An investigation of the relationships between rate and driving force in simple uncatalysed and enzyme-catalysed reactions with applications of the findings to chemiosmotic reactions

Clinton D. STONER

Department of Surgery, Ohio State University, Columbus, OH 43210, U.S.A.

Both the rate and the driving force of a reaction can be expressed in terms of the concentrations of the reactants and products. Consequently, rate and driving force can be expressed as a function of each other. This has been done for a single-reactant, single-product, uncatalysed reaction and its enzyme-catalysed equivalent using the van't Hoff reaction isotherm and Haldane's generalized Michaelis–Menten rate equation, the primary objective being explanation of the exponential and sigmoidal relationships between reaction rate and $\Delta\tilde{\mu}_{H^+}$ commonly observed in studies on chemiosmotic reactions. Acquisition of a purely thermodynamic rate vs. driving-force relationship requires recognition of the intensive and extensive variables and maintenance of the extensive variables constant. This relationship is identical for the two reactions and is hyperbolic or sigmoidal, depending on whether the equilibrium constant is smaller or larger than unity. In the case of the catalysed reaction, acquisition of the purely thermodynamic relationship requires the assumption that the enzyme be equally effective in catalysing the forward and backward reactions. If this condition is not met, the relationship is modified by the enzyme in a manner which can be determined from the ratio of the Michaelis constants of the reactant and product. Under conditions of enzyme saturation in respect to reactant + product, the rate vs. driving-force relationship is determined exclusively by the thermodynamics of the reaction and a single kinetic parameter, the magnitude of which is determined by the relative effectiveness of the enzyme in catalysing the forward and backward reactions. In view of this finding, it is pointed out that, since the catalytic components of chemiosmotic reactions appear to be saturated with respect to the reactant–product pair that is varied in experimental rate vs. $\Delta\tilde{\mu}_{H^+}$ determinations, and that, since many complex enzymic reactions conform to the simple Michaelis–Menten equation with respect to a single reactant–product pair when the concentrations of all other reactants and products are maintained constant, one might expect to be capable of simulating the experimental relationships simply from knowledge of the thermodynamics of the reaction and the relative effectiveness of the catalytic component in catalysing the forward and backward reactions using the simple Michaelis–Menten equation. That this expectation appears to be largely correct is demonstrated with model reactions. It is pointed out that experimental observations suggesting that the natural ATPase inhibitor inhibits ATP synthesis in chloroplasts and mitochondria can be explained on the basis of rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships with the assumption that the inhibitor inhibits only ATP hydrolysis.

INTRODUCTION

According to the chemiosmotic theory, the immediate driving force in the synthesis of ATP from ADP and P_i in mitochondria and similar systems is an electrochemical potential difference of protons ($\Delta\tilde{\mu}_{H^+}$) across the coupling membrane. Numerous experimental studies [1–20] have indicated that the relationship between the rate of ATP synthesis and $\Delta\tilde{\mu}_{H^+}$ is either exponential or sigmoidal, a consequence of which, in either case, is that small changes in $\Delta\tilde{\mu}_{H^+}$ are associated with large changes in rate, and vice versa. Similar relationships have been observed to exist between the rate of respiration and $\Delta\tilde{\mu}_{H^+}$ in mitochondria [13,21–29]. It is now generally recognized that the exponential rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships are inherently sigmoidal and that the observation of exponential relationships in many of the studies was due to insufficient extension of the range of $\Delta\tilde{\mu}_{H^+}$.

The physical basis of the sigmoidal relationships has been a subject of much discussion in recent years (e.g. see ref. [30] and reviews, refs. [31–36]). Early studies indicating discrepancies in the relationships depending on how $\Delta\tilde{\mu}_{H^+}$ is varied, and the steep dependence of reaction rate on $\Delta\tilde{\mu}_{H^+}$ at high values of $\Delta\tilde{\mu}_{H^+}$, which is characteristic of the observed exponential and sigmoidal relationships, have led some workers to question the validity of the chemiosmotic theory as originally formulated. However, in view of recent findings by Woelders *et al.* [37,38] and Davis &

Davis-van Thienen [39] indicating no discrepancies, the early discrepancies, which were the primary source of the scepticism concerning the validity of the chemiosmotic theory, now appear to have been due to experimental difficulties.

Attempts to explain the sigmoidal relationships have been few in relation to the number of experimental observations. In this regard, Pietrobon & Caplan [40] and Pietrobon [41] have examined a number of models of chemiosmotic reactions by the diagram method of Hill [42] and have demonstrated that, with appropriate assumptions, sigmoidal relationships between rate and driving force can be predicted for all such reactions. In the case of the ATP synthase reaction, a number of investigators have explained the sigmoidicity in terms of an allosteric effect of $\Delta\tilde{\mu}_{H^+}$ on the enzyme [8,12,14,19,32,43]. Although an allosteric effect of $\Delta\tilde{\mu}_{H^+}$ on H^+ -ATP synthases clearly occurs, its involvement in shaping rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships has not been clearly established.

The present study describes the rate vs. driving-force relationships expected of a single-reactant, single-product, uncatalysed reaction and its enzyme-catalysed equivalent and demonstrates that a simple equation that describes the rate vs. driving-force behaviour of the catalysed reaction under conditions of saturation of the enzyme with respect to reactant + product can be employed to simulate experimental rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships obtained with complex chemiosmotic systems. This is accom-

Fig. 1. Purely thermodynamic relationships between rate and driving force (30 °C) for uncatalysed reactions of the type (1) having the equilibrium constants (K_{eq}) indicated

The relationships are presented according to eqn. (3) in (a) and according to eqn. (4) in (b). 1 cal = 4.18 J, in all Figures.

plished by combining well-established and widely applicable principles of kinetics and thermodynamics through the fact that both can be expressed in terms of the concentrations of the reactant and product.

UNCATALYSED REACTION

As is generally recognized, equilibrium thermodynamics give little indication of the absolute rates of reactions occurring far from equilibrium. Although this is true of absolute rates, it is not true of the relative rates of the forward and backward components which, according to the principle of microscopic reversibility, are present in all reactions. This can readily be seen by examining the very simple, first-order, uncatalysed reaction:

in relation to the van't Hoff reaction isotherm [44,45]:

$$\begin{aligned} -\Delta G &= RT \ln K_{eq} - RT \ln (p/a) = RT \ln (k_1 a/k_2 p) \\ &= RT \ln (v_1/v_2) \end{aligned} \quad (2)$$

Here A and P are reactant and product in ideal solution in an open system at constant temperature and pressure, a and p are steady-state concentrations of A and P, k_1 and k_2 are the rate constants of the forward and backward components of the reaction, and v_1 and v_2 are the forward and backward velocities; $-\Delta G$ is the amount of Gibbs free energy dissipated in the conversion of 1 mol of A into 1 mol of P under a specific set of conditions of temperature, pressure, a and p , and may be considered to be the driving force of the reaction. It is important to note that $-\Delta G$ as defined here is identical with the 'affinity' of DeDonder [46] and with $-(\partial G/\partial \xi)_{T,P}$, the driving force of a reaction in a closed system in which the reaction is at a degree of advancement ξ [47,48].

Although eqn. (2) gives the relative velocities of the forward and backward components of reaction (1) in relation to the driving force, it is of little value for understanding how this force affects the net velocity v . This problem can be overcome by expressing v in relation to v_{max} , the maximum net velocity. In

doing so it is necessary to distinguish between the intensive and extensive variables of the system if a purely thermodynamic rate vs. driving-force relationship is to be obtained.

An intensive variable is one which is characteristic of the material present, not additive, and independent of the size of the system, whereas an extensive variable is one which is additive and a function of the system's size [49]. Although both are generally considered to be thermodynamic variables, it is convenient, appropriate, and consistent with present practice in the field of biochemistry to equate 'intensive' with 'thermodynamic' and 'extensive' with 'kinetic'. Considering the reactant and product to be individual systems within the overall system, the characteristic intensive variable of the individual systems A and P is the Gibbs potential, the difference between these systems in this regard being the driving force for net change in the overall system. The characteristic extensive variable is the amount of material undergoing reaction, which in the individual systems may be considered to be a and p , since the volumes of reaction systems of the type considered here are likely to be constant. Extensive variables being additive, the amount of material undergoing reaction in the overall system is then $a+p$. The intensive and extensive variables of the individual systems fix for each an energy to which the rate of conversion is proportional, the rate in system A being $k_1 a$, that in system P being $k_2 p$, and that in the overall system being $k_1 a - k_2 p$.

From this it is evident that expression of v/v_{max} exclusively in thermodynamic terms requires maintenance of $a+p$ constant (cf. [50–52]), a constraint inherent to closed systems such as those commonly used in initial-velocity studies. The need to do this is evident also from the fact that v will vary in direct proportion to $a+p$ at any particular non-zero driving force (i.e. from the fact that v can be varied exclusively by kinetic means). With the restriction that $a+p$ be constant, v_{max} for the forward direction is given by $k_1(a+p)$ and thus, for the forward direction:

$$v/v_{max} = \frac{k_1 a - k_2 p}{k_1(a+p)} = \frac{1 - (k_2/k_1)(p/a)}{1 + p/a} = \frac{1 - e^{\Delta G/RT}}{1 + K_{eq} e^{\Delta G/RT}} \quad (3)$$

Fig. 1(a) presents plots of v/v_{max} vs. driving force in accordance with eqn. (3) for various values of the equilibrium constant. Since

v is given relative to $v_{\max.}$ and nothing is given concerning the magnitude of $v_{\max.}$, the plots are useful primarily for observing the form of the rate vs. driving-force relationship and the magnitude of the driving force required to achieve a particular fraction of $v_{\max.}$. It may be noted, however, that this is often the only kind of information needed and that the only additional information required for conversion of the relative values into absolute values for a particular reaction is one of the two rate constants.

