Mitochondrial Respiratory Control: Energetics, Kinetics and Efficiency*

E. Gnaiger

Introduction: Controversies in Flux Control

Mitochondrial respiratory control mechanisms are central in regulating the flux of ATP turnover which mediates between catabolism and anabolism. Such control can be exerted by the catabolic driving force for ATP production or by the load on the ATP system due to the ATP demand for biosynthesis, ion pumps or muscle activities. The signals of drive and load are integrated in the energy status of the ATP/ADP system, expressed by the phosphorylation potential. Is metabolic flux regulated by energy demand or by energy supply? Do ATP/ADP ratios or ADP concentrations regulate the flux of mitochondrial oxidative phosphorylation? Different answers are obtained depending on metabolic conditions, but primarily depending on the particular model of flux control.

Despite the advances made in elucidating the chemiosmotic and enzymatic mechanisms of oxidative phosphorylation (Mitchell, 1961; Senior, 1988) we are left with controversies as to the regulatory aspects of oxygen flux in coupled mitochondria. The majority of biochemists direct their studies along the lines of enzyme kinetic theory. The most important kinetic variable in respiratory control is the concentration of substrates such as ADP which is high in State 3 at high flux, and low in State 4 at low flux (Chance and Williams, 1955). Such a model implies load-coupled regulation, exerted by the velocity of various ADP-generating reactions.

The phosphorylation potential, log ([ATP] / [ADP] × [P_i]), on the other hand, is a control variable based on the mass action ratio and represents the molar Gibbs energy (force) of phosphorylation. In nonequilibrium thermodynamic theory the molar Gibbs energy of reaction (*Gibbs force*) is considered as the driving force for the chemical flux, and hence control is thought to be exerted by logarithmic concentration ratios (Katchalsky and Curran, 1965). In a thermodynamic analysis various degrees of drive-coupled and load-coupled regulation are implied depending on which processes exert the predominating influence on the catabolic-anabolic coupled reaction (Gnaiger, 1987). An advanced mathematical method is available (Katcher and Porteous, 1987) which allows quantification of the relative importance of various control steps (Groen et al., 1983), and can be combined with either kinetic or thermodynamic concepts (Westerhoff and Van Dam, 1987).

Clearly, a reconciliation of these divergent traits of kinetics and nonequilibrium thermodynamics ("ergodynamics"; Gnaiger, 1987) is of great theoretical and practical importance. Such a reconciliation is proposed here on the basis of Einstein's diffusion equation. The new concept accounts for the effects exerted on flux by both the Gibbs force of reaction and the total concentration of all substrates and products. Its application to the study of chemical reactions as complex as mitochondrial oxidative phosphorylation provides new insights into the regulatory mechanisms of energy transformation, emphasizing the effect of efficiency on the control of flux.

*Dedicated to Wolfgang Wieser at his 65th birthday

Energetics and Kinetics of Diffusion and Chemical Reactions

The thermodynamic forces and flows are defined in strict relation to each other, in such a way that the product of conjugated flows and thermodynamic forces, $J\,X$, yields the rate of entropy production (Glansdorff and Prigogine, 1971). Defining the generalized forces as $-F = X\,T$, the product $J\,F$ is the dissipated power, P (per unit volume) [W m^{-3} = J s^{-1} m^{-3}] ,

$$P = J\,F \qquad (1)$$

In the case of diffusional flux, ${}_dJ$ [mol s^{-1} m^{-2}] , the conjugated force is the chemical potential gradient (in the z direction, ${}_dF$ = $d\mu/dz$ [J mol^{-1} m^{-1}] ; Einstein, 1905). The product (Eq. 1) yields the required dimension of power per volume [J s^{-1} m^{-3}] . The flux of chemical reaction, ${}_rJ$ [mol s^{-1} m^{-3}] , is scalar, as is the conjugated force or molar Gibbs energy ("Gibbs force") of reaction, ${}_rF$ = $\Delta_r G$ [J mol^{-1}]. Invariably, the product of flux and force (Eq. 1) yields the dissipated power.

The phenomenological law of nonequilibrium thermodynamics relates the generalized flux to the conjugated force (Glansdorff and Prigogine, 1971),

$$J = -L\,F \qquad (2)$$

It is assumed that near equilibrium the flux increases as a linear function of the force; the conductivity, L, is represented by the slope of the functional relationship. A more detailed perspective is obtained by reference to Einstein's diffusion equation,

$${}_dJ = -u\,c\,{}_dF = -u\,c\,d\mu/dz \qquad (3)$$

in which u is the mobility, or the reciprocal value of the frictional coefficient, and c is the concentration of the diffusing substance localized across the plane at which $d\mu/dz$ is measured. At steady state this force is counterbalanced by the frictional effects. The average *velocity* of particles (molecules) in the z direction then increases linearly with $-{}_dF$, and is equivalent to the steady state velocity of Newton's apple, which increases at constant u with the gravitational force. The *flux* (per unit area), however, does not solely depend on the force (per mole) but additionally on the number of molecules (apples) interacting with the force. At constant force, the flux varies with the number of particles moving across a plane of unit area. The Newtonian force and the number of bodies or particles (per unit volume) are complementary flux control variables. In Einstein's equation (3) both complementary control variables, concentration and force, are explicitely defined.

