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LINEAR RELATION BETWEEN RATE AND THERMODYNAMIC FORCE IN ENZYME-CATALYZED REACTIONS

R. VAN DER MEER, H.V. WESTERHOFF and K. VAN DAM

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam (The Netherlands)

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Summary

Starting from enzyme kinetics, it is shown that generally a linear rather than a proportional relationship exists between rate and free energy changes in biochemical processes. In the derivation the boundary condition of constant substrate plus product is used, which is appropriate for many cellular systems. An example is the ADP plus ATP concentration in mitochondrial oxidative phosphorylation, as is illustrated experimentally.

There are several ways to describe energy-transducing systems, each with its own specific advantage. Ordinary kinetics define reactions in terms of the rate constants of every single reaction. Michaelis-Menten kinetics use maximal velocities and half saturating concentrations as descriptive parameters.

A third important method, neither focussing on the characteristics of every elementary reaction, nor stressing saturation behaviour, relates the rate of a reaction to its thermodynamic willingness (affinity or the free energy difference) to take place [1]. Its strength lies in its ability to describe the complex constellations of reactions that often occur in bioenergetics, whilst still allowing the assessment of the relative importance of the different individual steps [2,3]. Furthermore, it gives a straightforward insight in the efficiency with which the overall process takes place.

The initial attempts to apply the third method to bioenergetics took the principles of thermodynamics of near-equilibrium processes as their starting point [4]. According to these principles the rate of a reaction is proportional to the extent (expressed as free energy difference) by which the reaction is out of equilibrium. Although the attempts led to interesting conclusions and

experiments [5—8, for review see Ref. 9], it was recognized that energy-transducing systems do not always operate so close to equilibrium as to allow the straightforward application of the laws of near-equilibrium thermodynamics. Also it was felt as a disadvantage that the specific properties of enzyme-catalyzed reactions could not be taken into account.

In view of all this, Rottenberg [10] derived thermodynamic equations for enzyme-catalyzed reactions. His equations show that for a kinetically irreversible reaction the rate is linear (though generally not proportional) to the free energy difference for a large range of the latter parameter, provided the product concentration is considered constant. For reversible reactions he showed that an analogous linear relation is found, if (but not only if) the substrate concentration is kept at a constant value, related to the Michaelis constant in a specific way.

Since these boundary conditions are not generally realistic for bioenergy transduction, we have investigated what would be the result of choosing a boundary condition more appropriate for oxidative phosphorylation. The mitochondrial oxidative phosphorylation machinery can be considered a conservative energy transducer: the intracellular amounts of adenine nucleotides [11], mitochondrial NAD [12] and components of the respiratory chain are constant. This implies that changes in the respiratory rate are accompanied by obligatory and reciprocal changes in substrate and product concentrations. Thus the choice of the boundary condition: substrate + product is constant, appeared appropriate.

The following simple enzyme-catalyzed reaction was considered as a model system:

$$S + E \rightleftharpoons SE \rightleftharpoons EP \rightleftharpoons E + P$$

The steady-state rate for this system is given by the equation (cf. Ref. 13)

$$v = \frac{(V_s \cdot S/K_s) - (V_p \cdot P/K_p)}{1 + S/K_s + P/K_p} \tag{1}$$

in which V_s and V_p are the maximum forward and reverse velocities, respectively; K_s and K_p the Michaelis-Menten constants for substrate and product and S and P the concentration of substrate and product, respectively. Furthermore, the system is considered to be conservative with respect to substrate and product, i.e. $S + P = C$ (see above). Using the Haldane equation $K_{eq} = V_s \cdot K_p/(V_p \cdot K_s)$ [13] Eqn. 1 can be written as:

$$\frac{v}{V_s} = \frac{S - ((C - S)/K_{eq})}{K_s + S + (V_s/V_p) \cdot ((C - S)/K_{eq})} \tag{2}$$

Since the affinity (i.e. the difference in chemical potential of substrate and product) is given by $A \equiv \mu_s - \mu_p = RT \ln (K_{eq} \cdot S/P)$, it follows that:

$$\exp (A/RT) = \frac{S}{C - S} \cdot K_{eq} \tag{3}$$

Substitution of Eqn. 3 into Eqn. 2 and rearrangement of the denominator results in:

$$\frac{v}{V_s} = \frac{\exp(A/RT) - 1}{((K_s/C) + 1)\exp(A/RT) + (V_s/V_p)((K_p/C) + 1)} \quad (4)$$