The question of how $v_{\max.}$ might be expected to vary with variation of the equilibrium constant cannot be answered at present, because doing so would require a thorough knowledge of the numerous factors that govern the absolute rates of reactions. Consequently, all that can be said in this regard is that $v_{\max.}$ is likely to be higher as the equilibrium constant is higher. Nevertheless, a more realistic impression of the relationship between $v_{\max.}$ and the equilibrium constant than that given by Fig. 1(a) can be obtained by presenting the rate vs. driving-force relationships for the forward direction relative to those for the backward direction as in Fig. 1(b) (cf. [53]), which was drawn in accordance with the equation:

$$v/v_{\max.} = \frac{k_1 a - k_2 p}{(k_1 + k_2)(a + p)} = \frac{1 - e^{\Delta G/RT}}{1 + K_{\text{eq.}} e^{\Delta G/RT}} \left(\frac{K_{\text{eq.}}}{1 + K_{\text{eq.}}} \right) \quad (4)$$

Here v is expressed relative to the sum of the maximum net velocities in the forward and backward directions (i.e. relative to the maximum $v_{\max.}$).

ENZYME-CATALYSED REACTION

As shown originally by Haldane [54], the general Michaelis-Menten rate equation for the enzyme-catalysed equivalent of reaction (1) is:

$$v = \frac{K_p V_1 a - K_a V_2 p}{K_a K_p + K_p a + K_a p} \quad (5)$$

in which K_a and K_p are the Michaelis constants of A and P, and V_1 and V_2 are the maximum velocities in the forward and backward directions respectively. At equilibrium $p/a = V_1 K_p / V_2 K_a = K_{\text{eq.}}$, a relation generally referred to as the Haldane relationship. By algebraic manipulation of eqn. (5) one can obtain:

$$v = \frac{\left[\frac{K_p V_1 - K_a V_2 (p/a)}{K_p + K_a (p/a)} \right] (a + p)}{(a + p) + \frac{K_a K_p (1 + p/a)}{K_p + K_a (p/a)}} \quad (6)$$

which facilitates separation of the thermodynamic (intensive) and kinetic (extensive) variables in respect to a and p . Since from eqn. (2) $p/a = K_{\text{eq.}} e^{\Delta G/RT}$, it appears from this equation that v depends solely on the driving force when the temperature and $a + p$ are constant (cf. [50–52]). However, as will be pointed out in detail below, the Haldane relationship indicates that with $a + p$ constant it is possible to vary the driving force without changing the effective amount of the enzyme, an extensive variable, only if $K_a = K_p$. By imposing this additional restriction and recognizing that in the forward direction v approaches $v_{\max.}$ as p/a approaches zero under a particular condition of $a + p$, one can obtain from eqn. (6) the relationship:

$$v/v_{\max.} = \frac{1 - (V_2/V_1)(p/a)}{1 + p/a} \quad (7)$$

which is equivalent to eqn. (3) for the uncatalysed reaction when $K_a = K_p$ and which thus indicates that the relationships of eqn. (4) and Fig. 1 are also applicable to the catalysed reaction when

$K_a = K_p$. This is expected from the fact that, through the ability of the catalyst to bind and thereby alter the effective amounts of the reactant and product, the amount of the catalyst is an extensive variable and thus not a factor in these purely thermodynamic relationships.

Since the condition $K_a = K_p$ is unlikely in catalysed reactions other than transport processes, it is evident from the above observations that it will not be generally possible to describe accurately the rate vs. driving-force behaviour of a catalysed reaction from thermodynamic information alone. However, this appears not to be a serious problem, because excellent techniques are available for determination of the kinetic parameters required for complete understanding of the steady-state rate vs. driving-force behaviour of many enzymic reactions, and there is no obvious practical need other than a pedagogic one for varying the rate of a reaction by thermodynamic means only.

As p is decreased to zero, eqns. (5) and (6) reduce to the purely kinetic simple Michaelis-Menten equation:

$$v = \frac{V_1 a}{a + K_a} \quad (8)$$

which gives v as a function only of the extensive variable a at maximum (infinite) driving force. Eqn. (6) is in a form similar to this and gives v for any particular driving force. Thus, eqn. (6) is in the form:

$$v = \frac{V_{\max.}(a + p)}{(a + p) + K_{a+p}} \quad (9)$$

in which $V_{\max.}$, the numerator term of eqn. (6) other than $(a + p)$, is the maximum net velocity at a particular driving force, and K_{a+p} , the denominator term of eqn. (6) other than $(a + p)$, is the value of $a + p$ at which $v = 0.5 V_{\max.}$. As the driving force is decreased from the maximum in the forward direction to the maximum in the backward direction, $V_{\max.}$ varies from V_1 to V_2 and K_{a+p} from K_a to K_p .

In contrast with the directly proportional relationship predicted for the purely kinetic uncatalysed reaction, the net velocity of the comparable catalysed reaction increases with increase in $a + p$ at constant driving force in accordance with a rectangular hyperbola, as is particularly obvious from eqn. (9). This of course is due to saturation of the enzyme, the amount of which at constant a and p and a non-zero driving force determines the velocity in a proportional manner over the range of validity of the steady-state assumption [55].

As is implicit in the Haldane relationship and generally recognized to be true, a catalyst cannot alter the equilibrium constant of a reaction. Nevertheless, the Haldane relationship indicates that the effectiveness of an enzyme in catalysing the forward and backward components of reaction (1) differs, depending on the relative magnitudes of K_a and K_p . Thus it indicates that if, for instance, K_p/K_a were somehow increased, V_1/V_2 would decrease, indicating a decrease in the effectiveness of the enzyme in catalysing the forward reaction relative to the backward reaction. At first glance, this seems to be inconsistent with the fact that an enzyme cannot alter the equilibrium constant of a reaction. However, the concern here is a property of the enzyme which upon modification alters the extent to which the absolute catalytic effectiveness of the enzyme changes as p/a is varied from one extreme to the other under the condition constant $a + p$, the absolute catalytic effectiveness being the effectiveness of the enzyme as a catalyst regardless of whether the reactant is A or P. If there were no change in this respect, the absolute catalytic effectiveness of the enzyme would be the same regardless of the value of p/a . According to the Haldane relationship, this condition obtains only when $K_a = K_p$ (i.e. only when $V_1/V_2 = K_{\text{eq.}}$).

It is implied above that a Michaelis constant is a property of the enzyme. That this is the case can readily be seen by noting that a Michaelis constant is often virtually a reciprocal affinity constant and that the affinity of an enzyme for a reactant is a property of the enzyme rather than of the reaction. A corollary of this is that V_1/V_2 is a property primarily of the reaction and secondarily of the enzyme. Thus, according to the Haldane relationship, V_1/V_2 is purely a thermodynamic quantity ($K_{eq.}$) when $K_a = K_p$ and, according to the above observations, is partially a kinetic one otherwise. For the purpose of illustration, the kinetic component might be considered to arise from a difference in affinity of the enzyme (E) for A and P when $K_a \neq K_p$ in a system in which K_a and K_p are reciprocal affinity constants (i.e. in a system in which the rates of formation and dissociation of EA and EP are rapid relative to the rates at which EA and EP interconvert). For example, if K_p/K_a were increased from unity by modifying the enzyme, V_1/V_2 would decrease as a result of EA becoming more stable relative to EP and of a tendency of the reaction to proceed in the direction of the more stable complex. At equilibrium this tendency presumably would be counteracted completely by the increase in $[EA]/[EP]$ that would occur consequent to the increase in K_p/K_a .

A major goal of these considerations is to identify and distinguish between the intensive and extensive variables of the enzyme-catalysed equivalent of reaction (1). In the case of the uncatalysed reaction, recognition of these variables is easily achieved because the effective concentrations of A and P are a and p and the equilibrium constant of the reaction fully describes the relative tendencies of A and P to interconvert. In the case of the catalysed reaction, the effective concentrations of A and P are $[EA]$ and $[EP]$ and the relative tendencies of A and P to interconvert depend not only on the equilibrium constant of the reaction, but also on the ability of the enzyme to catalyse the conversion of A into P relative to its ability to catalyse the conversion of P into A.

Since the Haldane relationship predicts that $K_{eq.} = V_1/V_2$ when $K_p/K_a = 1$ and that $K_{eq.} = K_p/K_a$ when $V_1/V_2 = 1$, one might suppose that the enzyme is equally capable of catalysing the forward and backward reactions when either $V_1/V_2 = 1$ or $K_p/K_a = 1$. However, only K_p/K_a can be clearly identified as a property of the enzyme. Furthermore, acquisition of eqn. (7), the purely thermodynamic rate equation for the catalysed reaction, requires the assumption $K_a = K_p$, indicating clearly that the enzyme is equally capable of catalysing the forward and backward reactions only when $K_p/K_a = 1$ and that when $K_p/K_a \neq 1$, variation of the intensive variable (p/a) is accompanied by variation of an extensive variable. Why this extensive variable remains constant when $K_a = K_p$ can readily be seen by assuming, as before, that K_a and K_p are reciprocal affinity constants and noting that, if the affinity of the enzyme for A and P were equal, $[EP]/[EA]$ would always equal p/a and the 'effective extensive variable' $[EA] + [EP]$ would not change as p/a is varied at constant $a+p$. The extensive variable does not remain constant when $K_p/K_a \neq 1$ because the enzyme is not equally capable of catalysing the forward and backward reactions and, in consequence, varying p/a from one extreme to the other at constant $a+p$ results in a continuous change in the absolute catalytic activity of the enzyme, owing to a continuous change in the absolute effectiveness of the enzyme as a catalyst. Since the Michaelis-Menten equations assume direct proportionality between enzyme activity and enzyme concentration [54,55], this continuous change in activity may be thought of as a continuous change in the effective amount of the enzyme. In fact it seems preferable to do so because of the relative ease of recognizing enzyme amount as an extensive variable.