In the phenomenological equation (Eq. 2) the nature of particles comprising the flow is "hidden" in L, thereby obscuring the concentration term (Eq. 3) with various other properties of L such as viscosity, catalytic effects etc. This restricts the analytical strength and applicability of nonequilibrium thermodynamics. Irrespective of the proximity to equilibrium, the phenomenological equation of nonequilibrium thermodynamics should be generally replaced by the "ergodynamic" equation, as deduced from Einstein's diffusion equation (3),

$$J = -u\,\alpha\,F \qquad (4)$$

α is the generalized concentration (= *free activity*). If α is constant, then the flux increases linearly with the force (Fig. 1; sloping arrow). But the flux changes at constant force with a change of free activity, (Fig. 1; vertical arrow). A nonlinear flux-force relation indicates a change of both force and free activity (Fig. 1; dashed line). The flux may

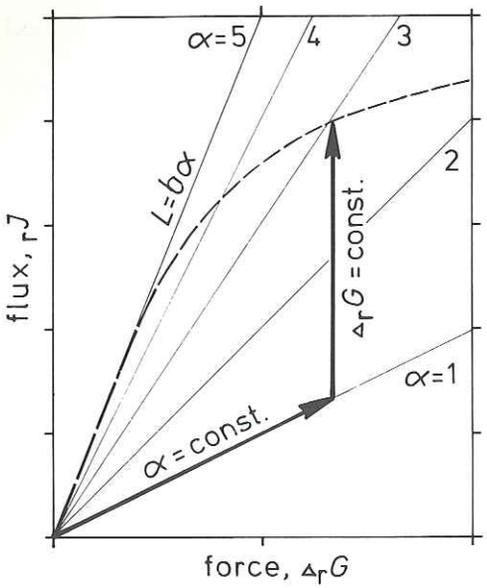


Fig. 1 The flux of a chemical reaction, $_rJ$ [mol s^{-1} m^{-3}], is a function of both, the molar force, Δ_rG, and the molar amount of substance interacting with the force (free activity α, numbers 1 to 5). *Sloping arrow* and *thin lines* - at constant free activity α, the flux-force relation is linear if the reaction coefficient (comp. mobility), *b*, is also constant ($L = b\alpha$ = const.). *Vertical arrow* - at constant molar force the flux increases with an increase of the molar free activity. *Broken line* - a nonlinear flux-force relation is due to the simultaneous change of force and free activity.

even decrease with increasing force, if the decrease of α overcompensates the increase of $-F$ (Eq. 4).

The product of the two complementary flux control variables, αF, is a function of state. For diffusion, the product of free activity, $\alpha = c$ [mol m^{-3}], and molar force, $_dF = d\mu/dz$ [J mol^{-1} m^{-1}], yields the gradient of *chemical pressure*, $d_d\Pi/dz$ [J m^{-3} m^{-1} = N m^{-2} m^{-1} = Pa m^{-1}],

$$d_d\Pi/dz = RT\, dc/dz = c\, d\mu/dz = \alpha\, {}_dF \qquad (5)$$

To familiarize ourselves with the extended concept of "chemical pressure", we should recall the definition of pressure by the ideal gas law, as generalized by Van't Hoff for osmotic pressure,

$$\Pi = RT\, n/V = RT\, c \qquad (6)$$

The concept of diffusion pressure and its linear relation to flux is implicit in Fick's First Law of diffusion, $_dJ = -D\, dc/dz$. Now if the mobility is substituted for the diffusion coefficient, $D = u\,RT$ (Einstein, 1905), $RT\, dc$ is recognized as $d_d\Pi$, the chemical pressure.

Instead of a linear flux-force relation (Glansdorff and Prigogine, 1971) Fick's First Law of diffusion implies a linear flux-pressure relation, $_dJ = -u\, d_d\Pi/dz$. Fick's law is also applicable to discontinuous systems (Hitchman and Gnaiger, 1983) where gradients are replaced by differences across the length of the diffusion path, *l*. Einstein's diffusion equation, however, requires special consideration when applied to discontinuous systems,

$$\begin{aligned} {}_dJ &= -u\,\alpha\,\Delta\mu/l \\ &= -u\,\Delta_d\Pi/l = -u\,RT\,\Delta c/l \end{aligned} \qquad (7)$$

In a discontinuous system α is not simply the average or equilibrium concentration (compare Katchalsky and Curran, 1965; Westerhoff and Van Dam, 1987). Free activity is a distribution function, $\alpha = \Delta c/\Delta \ln c$ (Eq. 12). Here the analogy with systems of classical mechanics (number of apples) fails. In the study of chemical systems we are in the realm of statistical mechanics, hence statistical arguments for a definition of free activity are implicated.

This "ergodynamic" analysis of flux control combines thermodynamic and kinetic aspects. The kinetic theory of ideal gases and ideal dilute solutions (activities equal concentrations) describes the passive flow of molecules across a semipermeable membrane as the net result of molecular movements in both directions,

$$_dJ = k_1\, c_A - k_{-1}\, c_B \qquad (8)$$

c_A and c_B are the concentrations of the diffusing substance (a nonelectrolyte) in the compartments A and B, and k_1 and k_{-1} are the kinetic coefficients from A to B and from B to A, respectively. The ratio k_1/k_{-1} is the equilibrium constant, which in this case is unity. Substituting in Eq. (8) for $k_1 = k_{-1} = D/l = uRT/l$, and $c_B - c_A = \Delta c$, we obtain: $_dJ = -D\,\Delta c/l$ (Eq. 7). Derived in this manner, Fick's First Law is a kinetic law. In mathematical, but not in physical terms it resembles the linear thermodynamic flux-force relation (Eq. 2) which has led to confusion. The product of $_dJ$ and $\Delta c/l$ (compare Eq. 1) does not yield the dissipated power. With $_dJ$ defined in Eq. (7), the conjugated force (Eq. 1) is $_dF = \Delta\mu/l$. The chemical potential difference across the membrane is,

$$\begin{aligned} \Delta\mu &= RT \ln(c_B/c_A) = RT\,(\ln c_B - \ln c_A) \\ &= RT\,\Delta \ln c \end{aligned} \qquad (9)$$