Thus for a given value of C the rate equation is solely dependent on one variable: the affinity of the reaction. In Fig. 1 Eqn. 4 is plotted at two different values of the sum concentration C for an enzyme system with a small standard free energy change (Fig. 1A) and for a system with a large standard free energy change (Fig. 1B). Colloquially these systems are referred to as kinetically reversible and kinetically irreversible, respectively. It is clear that over a large part of the total velocity range the rate in both types of enzymic reaction is almost linearly dependent on the affinity, with a slope dependent on the total concentration of substrate plus product and the kinetic parameters of the enzyme.

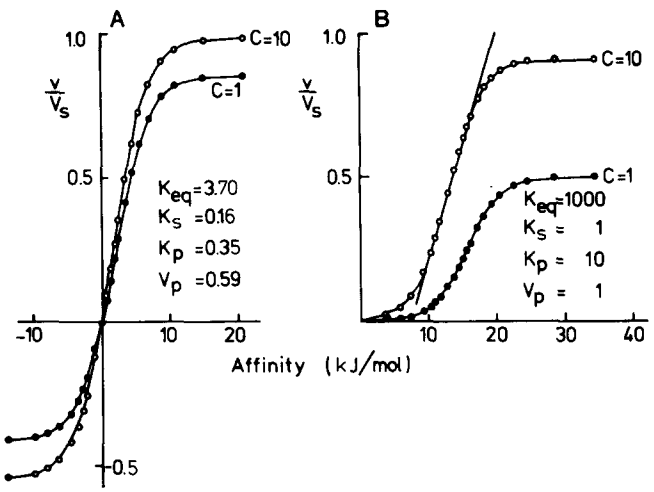

Fig. 1. Theoretical relationship between rate and affinity for enzyme-catalyzed reactions. A. Glucose-phosphate isomerase reaction at total substrate and product concentration of 1 and 10 mM. The kinetic constants (in mM) are taken from Ref. 10. B. Theoretical reaction with kinetic constants (in mM) as indicated in the figure, for C equal to 1 and 10 mM, respectively. Affinity was calculated using $A \equiv \mu_S - \mu_P = RT \ln (K_{eq} \cdot S/P)$ at $T = 298$ K. Rates were calculated using Eqn. 4.

An equation for the linear regions shown in Fig. 1 can be derived via a mathematical treatment of Eqn. 4. This treatment can be briefly summarized as follows. (A detailed treatment is available from the authors on request.) The inflexion point of the curve can be obtained using the condition that the second derivative of Eqn. 4 with respect to A equals zero. This results in the following coordinates:

$$A^{\star} = RT \ln \frac{\nu_s}{\nu_p} \; ; \quad v^{\star} = \tfrac{1}{2}(\nu_s - \nu_p) \quad (5)$$

in which $\nu_s \equiv V_s/((K_s/C) + 1)$ and $\nu_p \equiv V_p/((K_p/C) + 1)$ i.e. the Michaelis-Menten rates for $P = 0$ and $S = 0$, respectively. Subsequently, Eqn. 4 can be re-

written as a function centred around this inflexion point, and by developing a Taylor series the linearity around this inflexion point can be ascertained. With a maximal deviation of 10% relative to the velocity in the inflexion point ($|A - A^{\star}| \leqslant 1.1\ RT$), 55% of the total velocity range ($\equiv \nu_s + \nu_p$) can be described by the linear equation

$$v = \frac{1}{4\ RT}\ (\nu_s + \nu_p) \cdot A - \tfrac{1}{4}(\nu_s + \nu_p) \ln \frac{\nu_s}{\nu_p} + \tfrac{1}{2}(\nu_s - \nu_p) \qquad (6)$$

With a maximum deviation of 15% ($|A - A^{\star}| \leqslant 1.5\ RT$) this equation describes 75% of the total velocity range. The size of this linear domain is independent of the values of the different kinetic parameters and of the sum of substrate and product concentrations (contrast Ref. 10). It should be stressed that Eqn. 6 is centred around an inflexion point so that the deviation from Eqn. 4 is even less if the line is calculated by the method of least squares. This treatment shows that over a large velocity range the rate of an enzyme-catalyzed reaction can be described with a very good degree of approximation by the linear equation

$$J = L \cdot A - b \qquad (7)$$

in which $L = (1/4\ RT)(\nu_s + \nu_p)$, $b = \tfrac{1}{4}(\nu_s + \nu_p) \ln (\nu_s/\nu_p) - \tfrac{1}{2}(\nu_s - \nu_p)$