It may be noted that variation of the effective amount of the

Fig. 2. Relationship of V_1/V_2 and K_p/K_a to the driving force (30 °C) required to achieve $0.5 v_{max}$. ($-\Delta G_{0.5}$) of an enzyme-catalysed equivalent of reaction (1), assuming that $K_{eq.} = 1$ and that the reaction occurs according to eqn. (5)

v_{max} is the maximum net velocity under a particular condition of a or $a+p$ and is presented relative to V_1 to indicate different degrees of enzyme saturation. The results for conditions of constant a (-----) and constant $a+p$ (—) are identical when $v_{max}/V_1 = 1$.

enzyme is unavoidable while varying $a+p$ at constant p/a , since the amount of the enzyme is in effect zero when $a+p = 0$ and is maximal when $a+p$ is saturating. However, this does not present a problem in respect to the generalized purely kinetic eqn. (9), because both $a+p$ and the effective amount of the enzyme are extensive variables.

According to the above considerations, K_p/K_a is a property of the enzyme and an indicator of the relative effectiveness of the enzyme in catalysing the forward and backward reactions. Since enzymes can be modified, it is therefore appropriate to consider some consequences of changing K_p/K_a with regard to rate vs. driving-force relationships. As noted above, when $a+p$ is a constant and $K_a = K_p$, the rate vs. driving-force behaviour of an enzyme-catalysed equivalent of reaction (1) is as indicated by Fig. 1 and eqns. (3), (4) and (7). According to eqns. (2) and (5), varying K_p/K_a under zero-order conditions (i.e. under conditions of $a+p$ being much greater than K_{a+p}) would have the same effect on the form of the rate vs. driving-force relationship as variation of the equilibrium constant in the opposite direction. For instance, a 10-fold increase in K_p/K_a under these conditions would have the same effect on V_1/V_2 as a 10-fold decrease in the equilibrium constant. This can be seen by comparing $-\Delta G_{0.5}$ vs. $K_{eq.}$ in Fig. 1 with Fig. 2, which presents plots of $-\Delta G_{0.5}$ vs. K_p/K_a and V_1/V_2 for a particular reaction ($K_{eq.} = 1$), $-\Delta G_{0.5}$ being the driving force required to achieve $0.5 v_{max}$ under a particular condition of a or $a+p$. The constant- a condition is included here because under conditions of zero order it yields the same results as constant $a+p$, and will be considered below.

Fig. 2 also shows how the relationships change with changes in the degree of enzyme saturation as indicated by v_{max}/V_1 (i.e. as indicated by the velocity when $p = 0$ and a is non-saturating relative to the velocity when $p = 0$ and a is saturating). In the case of constant $a+p$, the relationship between $-\Delta G_{0.5}$ and

Fig. 3. Mixed thermodynamic and kinetic relationships between rate and driving force (30 °C) for the uncatalysed reaction (1) (a) and its enzyme-catalysed (Michaelis-Menten) equivalent (b), assuming in each case that $K_{eq} = 10$

In calculating the curves, a and p were varied as indicated (arbitrary units), assuming in the uncatalysed case that $k_1 = 1$ and in the catalysed case that $K_a = K_p = V_1 = 1$.

V_1/V_2 differs under mixed-order conditions depending on the equilibrium constant of the reaction. This is due to the facts that K_p/K_a and V_1/V_2 differ depending on the equilibrium constant and that, in the case of constant $a+p$, changes in the degree of enzyme saturation do not affect $-\Delta G_{0.5}$ when $K_p/K_a = 1$. In the case of constant a , the relationships between $-\Delta G_{0.5}$ and V_1/V_2 are the same as those shown in Fig. 2 regardless of the equilibrium constant and K_p/K_a . These observations are important because they point out that rate vs. driving-force relationships of the enzyme-catalysed reaction depend greatly on the relative effectiveness of the enzyme in catalysing the forward and backward reactions and that the relationships are thus subject to modification. In addition, they point out that V_1/V_2 may be used as an indicator of the form of the relationship, much as the equilibrium constant may be used for this purpose in the case of the purely thermodynamic reaction. In fact, under conditions of enzyme saturation (zero-order conditions), a given value of V_1/V_2 indicates the same relationship as does an equilibrium constant of equal value in the case of the purely thermodynamic reaction.

A relevant question at this point is what might one expect of the absolute magnitudes of V_1 and V_2 as V_1/V_2 is changed by modification of K_p/K_a . In this regard it is important to note that the equations of this study that include ratios of the above sort say nothing about absolute magnitudes. Accurate prediction of the effects on V_1 and V_2 of modifying the K_p/K_a ratio of a real enzyme-catalysed reaction is likely to require detailed knowledge of the mechanism of catalysis.

MIXED KINETIC AND THERMODYNAMIC RELATIONSHIPS

As is well known and indicated above to be largely unavoidable in the case of the catalysed reaction, the limitation or control of reactions in natural systems occurs by both thermodynamic and kinetic means. Reactants and products other than coenzymes (transfer-type intermediates) are not constrained to vary in constant ratio or constant sum as was done above to distinguish between the intensive and extensive variables. These variables can be expected normally to vary simultaneously to achieve

specific purposes. It seems appropriate, therefore, to represent rate vs. driving-force relationships as series of constant- a and constant- p curves as in Figs. 3(a) and 3(b) for the uncatalysed and catalysed reactions respectively (cf. [56]). As may be seen, this results in there being a marked difference between the two reactions, particularly with respect to $-\Delta G_{0.5}$.

With regard to the uncatalysed reaction constrained to the constant- a condition, since $v_{max.} = k_1 a$, one's choice of a determines $v_{max.}$ for a particular reaction. In contrast with the constant- $(a+p)$ case, when the driving force is increased at constant a , $-\Delta G_{0.5}$ is minimal [1.76 kJ (0.42 kcal)/mol at 30 °C], regardless of the equilibrium constant of the reaction and one's choice of a . As can be expected from the fact that enzyme-catalysed reactions exhibit first-order rate behaviour when the concentrations of the reactants and products are much lower than the Michaelis constants, the same is true of the catalysed reaction when these conditions obtain. However, as a is increased to values much greater than K_a , the enzyme becomes saturated and $-\Delta G_{0.5}$ increases, linearly when V_1/V_2 is less than unity and exponentially when V_1/V_2 is greater than unity, toward the same $-\Delta G_{0.5}$ value that is obtained when a and p are varied in constant sum (Fig. 2). This transition occurs because, under the zero-order conditions of enzyme saturation with A and P, the rate of the reaction is influenced only by changes in the ratio of a and p . In other words, as a result of the limited capacity of the enzyme to bind A and P, the extensive variable $a+p$ is in effect virtually constant throughout the range of $a+p$ over which the enzyme may reasonably be considered to be saturated, regardless of whether or not it is in fact maintained constant. This means that the catalysed reaction occurring under zero-order conditions would be governed with respect to a and p exclusively by the thermodynamics of the reaction if the effective amount of the enzyme were not to change in response to changes in p/a (i.e. if K_a were to equal K_p).

As noted in the preceding section, under conditions of zero order V_1/V_2 indicates the same form of rate vs. driving-force relationship as does an equilibrium constant of equal value in the case of the purely thermodynamic reaction, regardless of the value of K_p/K_a . This and the fact that when V_1/V_2 is greater than

unity, $-\Delta G_{0.5}$ increases exponentially toward the zero-order value as the degree of enzyme saturation increases (Fig. 2) account for the considerable similarity of the constant- a curves of Fig. 3(b) to the purely thermodynamic relationships of Fig. 1 and point out that relationships similar to the purely thermodynamic ones can be expected under the conditions that one might expect to find in living systems.

The difference between the uncatalysed and catalysed reactions (Fig. 3) with regard to $-\Delta G_{0.5}$ is of course greater as the equilibrium constant is higher. Whereas removal of the restriction that $a+p$ be constant allows an uncatalysed reaction of the type (1) having a high equilibrium constant to operate at half-maximum velocity at the minimum distance from equilibrium (i.e. at the minimum $-\Delta G$), a catalysed equivalent reaction having a high equilibrium constant can do so only if K_p/K_a is sufficiently high to result in V_1/V_2 being very low (Fig. 2). This comparison is important because it points out that a serious potential disadvantage of a catalysed equivalent reaction having a high equilibrium constant can be compensated for by the enzyme having a high K_p/K_a ratio, the potential disadvantage being that, if K_p/K_a were low, such a reaction could proceed at a reasonable fraction of its potential velocity only by operating at high $-\Delta G$ (i.e. only by operating at low p/a). It should be noted, however, that since a low $-\Delta G_{0.5}$ may mean that the reaction is thermodynamically unfavourable and in consequence has a strong tendency to proceed in the backward direction, there are instances in which a low $-\Delta G_{0.5}$ is potentially disadvantageous. In such a case, a low equilibrium constant could be compensated for by the enzyme having a low K_p/K_a ratio. Examples of chemiosmotic reactions in which these considerations appear to be very important with respect to energy conservation will be given below.