For chemical reactions, the analogous force is the Gibbs force,

$$\Delta_rG = \Delta_rG^o + \Delta_rG^+ = RT \ln(M/K) \qquad (10)$$

where Δ_rG^o and Δ_rG^+ are the concentration-independent and concentration dependent terms of the Gibbs force, and *M* and *K* are the molar mass action ratio and the equilibrium constant, respectively. As emphasized above (Eq. 1), the flux and conjugated force of a chemical reaction are not directed in space but flux proceeds along energetic discontinuities, defined by the energetic states of substrates and products. Analogously to Eq. (7), therefore, Eq. (4) for chemical reactions is,

$$\begin{aligned} {}_rJ &= -b\,\alpha\,\Delta_rG && (11.a) \\ &= -b\,\Delta_r\Pi && (11.b) \end{aligned}$$

Chemical flux varies as a function of free activity and force (Fig. 1). The product of the two flux control variables is the *reaction pressure*, $\Delta_r\Pi = \alpha\Delta_rG$ [J m^{-3} = Pa]. The slope of a flux-pressure plot is the reaction coefficient, *b*. For extending the ergodynamic concept to chemical reactions, the challenge is the quantification of α, on the basis of the activities of all substrates and products. In analogy to a chemical reaction, the concentration of a diffusing solute on one side, A, can be viewed as the substrate activity, $_sa = c_A$, and the concentration on side B as the product activity, $_pa = c_B$. For chemical processes in general, free activity is,

$$\alpha = \frac{{}_pa - {}_sa}{\ln({}_pa/{}_sa)} \qquad (12)$$

How is free activity calculated when Δ_rG^o is added to the force term and there is more than one substrate, such as in the reaction A+B = C? A phenomenological solution can be obtained by comparison with experimental results.

Alternative Models of Chemical Dynamics

Kinetic rate equations constitute the classical model of chemical dynamics. Nonequilibium thermodynamics, however, has not yet generated a theory on chemical dynamics beyond kinetics. In theoretical terms, linear relations are either inadequate for describing flux far from equilibrium (Glansdorff and Prigogine, 1971) or are redundant approximations of a kinetic description in a restricted range irrespective of the equilibrium position (Westerhoff and Van Dam, 1987). To be applicable to living systems, the ergodynamic approach must overcome this restriction. By abandoning entirely the quest for linear approximations, and by appreciation of the fundamental significance of Einstein's diffusion equation generalized in Eqs. 11 and 12, it can be shown that thermodynamics merges with reaction kinetics. A concise derivation of this claim is presented in Appendix A1. Importantly, and in contrast to

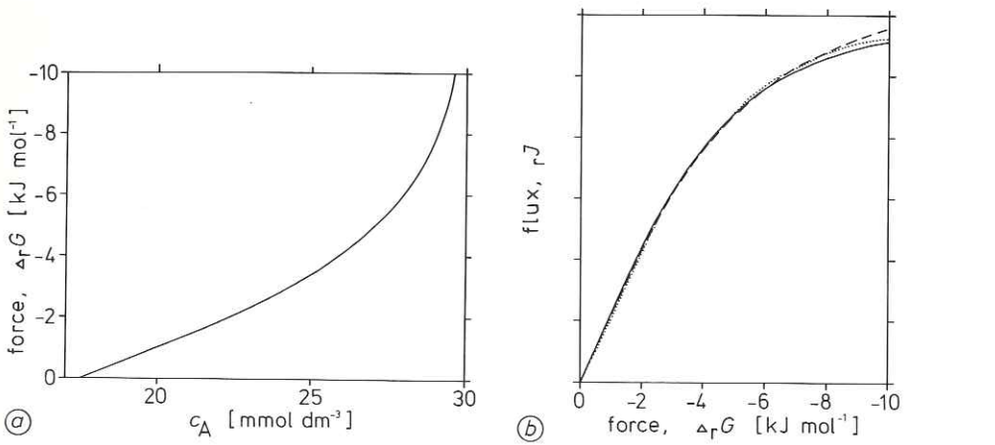


Fig. 2 Kinetic and ergodynamic flux control models for a third-order reaction, A+B=C, proceeding in a closed reaction system. The initial conditions are 30 and 110 mmol dm^{-3} for substrate A and B, respectively and C is initially not present. While A and B are consumed and their concentrations decrease, C accumulates and its concentration increases stoichiometrically (to 12.7 mmol dm^{-3} at equilibrium). The standard Gibbs force, $\Delta_r G^\circ$, is -5 kJ mol^{-1}.

a Gibbs force, $\Delta_r G$, as a function of the decrease from right to left of the concentration of substrate A, c_A. Kinetics predicts a linear increase of flux with concentration, but force increases linearly only up to ca. -2.5 kJ mol^{-1}.

b Normalized flux, $_rJ$, as a function of Gibbs force, $\Delta_r G$. *Full line* - predicted from the kinetic model (Eqs. A1-A4). *Dotted line* - first alternative model defining the partial substrate activity as $\sqrt{c_A c_B}$ and product activity as c_C in Eq. (A5) for calculation of flux according to Eq. 11a. *Broken line* - second alternative model using Eq. (12) with the partial substrate and product activity as defined in the first alternative model (Appendix A.2).

previous claims (Katchalsky and Curran, 1965; Westerhoff and Van Dam, 1987), the conductivity, $L = u\alpha$ or $L = b\alpha$ (Eq. 2), is an explicit function of the force, $\Delta_r G = RT \ln(M/K)$, as derived from kinetics in Eq. A4.

