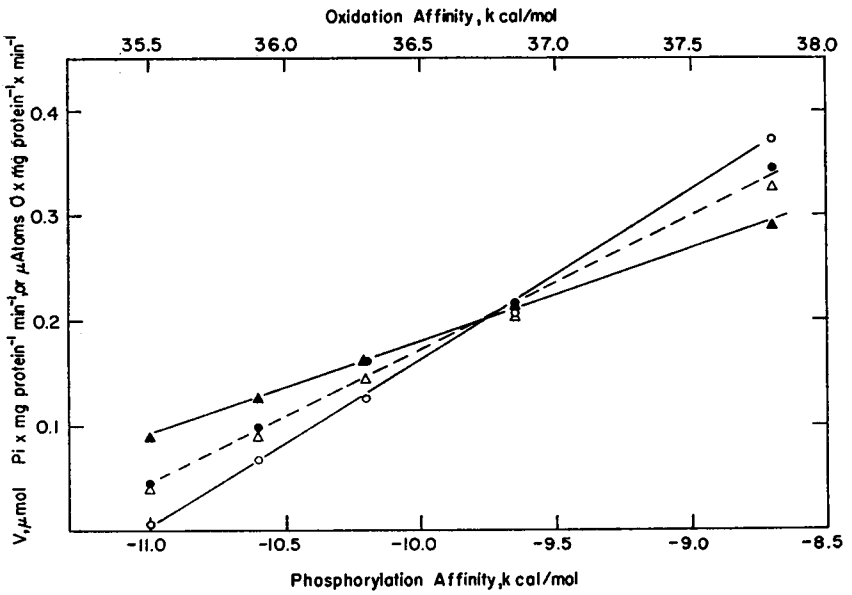

FIGURE 3 The rates of oxidative phosphorylation as a function of the reaction affinities. Rat liver mitochondria were incubated in a medium that contained 50 mM sucrose, 50 mM KCl, 3 mM Mg, 1 mM ATP, and 0.5 mM fumarate. The oxidation affinity was changed by various concentrations of succinate and the phosphorylation affinity, by various concentrations of phosphate. 0.5 mM ADP was added to start the phosphorylation. The oxygen consumption was measured by oxygen electrode and the phosphorylation rate was calculated from the period of the stimulation of oxidation that occurs on addition of ADP. The open circles (○) show the rate of the phosphorylation as a function of the phosphorylation affinity (when the oxidation affinity is constant at 36.7 kcal), and the closed circles (●) show the rate of this reaction as a function of the oxidation affinity (when the phosphorylation affinity is constant at 9.7 kcal/mol). The open triangles (△) show the rate of oxygen consumption as a function of the phosphorylation affinity, while the closed triangles (▲) show the rate of oxygen consumption as a function of the oxidation affinity.

These could be transformed into linear equations as described above. However, a probable reason for proportionality here is the fact that each of the reactions studied are actually series of enzymatic reactions, and when changing substrate concentration one does not change the affinity of all the reactions involved. These aspects of the system will be discussed in more detail elsewhere.[3]

It might seem to the reader that the restrictions in which linearity and proportionality are obeyed are too prohibitive for application in biological studies. However, the most important requirement, that the substrate concentration is of the same order of the K_m, is usually satisfied both in vivo and in vitro. The conditions which require that the concentration of one of the reactants be kept constant can be easily obtained in vitro, and may often occur in vivo as well due to homeostatic mechanisms.

A recent review (8), points to various phenomena in bioenergetics in which

linearity is observed or implied. In particular, the linearity of the reaction rate with affinity which was observed in a model system that coupled transport to enzymatic reaction probably could be explained on the basis of our treatment (9). Thus, if indeed biochemical reactions, which are the driving force in most biological energy conversions, are linear with the affinity, the theory of irreversible thermodynamics, and in particular the linear phenomenological equations, could provide a very useful tool for studies in bioenergetics.

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REFERENCES

1. De Donder, Th., and P. Van Rysselberghe. 1936. Thermodynamic Theory of Affinity. Stanford University Press, Stanford, Calif.
2. Prigogine, I., P. Outer, and C. L. Herbo. 1948. *J. Phys. Colloid Chem.* **52**:321.
3. Michaelis, L., and M. L. Menten. 1913. *Biochemistry.* **2**:49, 333.
4. Haldane, J. B. C. 1930. Enzymes. Longmans, Green & Co., Inc., New York.
5. Pigman, W. 1957. The Carbohydrates. Academic Press, Inc., New York. 523.
6. Dyson, J. D., and E. A. Noltman. 1968. *J. Biol. Chem.* **243**:1401.
7. Rottenberg, H., S. R. Caplan, and A. Essig. 1970. *In* Membranes and Ion Transport. E. E. Bittar, editor. John Wiley & Sons, Inc.-Interscience, New York. **1**:165.
8. Caplan, S. R. 1971. *In* Current Topics in Bioenergetics. R. Sanadi, editor. Academic Press, Inc., New York. **4**:1.
9. Blumenthal, R., S. R. Caplan, and O. Kedem. 1967. *Biophys. J.* **7**:735.