PROSPECTS FOR APPLICATION OF THE ABOVE FINDINGS TO CHEMIOSMOTIC REACTIONS

The above findings indicate that if reaction (1) were catalysed by an enzyme in accordance with Michaelis-Menten kinetics under conditions of $a+p$ being much greater than K_{a+p} (i.e. under conditions of saturation of the enzyme with respect to reactant + product), the form of the rate vs. driving-force relationship would be determined exclusively by the thermodynamics of the reaction (indicated by K_{eq} and $-\Delta G$) and the relative effectiveness of the enzyme in catalysing the forward and backward reactions (indicated by K_p/K_a). This can readily be seen through algebraic manipulation of the generalized Michaelis-Menten eqn. (5) and substitution of some of the terms with equivalent terms from the van't Hoff reaction isotherm (eqn. 2) and the Haldane relationship:

$$v/V_1 = \frac{K_p/K_a - (V_2/V_1)(p/a)}{K_p/a + K_p/K_a + p/a} = \frac{(K_p/K_a)(1 - e^{\Delta G/RT})}{K_p/a + K_p/K_a + K_{eq}e^{\Delta G/RT}} \quad (10)$$

It is evident from these modified forms of eqn. (5) that, presumably as a result of the reactant and product and their Michaelis constants not being independent of one another, one need assume only that K_p/a is negligibly small for v/V_1 to become a function only of the thermodynamics of the reaction and the relative effectiveness of the enzyme in catalysing the forward and backward reactions. Accordingly, K_p/a of eqn. (10) can be replaced with any one of three equivalent terms which include K_a/a , K_a/p or K_p/p . Note that, with the additional assumption that $K_a = K_p$, eqn. (10) becomes equivalent to the purely thermodynamic rate equations (3) and (7).

Since many complex enzymic reactions appear to obey simple Michaelis-Menten (saturation) kinetics with respect to a single reactant-product pair under conditions of constant concen-

tration of all other reactants and products [57], and since rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships of chemiosmotic reactions are generally determined by varying the concentration or activity only of H_{in}^+ or H_{out}^+ , the above findings suggest the possibility of simulating experimentally determined rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships with the use of eqn. (10) on the assumption that $K_p/a \approx 0$ (i.e. simply from knowledge of the reaction thermodynamics and of the relative effectiveness of the catalytic component in catalysing the overall forward and backward reactions). Although little is known of the catalytic components of chemiosmotic reactions as to their relative effectiveness in catalysing forward and backward reactions, various values can be assumed for this property to show what to expect of changes and to give some idea as to what the capabilities of the catalytic components might be in this regard.

Most of the experimental determinations of rate vs. $\Delta\tilde{\mu}_{H^+}$ were made in tests of the chemiosmotic theory in which the rate of respiration in mitochondria and the rate of ATP synthesis in mitochondria and similar systems were determined by the initial-velocity method as a function of $\Delta\tilde{\mu}_{H^+}$ or one of its components across the coupling membrane. The determinations were carried out by varying only the activity or concentration of H_{in}^+ or H_{out}^+ . The concentrations of all other reactants and products were maintained essentially constant, and the reactants were generally employed at saturating concentrations to minimize the rate-dependence of the reaction on factors other than $\Delta\tilde{\mu}_{H^+}$. Consequently, the reactions were essentially either $nH_{in}^+ \rightleftharpoons nH_{out}^+$ or $nH_{out}^+ \rightleftharpoons nH_{in}^+$ from the standpoint of Michaelis-Menten kinetics, n being the number of protons transported in one turnover of the overall reaction. This being the case, one need be concerned only about whether or not eqn. (10) is applicable to these proton-transport reactions and whether or not the catalytic component of the reaction is sufficiently saturated with respect to $H_{in}^+ + H_{out}^+$ to allow the assumption that K_p/a of eqn. (10) is negligibly small. With regard to this assumption, observations on ATP synthesis in chloroplasts, indicating that the rate at a particular $\Delta\tilde{\mu}_{H^+}$ is largely independent of pH_{out} over the range 7–9 [6,8,58], suggest that it is valid for the ATP synthase reaction. That it is valid also for the mitochondrial respiratory-chain reactions is suggested by the ability of mitochondria to catalyse rapid oxidative phosphorylation over a wide range of pH with little or no effect of pH on the $P/2e^-$ stoichiometry.

As for whether or not eqn. (10) is applicable to the H^+ -transport component of a chemiosmotic reaction, a number of questions need consideration. One such question is how to deal with the fact that n , the number of protons transported per turnover, is greater than unity. According to the Law of Mass Action, if a and p of eqn. (10) are to represent $[H_{in}^+]$ and $[H_{out}^+]$, for example, they must be given to the power n . Assuming that the velocities in eqn. (10) are expressed in terms of a reactant-product pair other than $H_{in}^+ - H_{out}^+$, as is ordinarily the case in studies on chemiosmotic reactions, this and expression of K_a and K_p in terms of $[H_{in}^+]^n$ and $[H_{out}^+]^n$ are the only changes needed if the overall reaction is catalysed by a single enzyme and the concentrations of all reactants and products other than H^+ are maintained constant. This is due to the fact that, since neither the n reactants nor the n products of a reaction such as $nA \rightleftharpoons nP$ can be distinguished from one another, relationships identical with those of eqn. (10) are obtained for a single-enzyme reaction, regardless of the value of n , as long as a and p and all the parameters expressed in terms of a and p are given in terms of a^n and p^n . Consequently, if the H^+ -transport component of a chemiosmotic reaction were catalysed by a single enzyme and the concentrations of all reactants and products other than H_{in}^+ or H_{out}^+ were maintained constant, one could expect the ratio of the apparent Michaelis constants of H_{in}^+ and H_{out}^+ , expressed in terms

of $[H_{in}^+]^n$ and $[H_{out}^+]^n$, to indicate the relative effectiveness of the catalytic component in catalysing the forward and backward components of the overall reaction under the conditions employed, as in any multireactant, multiproduct, single-enzyme reaction that is simplified by maintaining the concentrations of all but a single reactant-product pair constant for the purpose of applying the simple equations of saturation kinetics. This being the case, acquisition of the ratio of the Michaelis constants in terms of actual H^+ concentrations would require determination of the n th root of the ratio. Regardless of how it is expressed, the ratio would not give a reliable indication of the relative affinity of the enzyme for H_{in}^+ and H_{out}^+ , because the Michaelis constants would represent all of the reactants and products.

With regard to the question of whether the complex catalytic components of chemiosmotic reactions can be expected to be equivalent to single enzymes from the standpoint of saturation kinetics, Hearon [59] and Hearon *et al.* [60] have shown that Michaelis-Menten kinetics cannot distinguish between reactions which differ in the number of first-order steps. Therefore, as a first approximation, the H^+ -transport component of a chemiosmotic reaction catalysed by a multienzyme complex might be expected to conform more or less exactly to Michaelis-Menten kinetics if the various steps within the overall reaction are not connected by freely diffusible intermediates. The $\Delta\tilde{\mu}_{H^+}$ -driven ATP synthase reaction as ordinarily examined in chloroplast lamellae and other systems of similar orientation appears to be a multienzyme reaction of this type. Consequently, from this standpoint the prospects for valid simulation of the experimental rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships seem particularly good in this case. However, there is considerable evidence suggesting that $\Delta\tilde{\mu}_{H^+}$ has a marked allosteric effect on H^+ -ATP synthases which is particularly noticeable in the case of the chloroplast enzyme because of its relatively critical dependence on $\Delta\tilde{\mu}_{H^+}$ for catalysis of the backward reaction. The allosteric effect is thought to occur through $\Delta\tilde{\mu}_{H^+}$ -dependent displacement of an inhibitor protein component and/or of a tightly bound ADP (for reviews, see [61-66]).

Most of the studies on the allosteric effect of $\Delta\tilde{\mu}_{H^+}$ have been done with mitochondrial and chloroplast systems. Although the ATP synthases of both have an inhibitor protein as well as a site which binds ADP very tightly under conditions of low $\Delta\tilde{\mu}_{H^+}$ and which appears to have a regulatory function, the allosteric effect of $\Delta\tilde{\mu}_{H^+}$ on the mitochondrial synthase is more often considered to be mediated through the inhibitor protein, whereas that on the chloroplast synthase is more often considered to be mediated through the tightly bound ADP. In view of the considerable similarity in the actions attributed to these effectors, it seems unnecessary to distinguish between them here. In any case, for the sake of brevity, no attempt will be made to do so.

The inhibitor protein of the mitochondrial synthase has been studied extensively and was originally thought to inhibit only the backward component of the ATP synthase reaction [67,68]. Inhibition only of the backward reaction smacks of a Maxwell demon and thus seemed to some investigators to be inconsistent with the laws of thermodynamics. This problem appeared to be resolved with the discoveries, made with submitochondrial particles and chloroplast lamellar vesicles, that inhibition of the backward reaction is subject to partial relief by prior energization of the membrane [69-71] and that achievement of a steady-state rate of ATP synthesis initiated by energization of the membrane is often preceded by a significant lag period which is subject to elimination by prior energization of the membrane [72-76] and by displacement or removal of the inhibitor protein [71,73-77]. These findings have been interpreted by a number of workers [14,17,19,32,43,71,73,74,77,78] to indicate that the inhibitor totally inactivates the enzyme and thus that $\Delta\tilde{\mu}_{H^+}$ not only drives

ATP synthesis but also activates the enzyme for both ATP synthesis and ATP hydrolysis. However, studies on submitochondrial particles have shown that maximum $\Delta\tilde{\mu}_{H^+}$ -induced relief of inhibition of ATP hydrolysis requires periods of time much longer than those required for maximum relief of inhibition of ATP synthesis [64,75,76], and that maximum relief of inhibition of ATP hydrolysis involves displacement of the inhibitor protein from only a small fraction of the ATP synthases [76]. Furthermore, $\Delta\tilde{\mu}_{H^+}$ -induced displacement of the inhibitor from submitochondrial particles has been shown to be strongly counteracted by ATP [79]. These findings suggest that the inhibitor normally remains bound to the synthase in intact mitochondria and thus that its control by $\Delta\tilde{\mu}_{H^+}$ and other factors may be graded rather than all-or-none. As will be pointed out below, the observed initial inhibition of ATP synthesis can be explained on the basis of rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships with the assumptions that the inhibitor inhibits only ATP hydrolysis and that $\Delta\tilde{\mu}_{H^+}$ decreases the effectiveness of the inhibitor gradually.