However, linear or quasi-linear flux-force relations of chemical reactions frequently span a larger range of forces than expected on the basis of kinetic theory (Prigogine et al., 1948). Moreover, extended flux-force linearity is observed in many biochemical processes, including oxidative phosphorylation (reviewed by Caplan and Essig, 1983; Westerhoff and Van Dam, 1987). A possible solution of this puzzling phenomenon is suggested below.

The kinetic model is only one of several plausible interpretations of the ergodynamic formalism (Eqs. 11 and 12; see Appendix A.2). Any alternative energetic model of chemical dynamics must correspond to the kinetic rate equations in those cases where kinetics is well tested, particularly in simple reactions.

Consider the third-order reaction A + B = C as it proceeds to equilibrium in a closed system. According to the kinetic rate law, $_rJ = k_1 c_A c_B - k_{-1} c_C$ (see Eq. A1), the flux increases linearly with the concentration c_A. In contrast, the force varies in a strictly non-linear manner with c_A (Eq. 10; Fig. 2a). Therefore the kinetic rate law predicts a non-linear relation between flux and force (Fig. 2b; full line). Two alternative nonlinear energetic models (Appendix A.2) are in excellent agreement (Fig. 2b; dotted and dashed lines).

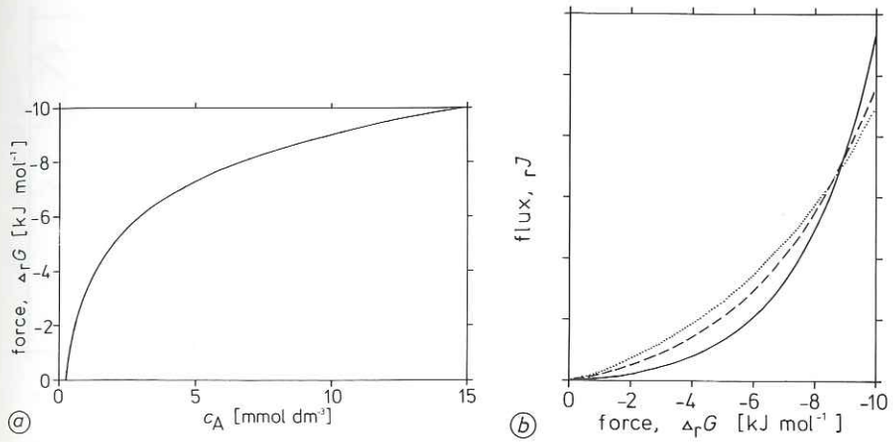


Fig. 3 Kinetic and ergodynamic flux control models for the reaction described in Fig. 2, but with substrate A added in increasing concentration (0.2 to 15 mmol dm^{-3}). The substrate B and product C are buffered at constant concentrations (50 mmol dm^{-3} and 0.1 mmol dm^{-3}, respectively).

a: Gibbs force, $\Delta_r G$, as a function of concentration of substrate A, c_A.

b: Normalized flux, $_rJ$, as a function of Gibbs force, $\Delta_r G$. The three lines correspond to the kinetic and ergodynamic models as described in Fig. 2b.

The same reaction is now examined under the conditions of a typical kinetic experiment (Fig. 3), when the flux is measured at increasing concentration of substrate A, a fixed high concentration of substrate B, and a fixed low concentration of product C. Simultaneously with the increase in force the free activity increases due to the addition of A in successively higher concentration (Fig. 3a). Therefore a non-linear increase of flux with force (Fig. 3b) is expected on the basis of Eq.(11.a). The curvature of the flux-force relation is the opposite of that obtained under the previous reaction conditions (compare Fig. 2b and 3b). Nevertheless, a reasonable agreement between the kinetic predictions and the ergodynamic concept (Eq. 11.a) is obtained when the substrate activity is defined as the geometric mean concentration, $_sa = \sqrt{c_A c_B}$; and $_pa = c_C$ (Fig. 3b). Therefore even if this alternative flux-pressure relation were true the kinetic model would not cause conflict.

An "approximately linear" region between flux and force is recognized near equilibrium, but even in this restricted range of "trivial" linearity the slope L depends on the way in which the force is varied (Fig. 2b and 3b). Kinetic models are well tested for simple reactions as depicted in the present comparison, when the agreement with alternative ergodynamic equations is good. With more complex reactions, significant deviations between the kinetic and ergodynamic approaches are obtained, and comparison with experimental data is required to decide which concept yields the superior results (Appendix A2). It is important to note that a linear flux-pressure relation can hold only as long as the general effects of free activity