It is clear that the slope (L) and the intercept (b) are solely determined by enzyme kinetic parameters and the total concentration of substrate and product. The free energy difference around which the linear region is centred is independent of this total concentration, though it still depends on kinetic parameters (see Eqn. 5). The slope increases with C, as long as the total concentration does not vastly exceed the largest K_m. It can easily be seen that for kinetically reversible reactions (i.e. $\nu_s \simeq \nu_p$) the expression for b in Eqn. 7 vanishes. This leads to a linearly proportional equation:

$$J = L \cdot A \qquad (8)$$

where $L = (1/4\ RT)(\nu_s + \nu_p)$ which is a special case of Eqn. 7. Thus for conservative systems Eqn. 7 gives the general description for the linear regions in both reversible and irreversible systems, without any restriction with regard to constancy of either substrate or product concentration separately. This is an important extension of the earlier finding by Rottenberg [10].

The extra insight gained by our treatment, can best be illustrated by an example. As already mentioned, the magnitude of the proportionality constant L depends on the total substrate plus product concentration and on the kinetic parameters of the enzyme reaction. Experimentally therefore one should find largely linear velocity versus affinity plots, if the sum of substrate plus product concentration is kept constant. The slope of the line in this plot should increase with an increase in this sum concentration.

We tested if these considerations would still apply to the more complex system of mitochondrial oxidative phosphorylation. The respiratory rate was varied by adding different amounts of dialyzed hexokinase (in the presence of glucose and adenine nucleotides). The oxidation affinity was kept constant at 164 kJ/mol (succinate as the substrate). Fig. 2 shows that both the prediction

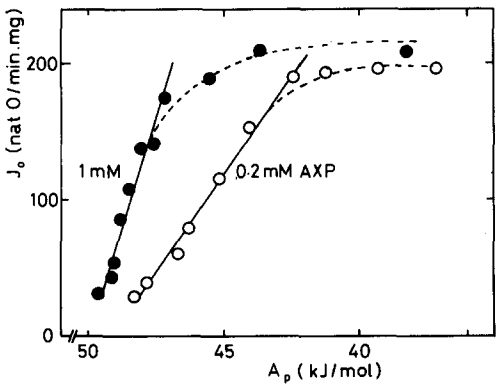

Fig. 2. Relationship between mitochondrial oxygen uptake and affinity of phosphorylation with its dependence on the total adenine nucleotide concentration. Mitochondria [15] (1 g protein/l) were incubated in 1.4 ml of the following medium (air saturated, pH 7.0, 25° C): 250 mM sucrose, 10 mM KCl, 10 mM Tris-phosphate, 1 mg/l rotenone, 1 mM sodium EGTA, 1 mM potassium malate, 10 mM glucose, 7 mM $MgCl_2$ and 0.2 mM (○—○) or 1.0 mM (●—●) sodium ATP. The reaction was started by adding 20 mM Tris-succinate followed shortly thereafter by the hexokinase. At an oxygen concentration of 85 μM the adenine nucleotide concentration was determined essentially as described [16]. $\Delta G'_{OP}$ was valued as −28 kJ/mol [17]; except for the points with phosphate potential lower than 40 kJ/mol, AMP concentrations were lower than ten percent of ATP + ADP.

of linearity and that of the increase in slope with increased ADP/ATP concentration are verified. The finding agrees with and gives an explanation for the findings of Davis and Lumeng [14] that the extramitochondrial ATP/ADP ratio at 50% state 3 respiration increases with the total concentration of adenine nucleotides.

Thus the approximation of linearity (but not that of proportionality) between rate and affinity of enzyme-catalyzed reactions appears justified even in complex systems far away from equilibrium. Care must, however, be taken in choosing the proper experimental variations in order to allow the use of the linear equations: depending on the experimental system and its physiological context one may fix either the substrate concentration, the product concentration [10] or their sum.

The conclusion that generally linear rather than proportional equations should be used in describing enzymatic reactions does have implications for descriptions that have been developed earlier (e.g. Refs. 4, 9, 2 and 3).

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