A common observation with respect to experimental rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships is that simple and complex chemiosmotic systems yield relationships of similar form even though the complex systems catalyse overall reactions that are known to consist of several H^+ -transport and other individual reactions linked by freely diffusible intermediates. A conceivable explanation for this is that the H^+ -transport reactions are catalysed by saturable enzymes having comparable affinities for H^+ , and that it does not matter greatly how many individual reactions are involved, provided that the steady-state rate of the overall reaction is determined as a function only of $[H_{in}^+]$ or $[H_{out}^+]$ under conditions of saturation of the 'enzyme' with respect to $H_{in}^+ + H_{out}^+$. In fact the exponential form of eqn. (10) is clearly consistent with this, in that it states that reaction velocity under conditions of enzyme saturation with respect to reactant + product is a function only of the driving force and a single kinetic parameter, the magnitude of which is determined by the relative effectiveness of the enzyme in catalysing the forward and backward reactions. It does not seem unreasonable to suppose that this applies to all enzyme-catalysed reactions that obey saturation kinetics with respect to the varied reactant-product pair, regardless of their complexity. Thus all reactions are driven by a thermodynamic force, and all enzymes or systems of enzymes can be expected to possess certain abilities to catalyse the forward and backward components of the reactions they catalyse. The only obvious problems in dealing with complex reactions consisting of several individual reactions linked by freely diffusible intermediates appear to be (i) that it may be difficult to achieve a valid steady-state analysis of the overall reaction, and (ii) that it may be difficult or impossible to determine the relative effectiveness of the system in catalysing the overall forward and backward reactions from rate vs. reactant-concentration studies. The first of these problems tends to be minimal for chemiosmotic reactions, because most, if not all, of the freely diffusible intermediates are likely to be confined to very small spaces and consequently are likely to approach a steady-state condition very rapidly [81]. On the other hand, the second of these problems is particularly serious with chemiosmotic reactions, because determination of the apparent Michaelis constants for H_{in}^+ and H_{out}^+ would require variation of reactant H^+ activity under conditions of product H^+ activity much lower than the apparent Michaelis constant of product H^+ , and if one were to attempt this the catalytic system would be destroyed. However, as indicated above, some idea as to the relative effectiveness of chemiosmotic systems in catalysing their forward and backward reactions can be obtained by assuming various values for this parameter on the assumption that the velocity of the overall reaction is in fact governed with respect to $[H_{in}^+]^n$ and $[H_{out}^+]^n$ in

accordance with saturation kinetics under conditions of constant concentration of all other reactants and products. As is demonstrated below, this is possible because the driving forces and rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships of many chemiosmotic reactions are known.

SIMULATION OF RESPIRATION-RATE VS. $\Delta\tilde{\mu}_{H^+}$ RELATIONSHIPS

Since many valid steady-state determinations of rate vs. $\Delta\tilde{\mu}_{H^+}$ have been done with intact mitochondria, and since the relationship of $\Delta\tilde{\mu}_{H^+}$ to the rates of both $\Delta\tilde{\mu}_{H^+}$ -forming and $\Delta\tilde{\mu}_{H^+}$ -consuming reactions have been determined with this system, consideration of intact mitochondrial systems will be included in attempts to simulate experimental relationships. Experimental respiration-rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships have been obtained by varying the activity only of H_{in}^+ either with an uncoupling agent or through activation or inactivation of the energy-conserving reactions normally driven by $\Delta\tilde{\mu}_{H^+}$. Theoretically it should not matter which of these means of varying $\Delta\tilde{\mu}_{H^+}$ is employed. With intact mitochondria, however, it often does matter, because uncouplers can inhibit respiration in these organelles by number of mechanisms [82,83]. In the attempt at simulation, the reaction:

will be employed. Here βOHB and AcAc are extramitochondrial β -hydroxybutyrate and acetoacetate. The oxidation of βOHB by O_2 is the driving (redox) reaction, and the transport of H_{in}^+ to H_{out}^+ is the driven reaction. It will be assumed that oxidation of βOHB beyond AcAc is prevented and that the reaction is carried out under conditions of 25 °C, $\text{pH}_{out} = 7$, and complete coupling of H^+ transport to the redox reactions. With complete coupling the value of n is very likely 10 [84] and will be assumed to be so. Since the βOHB and AcAc in reaction (11) are extramitochondrial, varying $\Delta\tilde{\mu}_{H^+}$ at constant pH_{out} would not affect the Gibbs potential difference of the $\text{AcAc}/\beta\text{OHB}$ redox couple. Recent findings [84] suggesting that O_2 is reduced at the outer side of the inner membrane suggest that this would be true also of the oxygen/water couple.

The driving force of reaction (11) is given by:

$$-\Delta G = -\Delta G_R - n\Delta\tilde{\mu}_{H^+} \quad (12)$$

in which ΔG_R is the sum of the chemical potentials of the products minus that of the reactants of the redox reaction and $\Delta\tilde{\mu}_{H^+}$ is the electrochemical potential of H_{out}^+ minus that of H_{in}^+ . The standard Gibbs potential difference between the reactants and products of the redox reaction is given by the relation $\Delta G_R^{\circ'} = -2F\Delta E_m$, in which 2 is the number of equivalents of electrons transferred from βOHB to O_2 per mol of βOHB oxidized, F is the Faraday constant, and ΔE_m is the algebraic difference between the midpoint potentials of the $\text{AcAc}/\beta\text{OHB}$ and oxygen/water redox couples. Under conditions of pH 7 and 25 °C, the midpoint potential of the $\text{AcAc}/\beta\text{OHB}$ couple is -0.266 V [85] and that of the oxygen/water couple is 0.815 V [86]. These values yield a $\Delta G_R^{\circ'}$ of -209 kJ (-49.9 kcal)/mol.

As indicated above, reaction rates are ordinarily determined by the initial-velocity method starting with saturating concentrations of reactants. Accordingly, it will be assumed that the concentration of AcAc is initially zero and that the average concentrations of βOHB , AcAc and O_2 during the period required for rate measurement are 10 mM, 5 μM and 20 kPa (0.2 atm) respectively. From these values, a ΔG_R of -226 kJ (-53.9 kcal)/mol is obtained. Since ΔG_R is to be maintained constant, and since eqn. (10) is to be used in conjunction with an H^+ -transport reaction that includes the redox component of reaction (11), ΔG_R is the contribution of the redox component to the apparent ΔG° of the H^+ -transport reaction that includes the

redox reaction. It will be assumed that the ΔG° of the H^+ -transport component of reaction (11) is zero, and thus that the apparent ΔG° of the H^+ transport reaction is -226 kJ (-53.9 kcal)/mol (i.e. ΔG_R°). This value yields an apparent equilibrium constant for the H^+ -transport reaction of 3.14×10^{39} .

Fig. 4 presents relationships of rate vs. $\Delta\tilde{\mu}_{H^+}$ and $-\Delta G$ for reaction (11) according to eqn. (12) and the exponential form of eqn. (10), assuming that K_p/a is negligibly small and that the equilibrium constant of the reaction is 3.14×10^{39} . Relationships with K_p/K_a values ranging from 1 to 10^{30} are compared. The rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships are plotted as most experimental respiration-rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships have been plotted. Thus $\Delta\tilde{\mu}_{H^+}$ is presented on the ordinate as Δp (i.e. as $\Delta\tilde{\mu}_{H^+}/F$) in millivolts, and the rate is given on the abscissa. The relationship obtained by assuming $K_p/K_a = 1$ ($V_1/V_2 = 3.14 \times 10^{39}$) indicates that if the mitochondria were equally effective in catalysing the forward and backward components of reaction (11), the net forward velocity at the ΔG_R employed would be virtually zero at a Δp of 20 mV and the redox reaction would require complete uncoupling from the H^+ -transport reaction to achieve half its potential maximum velocity. In other words, the $K_p/K_a = 1$ relationship indicates that, if the mitochondria were equally capable of catalysing the forward and backward components of reaction (11), the redox reaction could not maintain Δp above 20 mV at a measurable rate. Of course this would be due to the products of the individual reactions being able to compete very effectively with the reactants for the enzymes.

Experimental respiration-rate vs. Δp relationships indicate that mitochondrial redox reactions are readily capable of achieving their maximum potential net forward velocities when $-\Delta G_R$ is high and that they are capable of achieving half-maximal velocity at Δp values in the range 150 – 225 mV (e.g. see [21–29]). As may be seen from Fig. 4, to bring the model employed here into line with these results, one must assume that the mitochondria were more capable of catalysing the backward reaction relative to the forward reaction by factors ranging from 10^{20} ($V_1/V_2 = 3.14 \times 10^{19}$) to more than 10^{30} ($V_1/V_2 < 3.14 \times 10^9$). Although these are very large differences, they do not seem unreasonable when one considers the fact [87] that the Michaelis constant of O_2 is less than 10^{-7} M at a water concentration of approx. 55 M in mitochondria incubated under conditions similar to those employed in respiration-rate vs. $\Delta\tilde{\mu}_{H^+}$ determinations. It is obvious from this that the cytochrome oxidase component alone could account for a large proportion of the increase in K_p/K_a required to bring the model into accord with the experimental observations. The apparent irreversibility of the cytochrome oxidase reaction [87] indicates that the V_1/V_2 of this reaction is nevertheless very high.

It is important to note that the K_p/K_a values employed in the construction of Fig. 4 represent the ratio of the apparent Michaelis constants of H_{out}^+ and H_{in}^+ in terms of $[\text{H}_{out}^+]$ and $[\text{H}_{in}^+]$. Consequently, owing to the high n value in reaction (11), the K_p/K_a values of Fig. 4 other than unity would be much lower if K_a and K_p were expressed in terms of actual H^+ concentrations or activities. For example, a K_p/K_a value of 10^{30} corresponds to a $K_{H_{out}^+}/K_{H_{in}^+}$ value of 10^3 . This means that if experimental determination of the apparent Michaelis constants of H_{out}^+ and H_{in}^+ were possible for reaction (11) and a value for $K_{H_{out}^+}$ of 10^{-10} M were obtained, one could expect to find a value for $K_{H_{in}^+}$ of 10^{-13} M. As indicated above, these values could not be expected to be reliable indicators of the affinity of the H^+ -transport enzymes for H^+ , since the magnitude of $K_{H_{out}^+}/K_{H_{in}^+}$ would be a function of the relative effectiveness of all the enzymes of the system in catalysing the forward and backward components of their respective reactions under the conditions employed.