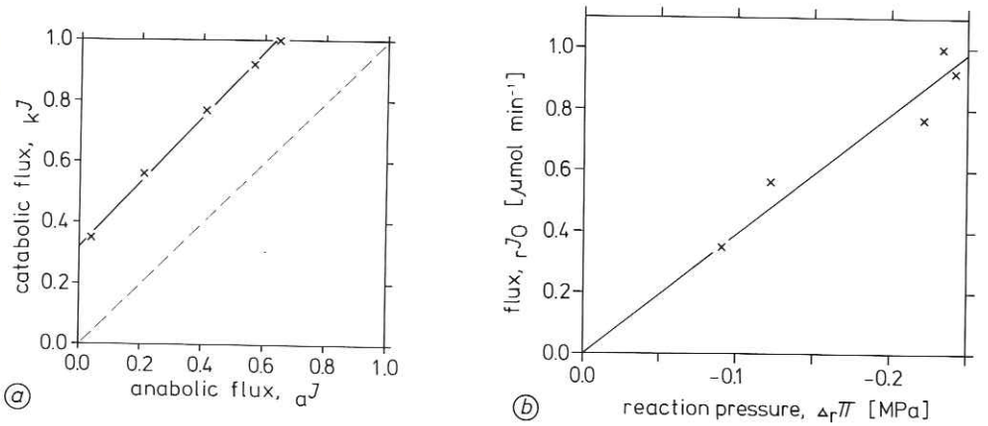


Fig. 4 Ergodynamic analysis of liver mitochondrial respiration. Original data (x) from Stucki (1980, Tab. 1A). The incubation medium contained on average 0.81 mmol dm^{-3} ATP+ADP, 4.8 mmol dm^{-3} P_i, pH 7.4, and 5 mmol dm^{-3} malate as catabolic substrate.

***a*:** **Catabolic and anabolic flux,** $_kJ$ and $_aJ$, normalized for the ATP/O_2 ratio of 6.0; measured as oxygen flux and formation of Glu-6-P, respectively. *Full line* - linear regression with a slope not signifiantly different from unity suggesting an ATP/O_2 ratio of 6.0 The intercept is the catabolic oxygen flux of maintenance at zero anabolic net flux. *Broken line* - line of ideal correspondence of a fully catabolic-anabolic coupled system.

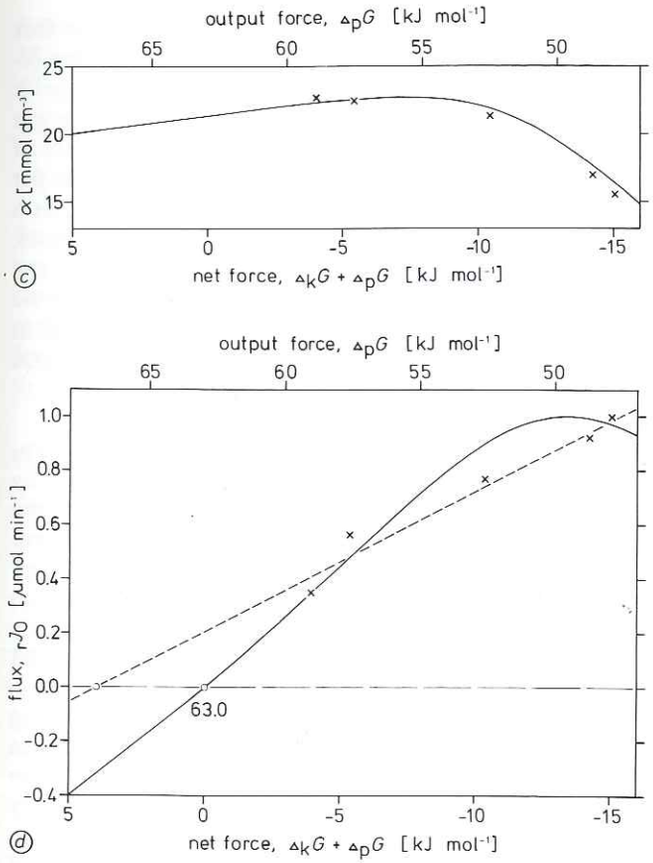
***b*:** Linear flux-reaction pressure relation.

and force are not altered by specific catalytic effects such as saturation or modulation of enzyme activity, which would change *b*.

Respiratory Control in Mitochondrial Oxidative Phosphorylation

The single most important process of energy transformation in animals is mitochondrial oxidative phosphorylation. The controversies as to kinetic control (Chance and Williams, 1955; Jacobus et al., 1982) or thermodynamic control (Stucki, 1980; Van der Meer et al., 1980) are unresolved. Complex regulatory mechanisms have been implied to explain the observed flux-force linearities (Pietrobon et al., 1982), or even unknown electrochemical network mechanisms have been postulated to rationalize the unexpectedly frequent occurence and wide range of linear flux-force relations (Berry et al., this volume).

Linear flux-force relations have become important in bioenergetics (Caplan and Essig, 1983), since such linearity and independence of *L* on Gibbs force are required for application of the Onsager reciprocity relations in the analysis of coupled processes and thermodynamic control. Biochemical reactions are energetically coupled if their stoichiometries are not fixed by the law of conservation of mass, but if they proceed simultaneously solely due to the operation of specific coupling mechanisms. The stoichiometry of an independent chemical reaction is defined by chemical laws, whereas the coupling stoichiometry of respiration and phosphorylation is the result of biological evolution and specific biochemical design constraints (Atkinson, 1977). The ATP/O_2 ratio must be determined experimentally, and be interpreted in terms of evolutionary energetics (Gnaiger, 1987).



***c*:** Free activity, as a function of phosphorylation potential, Δ_pG. The continuous line is calculated by varying the ATP/ADP ratio at constant sum concentration.

***d*:** Oxygen flux as a function of Gibbs force. *Full line* - calculated from free activity, net force and the slope (mobility) of the linear flux-pressure relation (*c*). *Broken line* - calculated on the assumption (Stucki, 1980) of a linear flux-force relation.

ATP production by isolated mitochondria is most conveniently measured as product formation in a coupled anabolic reaction, such as the phosphorylation of glucose catalyzed by hexokinase. However, the stoichiometry of this anabolic reaction and oxygen consumption, ≈P/O_2, is not necessarily equivalent to the ratio of total ATP turnover and respiration, ATP/O_2. In poorly coupled isolated liver mitochondria, the anabolic flux is zero at relatively high levels of catabolism (Stucki, 1980; Fig. 4a). This may be compared to an organism with high maintenance costs as measured by the intercept of oxygen flux and growth rate (Hawkins et al., 1987; Wieser, this volume). The linear slope of the anabolic versus catabolic flux is the net ATP/O_2 ratio. In Fig. 4a this ATP/O_2 ratio is not significantly different from the expected mechanistic stoichiometry of 6.0 when malate is the catabolic substrate (compare Lemasters, 1984). Therefore, the theory of fully coupled catabolism is applicable (Gnaiger, 1983, 1987) whereas the theory of partially coupled energy transformation, not only requires linear, but depends on the more restrictive symmetric and proportional flux-force relations (Kedem and Caplan, 1965).

If the observed flux-force relation is linear, then the free activity must be constant according to the concept of reaction pressure (Eq. 11). In a typical experiment on mitochondrial respiratory control, the oxygraph medium contains the catabolic substrates in saturating concentrations. Therefore, it is reasonable to calculate α as a first approximation on the basis of the adenylate and in-

organic phosphate concentrations for the reaction,

$$ADP + P_i + H^+ \rightarrow ATP + H_2O$$

For insertion into Eq. (12), the geometric mean for the partial substrate activity is (second alternative model in Appendix A.2),

$$_sa = \sqrt[3]{[ADP]\,[P_i]\,[H^+]}$$

and for the partial product activity,

$$_pa = \sqrt[2]{[ATP]\,[H_2O]}$$

From the measured adenylate and inorganic phosphate concentrations (Stucki, 1980; mitochondria isolated from rat liver) α was calculated as outlined above and plotted as a function of the phosphorylation potential in Fig. 4c.

The force of oxidative phosphorylation is a composite net force, including the phosphorylation of ADP to ATP as the output (positive Gibbs force, Δ_pG). This endergonic reaction is coupled via complex vectorial mechanisms to the catabolic electron transport which generates the input force (negative Gibbs force, Δ_kG). The catabolic force is normalized to the output and expressed in units of kJ per mol ATP turnover, such that the net force of the coupled reaction is $\Delta_rG = \Delta_kG + \Delta_pG$ [kJ mol^{-1} ATP].

The apparent catabolic force in isolated mitochondria is obtained experimentally by measurement of the phosphorylation potential at various steady state levels of oxygen flux. The slope, b, in the plot of oxygen flux and reaction pressure includes implicitely the ATP/O_2 ratio (Fig. 4b).

Alternatively, from a rearrangement of Eq.(11a), b is recognized as the slope of the rate-force relation,

$$_rJ_{O_2}/\alpha = -b\,(\Delta_kG + \Delta_pG) \qquad (13)$$

It is important to note that the *rate* is flux divided by free activity, with the proper dimension for a rate, that is per unit time ([s^{-1}]: compare the per capita growth rate, or per cent interest rate as opposed to the flux of actual growth, or total capital increase per unit of time). When the rate is plotted as a function of the phosphorylation potential; then the Y-intercept is $-b\,\Delta_kG$ (Eq. 13), and the X-intercept (Y-intercept divided by the slope) is the phosphorylation potential at zero flux equal to the negativ catabolic force per mol ATP turnover, Δ_kG.

The apparent Δ_kG is –63 kJ mol^{-1} ATP turnover as calculated according to Eq. (13) by linear regression analysis (r = 0.979; the same value results from the first alternative model in Appendix A.2). From α and the apparent net force, $\Delta_kG + \Delta_pG$, the reaction pressure is then obtained for each steady state condition in the oxygraph medium. The flux-pressure relation is linear in the observed range (Fig. 4*b*; r = 0.959), supporting the concept of reaction pressure. Whereas the rate-force relation is equivalent to the flux-pressure relation and is theoretically linear (Fig. 4*b*), the linearity of the flux-force relation depends on the variation of free activity with the force (Eq. 11a). Since α varies by less than ± 20 % in the range of observed forces (Fig. 4*c*), an approximately linear flux-force relation can be predicted well beyond the trivial near-equilibrium range (Fig. 4*d*; full line). From a simple phenomenological linear flux-force regression (Fig. 4*d*; stippled line; Stucki, 1980), a significantly different intercept is calculated. The latter approach, however, lacks theoretical foundation.

In aerobic respiration with glycogen as the substrate, the Gibbs force per mol oxygen is –480 kJ mol^{-1} (Eq. 10) which is nearly identical to the enthalpy of reaction or the oxycaloric equivalent (Gnaiger, 1983b). With 37 mol ATP formed per mol glycosyl-unit or an in vivo ATP/O_2 ratio of 6.2 (Crow and Kushmerick, 1982), we get Δ_kG = –480/6.17 = -78 kJ mol^{-1} ATP turnover (Gnaiger, 1983), the same value as for succinate to fumarate conversion in oxygraph medium. For the reaction from malate to oxaloacetate, Δ_kG is -67 kJ per mol ATP turnover under in vivo conditions specified by Stucki (1980; where a lower value is given possibly due to consideration of concentrations when activities should be used). The reason for a deviation of the apparent Gibbs force (Eq. 13) and the Gibbs force according to Eq. (10) is not known.