As for the form of the respiration-rate vs. Δp relationships,

Fig. 4. Simulation of experimental respiration-rate vs. Δp relationships for intact mitochondria catalysing the oxidation of β -hydroxybutyrate by O_2 under conditions of pH 7.0 and 25 °C

The relationships were simulated with the exponential form of eqn. (10), assuming $K_p/a \approx 0$, $K_{eq} = 3.14 \times 10^{39}$, and K_p/K_a values as indicated. Δp in relation to $-\Delta G$ was determined according to eqn. (12), assuming ΔG_R and n to be -226 kJ (-53.9 kcal)/mol and 10 respectively. See the text for explanation and additional details.

the model appears to agree very well with the experimental observations, particularly with those carried out using NAD-linked substrates [23,25]. Note that, owing to a lower electrogenic $H^+/2e^-$ stoichiometry, the slopes of the relationships at half-maximum velocity would be higher if succinate or glycerol 3-phosphate rather than an NAD-linked substrate were employed as the oxidizable substrate. This has been demonstrated experimentally by Johnson & Hansford [23].

SIMULATION OF RELATIONSHIPS BETWEEN RATE OF ATP SYNTHESIS AND $\Delta\tilde{\mu}_{H^+}$

ATP synthesis catalysed by intact mitochondria occurs according to the reaction:

in which ADP, P_i and ATP will be considered here to be extramitochondrial. The driving force of this reaction is given by

$$-\Delta G = -n\Delta\tilde{\mu}_{H^+} - \Delta G_p \quad (14)$$

in which $\Delta\tilde{\mu}_{H^+}$ is the electrochemical potential of H_{in}^+ minus that of H_{out}^+ and ΔG_p is the chemical potential of ATP minus the sum of the chemical potentials of ADP and P_i . It will be assumed that the mitochondria are incubated under conditions of pH 7.5, 25 °C, 1 mM-MgCl₂, and 0.1 M ionic strength. Under these conditions, ΔG_p° is 29.9 kJ (7.12 kcal)/mol [80]. The average concentrations of ADP, P_i and ATP during the period required for rate measurement will be assumed to be 1 mM, 10 mM and 10 μ M respectively. Using these values, a ΔG_p of 29.9 kJ (7.12 kcal)/mol is obtained. Assuming that the ΔG° of the H^+ -transport component of reaction (13) is zero, the apparent ΔG° of the H^+ -transport reaction to be used in conjunction with eqn. (10) is ΔG_p , which at 29.9 kJ (7.12 kcal)/mol yields an apparent equilibrium constant for the H^+ -transport reaction of 6.04×10^{-6} .

The value of n in reaction (13) and eqn. (14) is generally accepted to be 4 in intact mitochondria. Most of the experimental rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships for ATP synthesis have been obtained

with $n = 3$ systems such as inverted mitochondrial inner-membrane vesicles, chloroplast lamellar vesicles and bacterial chromatophores, in which the ATP synthase is accessible to ADP, P_i and ATP from the outside. Therefore, simulation of experimental relationships obtained with these systems will also be attempted. This requires modification of reaction (13) to show H_{in}^+ as a reactant and H_{out}^+ as a product and requires definition of $\Delta\tilde{\mu}_{H^+}$ in eqn. (14) as the electrochemical potential of H_{out}^+ minus that of H_{in}^+ .

Fig. 5 presents relationships of rate vs. $-\Delta p$ and $-\Delta G$ for reaction (13) determined according to eqn. (14) and the exponential form of eqn. (10), assuming that K_p/a is negligible and that the equilibrium constant of the reaction is 6.04×10^{-6} . Reactions with n values of 3 and 4 and K_p/K_a values ranging from 1 to 10^{-15} are compared. As may be seen, if one assumes that the catalytic component is equally effective in catalysing the forward and backward reactions (i.e. that $K_p/K_a = 1$ and $V_1/V_2 = 6.04 \times 10^{-6}$), the relationship between rate and $-\Delta p$ is steep and hyperbolic. Thus the net forward velocity increases very sharply toward a maximum as $-\Delta p$ is increased beyond the point at which $-n\Delta\tilde{\mu}_{H^+} = \Delta G_p$. Contrary to this, sigmoidal and exponential relationships similar in position and form to the $K_p/K_a = 10^{-10}$, $n = 3$, relationship and its lower exponential portion are most often observed experimentally. Assuming the model of Fig. 5 to be correct, this suggests that in most systems the catalytic component is much more effective in catalysing the forward reaction than the backward reaction.

A K_p/K_a ratio of 10^{-10} assumes that the enzyme is more effective in catalysing the forward reaction by a factor of 10^{10} and that $V_1/V_2 = 6.04 \times 10^4$ when $\Delta G_p = 29.9$ kJ (7.12 kcal)/mol. A $K_p/K_a = 10^{-10}$, $n = 3$, relationship corresponds to an $n = 4$ relationship having a K_p/K_a of about $10^{-13.5}$. If it were possible to determine apparent Michaelis constants for H_{in}^+ and H_{out}^+ with systems conforming to these relationships, one could expect to

Fig. 5. Simulation of experimentally determined relationships between rate and $-\Delta p$ for ATP synthesis in chemiosmotic systems having H^+ /ATP stoichiometries (n values) of 3 (----) and 4 (—)

The relationships were simulated with the exponential form of eqn. (10), assuming $K_p/a \approx 0$, $K_{eq} = 6.04 \times 10^{-6}$, and K_p/K_a values as indicated. $-\Delta p$ in relation to $-\Delta G$ was determined according to eqn. (14), assuming ΔG_p to be 29.9 kJ (7.12 kcal)/mol. See the text for explanation and additional details.

find apparent $K_{H_{in}^+}/K_{H_{out}^+}$ values of $(10^{-10})^{-\frac{1}{3}}$ and $(10^{-13.5})^{\frac{1}{4}}$ for the $n = 3$ and $n = 4$ ATP synthase reactions respectively. Note that this mitochondrial ($n = 4$) value for $K_{H_{in}^+}/K_{H_{out}^+}$ is approximately the same as that indicated by experimentally determined respiration-rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships when it is expressed in the same manner (i.e. when it is expressed as $K_{H_{out}^+}/K_{H_{in}^+}$).

As noted above, relatively few relationships between rate and $-\Delta p$ have been determined for ATP synthesis in intact mitochondrial ($n = 4$) systems. Zoratti *et al.* [11] and Mandolino *et al.* [13] have conducted studies of this sort using mitochondria from rat liver and artichoke tubers respectively. Both yielded relationships which would correspond in both position and form to the lower exponential portion of an $n = 4$ relationship in Fig. 5 having a K_p/K_a of about $10^{-13.5}$. Presumably, the exponential forms of the observed relationships came about as a result of the maximal $-\Delta p$ which the mitochondria were capable of generating under conditions favourable for ATP synthesis (180–200 mV) not being sufficient to achieve a maximum rate comparable with V_1 in eqn. (10).

Studies of rate vs. $-\Delta p$ on $n = 3$ systems [2,4,5,12,14,15,17,19,88,89] have generally yielded relationships similar in position and form to the lower exponential portion of the $K_p/K_a = 10^{-10}$, $n = 3$, relationship of Fig. 5. In the case of chloroplast lamellar vesicles incubated under conditions of low ΔG_p , it is possible to vary the position and form of the relationship by pretreating the vesicles with a thiol such as dithiothreitol in the presence of light. For example, Junesch & Gräber [17] have shown such pretreatment to result in a leftward shift in the relationship, with no change in the maximum rate, but with a change in the form of the relationship from sigmoidal to steep and hyperbolic much as would be indicated in Fig. 5 by an increase in K_p/K_a from about 10^{-10} ($V_1/V_2 \approx 6 \times 10^4$) to about 10^{-5} ($V_1/V_2 \approx 0.6$). However, the observed shift was not this great in terms of $-\Delta p$, presumably because ΔG_p was higher than 29.9 kJ (7.12 kcal)/mol. Consistent with the model of Fig. 5, the pretreatment was observed to bring about a substantial increase in the ability of the lamellae to catalyse the uncoupled backward reaction [19]. In fact, a V_1/V_2 of about 4 is indicated by the maximal rates of ATP synthesis and hydrolysis observed with the pretreated lamellae [19].

Hangarter *et al.* [90] have reported results which suggest that thiol modulation of chloroplast lamellae does not affect the relationship between rate and $-\Delta p$ when ΔG_p is higher than about 51 kJ (12.2 kcal)/mol and that this value corresponds to the minimal $-\Delta p$ required for detectable ATP synthesis in unmodulated lamellae. These findings are in accord with earlier observations by Pick *et al.* [5] suggesting that varying ΔG_p up to 54.6 kJ (13 kcal)/mol has no effect on rate vs. $-\Delta p$ relationships in unmodulated chloroplast lamellae. A ΔG_p of 51 kJ (12.2 kcal)/mol in a chloroplast ($n = 3$) system corresponds to a $-\Delta p$ of 176 mV and suggests a rate vs. $-\Delta p$ relationship that would correspond to one in Fig. 5 having a K_p/K_a of about 10^{-10} . The model of Fig. 5 is in excellent accord with these findings in that it predicts there to be no effect on the relationship between rate and $-\Delta p$ of increasing ΔG_p up to the minimum $-\Delta p$ necessary for detectable ATP synthesis. The model indicates that increasing ΔG_p beyond this point alters the form of the relationship in the same way as does increasing K_p/K_a at constant ΔG_p (i.e. from sigmoidal to steep and hyperbolic, as was observed by Junesch and Gräber [17] to occur in chloroplast lamellae in response to thiol modulation). In accord with observations by Hangarter & Good [88], the model predicts that increasing ΔG_p to a point beyond that necessary to transform the relationship simply shifts the transformed relationship to higher $-\Delta p$.