Optimum Efficiency and Power

Optimization principles of efficiency in metabolic energy transformation can now be evaluated on new theoretical grounds. The control variables of kinetics (substrate concentrations; free activity) and nonequilibrium thermodynamics (Gibbs force) are combined in the concept of reaction pressure (Appendix A). Active control mechanisms increase the metabolic flux at any given Δ_pG/Δ_kG ratio (= force efficiency; Gnaiger, 1987). These active mechanisms are associated with specific "external costs" such as an increase of enzyme concentrations to increase the rate coefficient, b, or an increase of all metabolite concentrations to increase the free activity. On the other hand, the flux increases at constant Δ_kG with a decrease of Δ_pG (Fig. 4*d*) at the "internal cost" of diminished efficiency.

A previous concept, based on maximum (rather than optimum) efficiency within the restrictions of adjusted degrees of decoupling (Stucki, 1980), lacks the appropriate consideration of the cellular functions of ATP demand and optimum system performance (Gnaiger, 1987). Irrespective of the applicability of near-linear flux-force relations (Fig. 4*d*), the increase of total power output per unit power input (= efficiency) can be achieved either at the expense of external costs, or more economically by a reduction of metabolic power. This power-efficiency tradeoff is a general phenomenon in the region of high efficiency (Fig. 4*d*; the efficiency of ATP production and turnover increase with decreasing flux). ATP utilization is a multifunctional process in the living animal. Therfore, efficiencies calculated for specific functions such as growth or locomotion are comparatively low and tend to increase with increasing flux or power, without violation of the ergodynamic exclusion principle which prevents the simultaneous maximization of both, power and efficiency.

Optimization of system performance requires a variety of compromizes including the optimization of efficiency. Adjustment of forces and optimization of efficiency are important ergodynamic strategies in metabolic energy transformation. Allosteric enzyme kinetic and ergodynamic mechanisms interact as coordinated flux control mechanisms in highly evolved and economically regulated biological systems.

Supported by FWF Austria, project Nr. P7162-BIO.

Appendix

A.1. Identity of kinetics and thermodynamics

The mass action ratio of a reaction is $M = {}_pm/{}_sm$. In the reaction A+B = C for example, the mass action of products is $_pm = c_C$, and of substrates $_sm = c_A\,c_B$. At equilibrium we observe the constant, $M_{eq} = K = k_1/k_{-1}$. When the kinetic order and stoichiometric order of a reaction are identical, then

$$_rJ = -(k_{-1}\,{}_pm - k_1\,{}_sm) \qquad (A1)$$

Substituting for $k_{-1} = b\,RT$ and for $k_1 = k_{-1}\,K = b\,RT\,K$, we obtain (compare Eq. 7),

$$_rJ = -b\,RT\,({}_pm - {}_sm\,K) \qquad (A2)$$

Eq. (A2) and Eq. (11) are identical under the condition that reaction pressure is,

$$\Delta_r\pi = \alpha\,\Delta_rG = RT\,({}_pm - {}_sm\,K) \qquad (A3)$$

The definition in Eq.(A3) implies that the partial product activity and substrate activity are equivalent to ${}_pm$ and ${}_sm\,K$, since (Eq. 12),

$$\alpha = \frac{{}_pa - {}_sa}{\ln({}_pa/{}_sa)} = \frac{{}_pm - {}_sm\,K}{\ln(M/K)} = \frac{{}_pm - {}_sm\,K}{\Delta_rG/RT} \qquad (A4)$$

Multiplication of α with Δ_rG yields the reaction pressure, consistent with the definition in Eq.(A3). α, and hence the phenomenological conductivity L, is an explicit function of the force, in contradiction to current textbooks.

For the first time, a complete reconciliation of kinetics and thermodynamics is achieved. Modifications of the definition of α lead to phenomenological equations which may be successful beyond the established kinetic theory. The classic study on the dynamics of a gas reaction by Prigogine et al. (1948) provides an example of the limited predictive value of kinetics and is used below as a test case (compare Gnaiger, 1988).

A.2. Two alternative models

By noting that consistency with kinetics implies that the sum of partial activities is ${}_pa+{}_sa = {}_pm+{}_smK = {}_sm\,(M+K)$, we can rearrange Eq.(A4) as,

$$\alpha = ({}_pa + {}_sa)\,\frac{(M/K - 1)}{(M/K + 1)}\,\frac{1}{\ln(M/K)} \qquad (A5)$$

Why should the sum of partial activities, ${}_pa+{}_sa$, depend on the equilibrium constant, ${}_pm+{}_smK$? This problem does not arise in diffusion, since K =1. The use of a reaction coefficient defined as $b' = b\,K$ in Eq.(A2) would lead to the same equation if we redefine ${}_pa'+{}_sa' = {}_pm/K+{}_sm$. Such an interdependence of the reaction coefficient and free activity appears strange for the definition of α as a function of state, although it would justify to some extent the combination of terms in $L = b\,\alpha$. If it is postulated that the ratio ${}_pa/{}_sa = M/K$ remains (Eq.A4) but the sum of partial activities be independent of the equilibrium constant, then we may consider ${}_pa+{}_sa = {}_pm+{}_sm$. However, this yields poor agreement with kinetics in the model reaction in Fig. 2, and does not improve the fit of the data of Prigogine et al. (1948). Excellent results are obtained in both cases, however, by considering the sum of partial activities in Eq.A5 to be the geometric mean of products plus the geometric mean of substrates. For the 9 test data in Tab. 2 of Prigogine et al. (1948), this first alternative model improves the correlation coefficient from 0.93 (kinetic model) to 0.99, whereas for the reaction shown in Fig. 2 the model is indistinguishable from the kinetic equation.

Even the identity ${}_pa/{}_sa = M/K$ may be relaxed, by postulating that the product activity is the geometric mean of product concentrations and the substrate activity is the geometric mean of substrate concentrations. These partial activities are inserted into Eq.(12) for the calculation of free activity, α, which then does not lead to Eq.(A5). This second alternative model is compared with the kinetic rate equation in Figs. 2 and 3. It yields a better agreement with kinetics than the first model for the conditions in Fig. 3, and provides the same excellent fit to the data of Prigogine et al. (1948) (Gnaiger, 1988). As a working hypothesis, this model is applied to rationalize the paradoxically wide range of apparently linear flux-force relations in mitochondrial oxidative phosphorylation (Fig. 4).

References

Atkinson, D.E. 1977. Cellular Energy Metabolism and its Regulation. Academic Press, New York, pp. 293.

Berry, M. 1989. In: Energy transformations in cells and animals. Eds. W. Wieser and E. Gnaiger.

Caplan, S.R. and Essig, A. 1983. Bioenergetics and linear nonequilibrium thermodynamics. The steady state. Harvard Univ. Press, Cambridge, London, pp. 435.

Chance, B. and Williams, G.R. 1955. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J. Biol. Chem. 217, 383-393.

Crow, M.T. and Kushmerick, M.J. 1982. Chemical energetics of slow- and fast-twitch muscles of the mouse. J. Gen. Physiol. 79, 147-166.

Einstein, A. 1905. Über die Von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. Ann. Physik 4, XVII, 549-560.

Glansdorff, P. and Prigogine, I. 1971. Thermodynamic Theory of Structure, Stability and Fluctuations. Wiley.

Gnaiger, E. 1983. Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. J. Exp. Zool. 228, 471-490.

Gnaiger, E. 1983b. Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In: Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Eds. Gnaiger, E. and Forstner, H. Springer, Berlin, Heidelberg, New York, 337-345.

Gnaiger, E. 1987. Optimum efficiencies of energy transformation in anoxic metabolism. The strategies of power and economy. In: Evolutionary physiological ecology. Ed. P. Calow, London, Cambridge Univ. Press 7-36.

Gnaiger, E. 1988. The concept of chemical reaction pressure: Theoretical basis of linearity between chemical flux and force. BioThermoKinetics Workshop, Abstracts, Univ. Coll. Wales, Aberystwyth.

Groen, A.K., Wanders, R.J.A., Westerhoff, H.V., van der Meer, R. and Tager, J.M. 1982. Quantification of the contribution of various steps to the control of mitochondrial respiration. J. Biol. Chem. 257, 2754-2757.

Hawkins, A.J.S., Bayne, B.L. and Day, A.J. 1986. Protein turnover, physiological energetics and heterozygosity in the blue mussel *Mytilus edulis*: the basis of variable age-specific growth. Proc. R. Soc. Lond. Biol. B 229, 161-176.

Hitchman, M.L. and Gnaiger, E. 1983. A thermodynamic consideration of permeability coefficients of membranes. In: Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Eds. Gnaiger, E. and Forstner, H. Springer, Berlin, Heidelberg, New York 31-36.

Jacobus, W.E., Moreadith, R.W. and Vandegaer, K.M. 1982. Mitochondrial respiratory control. Evidence against the regulation of respiration by extramitochondrial phosphorylation potential or by ATP / ADP ratios. J. Biol. Chem. 257, 2397-2402.

Kacser, H. and Porteous, J.W. 1987. Control of metabolism: what do we have to measure? Trends Biochem. Sci. 12, 5-14.

Katchalsky, A. and Curran, P.F. 1965. Nonequilibrium thermodynamics in biophysics. Harvard Univ. Press, Cambridge, Mass.

Kedem, O. and Caplan, S.R. 1965. Degree of coupling and its relation to efficiency of energy conversion. Trans. Faraday Soc. 61, 189–1911.

Lemasters, J.J. 1984. The ATP-to-oxygen stoichiometries of oxidative phosphorylation by rat liver mitochondria. J. Biol. Chem. 259, 13123-13130.

Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191, 144-148.

Pietrobon, D., Zoratti, M. Azzone, G.F., Stucki, J.W. and Walz, D. 1982. Non-equilibrium thermodynamic assessment of redox-driven H^+ pumps in mitochondria. Eur. J. Biochem. 127, 483-494.

Prigogine, I., Outer, P. and Herbo, C. 1948. Affinity and reaction rate close to equilibrium. J. Phys. Colloid Chem. 52, 321-331.

Senior, A.E. 1988. ATP synthesis by oxidative phosphorylation. Physiol. Rev. 68, 177–231.

Stucki, J.W. 1980. The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. Eur. J. Biochem. 109, 269–283.

Van der Meer, R. Westerhoff, H.W. and Van Dam, K. 1980. Linear relation between rate and thermodynamic force in enzyme-catalyzed reactions. Biochim. Biophys. Acta 591, 488-493.

Westerhoff, H.V. and van Dam, K. 1987. Thermodynamics and control of biological free-energy transduction. Elsevier, Amsterdam, NewYork, Oxford, pp. 568.

Wieser, W. 1989. In: Energy Transformation in cells and animals. Eds. W. Wieser and E. Gnaiger.