The sigmoidicity and variability of the experimentally determined rate vs. $-\Delta\tilde{\mu}_{H^+}$ relationships obtained with chloroplast lamellae have been interpreted to indicate that there is a variable

threshold $-\Delta\tilde{\mu}_{H^+}$ at which the synthase is activated allosterically by $-\Delta\tilde{\mu}_{H^+}$ in respect to both ATP synthesis and ATP hydrolysis and that the form of the rate vs. $-\Delta\tilde{\mu}_{H^+}$ relationship reflects the degree of activation [14,17,19,32,43]. If this interpretation were entirely correct and the synthase were equally effective in catalysing the synthesis and hydrolysis of ATP, in view of the fact that the equilibrium constant of the ATP synthase reaction is approx. 10^{-5} , one might expect the forward reaction to be accompanied by a considerable backward reaction, even when the threshold $-\Delta\tilde{\mu}_{H^+}$ is high. However, studies on the various exchange reactions that can reasonably be expected to accompany simultaneous synthesis and hydrolysis clearly indicate this not to be the case [91,92]. Furthermore, one could expect simultaneous activation of the enzyme with respect to both synthesis and hydrolysis. Apparently this also is not observed, at least not always [90,93]. In view of these observations and of those by Junesch & Gräber [19], suggesting that even with thiol modulation the V_1/V_2 of the chloroplast ATP synthase reaction is higher than the equilibrium constant by a factor of more than 10^5 , a much better interpretation seems to be that the synthase is a much better catalyst of the forward reaction than the backward reaction and that its ability to catalyse the backward reaction is somewhat adjustable. This interpretation is in accord with views expressed by a number of workers [62,64–68,75,76,94] concerning the involvement of the inhibitor protein in modulating the activity of the mitochondrial ATP synthase and with the observation [17,19] that thiol modulation of the chloroplast enzyme does not affect the maximum rate of ATP synthesis despite inducing a marked shift in the rate vs. $-\Delta\tilde{\mu}_{H^+}$ relationship under conditions of low ΔG_p . As may be seen from Fig. 5, the shift can be explained by assuming that thiol modulation affects the enzyme only in respect to its ability to catalyse ATP hydrolysis.

The aforementioned lag in the approach of the ATP synthase reaction to a steady-state rate observed with chloroplast lamellar vesicles [71,72] and submitochondrial particles [73–77] can be explained on the basis of the effects of the ATPase inhibitor on the rate vs. $-\Delta\tilde{\mu}_{H^+}$ relationships that would be predicted if the model of Fig. 5 were correct and the inhibitor were to modulate the synthase slightly and gradually and only in respect to its ability to catalyse ATP hydrolysis. Thus the observed lags can be explained on this basis by assuming that the inhibitor was initially in a maximally effective state and that $-\Delta\tilde{\mu}_{H^+}$ allosterically decreased its effectiveness gradually, resulting in a gradual shift of the rate vs. $-\Delta\tilde{\mu}_{H^+}$ relationship to lower values of $-\Delta\tilde{\mu}_{H^+}$ and in a consequent increase in the rate to the maximum possible under the conditions employed. Owing to the steep dependence of the rate on $-\Delta\tilde{\mu}_{H^+}$, very little relaxation in the effectiveness of the inhibitor would be required to achieve a rate close to the maximum unless $-\Delta\tilde{\mu}_{H^+}$ were unusually low. In the case of lags observed with unmodulated chloroplast lamellae, slight relaxation in the effectiveness of the inhibitor is clearly evident from the fact that $-\Delta\tilde{\mu}_{H^+}$ generation elicits very little ATPase activity in this system. The observation [75,76] that activation of submitochondrial particles in respect to ATP hydrolysis continues long after the lag period for ATP synthesis is consistent with gradual $-\Delta\tilde{\mu}_{H^+}$ -induced relaxation of the effectiveness of the inhibitor and with the need for only a slight relaxation to achieve a maximal rate of ATP synthesis. It is also consistent with the interpretation [64,75,76] that the enzyme is affected only with respect to its ability to catalyse ATP hydrolysis.

The model of Fig. 5 does not specify the mechanism by which H^+ -ATP synthases favour the forward reaction over the backward reaction. In this regard, Feldman & Sigman [95] and Grubmeyer *et al.* [96], working with the isolated F_1 components of the chloroplast and mitochondrial enzymes respectively, have

shown that ATP binds at a catalytic site much more tightly than do ADP and P_i , and that in consequence the equilibrium constant of the ATP synthase reaction on the enzyme is close to unity. Similar findings have been made with de-energized chloroplast lamellae [97] and submitochondrial particles [98]. These observations are clearly in accord with the interpretation that the synthase strongly favours the forward reaction.

Presumably, the ATPase inhibitor functions by adjusting the affinity of the enzyme for reactants and products in response to fluctuations in $-\Delta\tilde{\mu}_{H^+}$. Of course its purpose very likely is to minimize wasteful hydrolysis of ATP and thereby to make possible the maintenance of a high ΔG_p . The common observations that large changes in the rate of ATP synthesis occur over very narrow ranges of $-\Delta\tilde{\mu}_{H^+}$ and that the threshold $-\Delta\tilde{\mu}_{H^+}$ for ATP synthesis in unmodulated systems is maintained close to the maximum $-\Delta\tilde{\mu}_{H^+}$ that the system is capable of generating, regardless of whether ΔG_p is high or low, suggest that the inhibitor can and does fulfil this purpose when $-\Delta\tilde{\mu}_{H^+}$ is high as well as when it is low.

CONCLUSION

This study demonstrates that the major features of the rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships ordinarily observed in experimental studies on complex chemiosmotic reactions can be simulated using simple relationships of equilibrium thermodynamics and saturation kinetics. The most perplexing feature of the rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships has been the association of large changes in rate with small changes in $\Delta\tilde{\mu}_{H^+}$ at high values of $\Delta\tilde{\mu}_{H^+}$. This feature is easily explained in view of the sigmoidal nature of the rate vs. driving-force relationships outlined in this study, and is particularly noticeable in rate vs. $\Delta\tilde{\mu}_{H^+}$ relationships of chemiosmotic reactions, because changes in driving force in these reactions correspond to much smaller changes in $\Delta\tilde{\mu}_{H^+}$ as a result of several equivalents of H^+ being transported per mol of other reactants undergoing transformation in the same reaction. As is particularly evident from the observed relationships between respiration rate and $\Delta\tilde{\mu}_{H^+}$, an important consequence of this feature is that it permits cells to vary their activity over a wide range while maintaining the high and constant ΔG_p necessary for homeostasis [21,33]. The corollary feature of large changes in driving force being associated with small changes in rate at very high driving forces is also important, and probably accounts in large part for the remarkable success and widespread applicability of the initial-velocity method of determining kinetic parameters.

I thank Dr. John Rieske for helpful discussions and Nancy Sally for drawing the figures. This work was supported in part by the Stoner Research Fund of the Ohio State University Development Fund.

REFERENCES

- Schwartz, M. (1968) *Nature* (London) **219**, 915–919
- Schuldinger, S., Rottenberg, H. & Avron, M. (1973) *Eur. J. Biochem.* **39**, 455–462
- Portis, A. R. & McCarty, R. E. (1974) *J. Biol. Chem.* **249**, 6250–6254
- Thayer, W. S. & Hinkle, P. C. (1974) *J. Biol. Chem.* **250**, 5336–5342
- Pick, U., Rottenberg, H. & Avron, M. (1974) *FEBS Lett.* **48**, 32–36
- Gräber, P. & Witt, H. T. (1976) *Biochim. Biophys. Acta* **423**, 141–163
- Sone, N., Yoshida, M., Hirata, H. & Kagawa, Y. (1977) *J. Biol. Chem.* **252**, 2956–2960
- Rumberg, B. & Heinze, T. (1980) *Ber. Bunsen-Ges. Phys. Chem.* **84**, 1055–1059
- Sorgato, M. C., Branca, D. & Ferguson, S. J. (1980) *Biochem. J.* **188**, 945–948
- Takabe, T. & Hammes, G. C. (1981) *Biochemistry* **20**, 6859–6864
- Zoratti, M., Pietrobon, D. & Azzone, G. F. (1982) *Eur. J. Biochem.* **126**, 443–451
- Clark, A. J., Cotton, N. P. J. & Jackson, J. B. (1983) *Biochim. Biophys. Acta* **723**, 440–453
- Mandolino, G., DeSantis, A. & Melandri, B. A. (1983) *Biochim. Biophys. Acta* **723**, 428–439
- Gräber, P., Junesch, U. & Schatz, G. H. (1984) *Ber. Bunsen-Ges. Phys. Chem.* **88**, 599–608
- Bokranz, E., Mörschel, E. & Kröger, A. (1985) *Biochim. Biophys. Acta* **810**, 332–339
- Sigalat, C., Haraux, F., DeKouchkovsky, F., Hung, S. P. N. & DeKouchkovsky, Y. (1985) *Biochim. Biophys. Acta* **809**, 403–413
- Junesch, U. & Gräber, P. (1985) *Biochim. Biophys. Acta* **809**, 429–434
- Davenport, J. W. & McCarty, R. E. (1986) *Biochim. Biophys. Acta* **851**, 136–145
- Junesch, U. & Gräber, P. (1987) *Biochim. Biophys. Acta* **893**, 275–288
- Matsuno-Yagi, A. & Hatefi, Y. (1987) *J. Biol. Chem.* **262**, 14158–14163
- Nicholls, D. G. (1974) *Eur. J. Biochem.* **49**, 573–583
- Nicholls, D. G. & Bernson, S. M. (1977) *Eur. J. Biochem.* **75**, 601–612
- Johnson, R. N. & Hansford, R. G. (1977) *Biochem. J.* **164**, 305–322
- Azzone, G. F., Pozzan, T., Massari, S. & Bragadin, M. (1978) *Biochim. Biophys. Acta* **501**, 296–306
- Küster, U., Letko, G., Kunz, W., Duszynski, J., Bogucka, K. & Wojtczak, L. (1981) *Biochim. Biophys. Acta* **636**, 32–38
- Wilson, D. F. & Forman, N. (1982) *Biochemistry* **21**, 1438–1444
- Duszynski, J., Bogucka, K. & Wojtczak, L. (1984) *Biochim. Biophys. Acta* **767**, 540–547
- Diolez, P. & Moreau, F. (1985) *Biochim. Biophys. Acta* **806**, 56–63
- Murphy, M. P. & Brand, M. D. (1987) *Biochem. J.* **243**, 499–505
- O'Shea, P. S. & Chappell, J. B. (1984) *Biochem. J.* **219**, 401–404
- Ferguson, S. J. & Sorgato, M. C. (1982) *Annu. Rev. Biochem.* **51**, 185–217
- Schlödter, E., Gräber, P. & Witt, H. T. (1982) in *Electron Transport and Photophosphorylation* (Barber, J., ed.), pp. 105–175, Elsevier, Amsterdam
- Ferguson, S. J. (1985) *Biochim. Biophys. Acta* **811**, 47–95
- Haraux, F. (1985) *Physiol. Vég.* **23**, 397–410
- Slater, E. C. (1987) *Eur. J. Biochem.* **166**, 489–504
- Matsuno-Yagi, A. & Hatefi, Y. (1988) *J. Bioenerg. Biomembr.* **20**, 481–502
- Woelders, H., Putters, J. & Van Dam, K. (1986) *FEBS Lett.* **204**, 17–21
- Woelders, H., Van Der Velden, T. & Van Dam, K. (1988) *Biochim. Biophys. Acta* **934**, 123–134
- Davis, E. J. & Davis-van Thienen, W. I. A. (1989) *Arch. Biochem. Biophys.* **275**, 449–458
- Pietrobon, D. & Caplan, S. R. (1985) *Biochemistry* **24**, 5764–5776
- Pietrobon, D. (1986) *Bioelectrochem. Bioenerg.* **15**, 193–209
- Hill, T. (1977) *Free Energy Transduction in Biology*, pp. 1–32, Academic Press, New York
- Mills, J. D. & Mitchell, P. (1984) *Biochim. Biophys. Acta* **764**, 93–104
- Caldwell, P. C. (1969) *Curr. Top. Bioenerg.* **3**, 251–278
- Wilson, D. F. (1980) *Biochim. Biophys. Acta* **616**, 371–380
- DeDonder, T. & Van Rysseberghe, P. (1936) *Photodynamic Theory of Affinity*, Stanford University Press, Menlo Park, CA
- Zemansky, M. W. (1974) *J. Chem. Educ.* **51**, 572–576
- Welch, G. R. (1985) *J. Theor. Biol.* **114**, 433–446
- Lewis, G. N. & Randall, M. (1961) *Thermodynamics*, 2nd edn., pp. 10–14, McGraw-Hill, New York
- Van der Meer, R., Westerhoff, H. V. & Van Dam, K. (1980) *Biochim. Biophys. Acta* **591**, 488–493
- Van Dam, K. & Westerhoff, H. V. (1984) in *Bioenergetics* (Ernster, L., ed.), pp. 1–27, Elsevier, Amsterdam
- Westerhoff, H. V. & Van Dam, K. (1987) *Thermodynamics and Control of Biological Free-Energy Transduction*, pp. 228–256, Elsevier, Amsterdam
- Wilkie, D. R. (1960) *Prog. Biophys.* **10**, 259–298
- Haldane, J. B. S. (1930) *Enzymes*, Longmans Green, London
- Briggs, G. E. & Haldane, J. B. S. (1925) *Biochem. J.* **19**, 338–339
- Rottenberg, H. (1973) *Biophys. J.* **13**, 503–511
- Cleland, W. W. (1970) *Enzymes* 3rd Ed. **2**, 1–65

58. Schlodder, E., Rögner, M. & Witt, H. T. (1982) *FEBS Lett.* **138**, 13–18
59. Hearon, J. Z. (1952) *Physiol. Rev.* **32**, 499–523
60. Hearon, J. Z., Bernhard, S. A., Fries, S. L., Botts, D. J. & Morales, M. F. (1959) *Enzymes* 2nd Ed. **1**, 49–142
61. Shavit, N. (1980) *Annu. Rev. Biochem.* **49**, 111–138
62. Pedersen, P. L., Schwerzmann, K. & Citrón, N. (1981) *Curr. Top. Bioenerg.* **11**, 149–199
63. Strotmann, H. & Bickel-Sandkötter, S. (1984) *Annu. Rev. Plant Physiol.* **35**, 97–120
64. Schwerzmann, K. & Pedersen, P. L. (1986) *Arch. Biochem. Biophys.* **250**, 1–18
65. Tuena de Gómez-Puyou, M., Martins, O. B. & Gómez-Puyou, A. (1988) *Biochem. Cell Biol.* **66**, 677–682
66. Hashimoto, T., Yoshida, Y. & Tagawa, K. (1990) *J. Bioenerg. Biomembr.* **22**, 27–37
67. Pullman, M. E. & Monroy, G. C. (1963) *J. Biol. Chem.* **238**, 3762–3769
68. Asami, K., Juntti, K. & Ernster, L. (1970) *Biochim. Biophys. Acta* **205**, 307–311
69. Avron, M. (1962) *J. Biol. Chem.* **237**, 2011–2017
70. Van de Stadt, R. J., de Boer, B. L. & Van Dam, K. (1973) *Biochim. Biophys. Acta* **292**, 338–349
71. Harris, D. A. & Crofts, A. (1978) *Biochim. Biophys. Acta* **502**, 87–102
72. Kahn, J. (1962) *Arch. Biochem. Biophys.* **98**, 100–103
73. Gómez-Puyou, A., Tuena de Gómez-Puyou, M. & Ernster, L. (1979) *Biochim. Biophys. Acta* **547**, 252–257
74. Harris, D. A., Von Tscharn, V. & Radda, G. K. (1979) *Biochim. Biophys. Acta* **548**, 72–84
75. Schwerzmann, K. & Pedersen, P. L. (1981) *Biochemistry* **20**, 6305–6311
76. Klein, G. & Vignais, P. V. (1983) *J. Bioenerg. Biomembr.* **15**, 347–362
77. Lippe, G., Sorgato, M. C. & Harris, D. A. (1988) *Biochim. Biophys. Acta* **933**, 1–11
78. Lohse, D., Thelen, R. & Strotmann, H. (1989) *Biochim. Biophys. Acta* **976**, 85–95
79. Lippe, G., Sorgato, M. C. & Harris, D. A. (1988) *Biochim. Biophys. Acta* **933**, 12–21
80. Rosing, J. & Slater, E. C. (1972) *Biochim. Biophys. Acta* **376**, 446–457
81. Stoner, C. D. & Sirak, H. D. (1979) *J. Bioenerg. Biomembr.* **11**, 113–146
82. Nicholls, D. G. & Lindberg, O. (1972) *FEBS Lett.* **25**, 61–64
83. Stoner, C. D. (1985) *J. Bioenerg. Biomembr.* **17**, 85–108
84. Stoner, C. D. (1987) *J. Biol. Chem.* **262**, 10445–10453
85. Hoff-Jorgensen, E. (1938) *Skand. Arch. Physiol.* **80**, 176–192
86. Clark, W. M. (1960) *Oxidation-Reduction Potentials of Organic Systems*, Williams and Wilkins, Baltimore
87. Bienfait, H. F., Jacobs, J. M. C. & Slater, E. C. (1975) *Biochim. Biophys. Acta* **376**, 446–457
88. Hangarter, R. & Good, N. E. (1982) *Biochim. Biophys. Acta* **681**, 397–404
89. Turina, P., Melandri, B. A. & Gräber, P. (1991) *Eur. J. Biochem.* **196**, 225–229
90. Hangarter, R. P., Grandoni, P. & Ort, D. R. (1987) *J. Biol. Chem.* **262**, 13513–13519
91. Jagendorf, A. (1975) in *Bioenergetics of Photosynthesis* (Govindjee, ed.), pp. 413–492, Academic Press, New York
92. Carmeli, C. (1977) in *Encyclopedia of Plant Physiology—Photosynthesis I* (Trebst, A. & Avron, M., eds.), vol. 5, pp. 492–500, Springer Verlag, Berlin
93. Biauudet, P. & Haraux, F. (1986) *Bioelectrochem. Bioenerg.* **16**, 111–123
94. Citrón, N. M., Hullihen, J., Schwerzmann, K. & Pedersen, P. L. (1982) *Biochemistry* **21**, 1878–1885
95. Feldman, R. I. & Sigman, D. S. (1982) *J. Biol. Chem.* **257**, 1676–1683
96. Grubmeyer, C., Cross, R. L. & Penefsky, H. S. (1982) *J. Biol. Chem.* **257**, 12092–12100
97. Feldman, R. I. & Sigman, D. S. (1983) *J. Biol. Chem.* **258**, 12178–12183
98. Penefsky, H. S. (1985) *J. Biol. Chem.* **260**, 13728–13734

Received 3 April 1991/7 August 1991; accepted 3 September 1991