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Changes in the apparent Michaelis constant for ADP during photophosphorylation are consistent with delocalised chemiosmotic energy coupling

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The apparent Michaelis constant (K_m) for ADP has been measured under various conditions of steady-state photophosphorylation in isolated thylakoid membranes. In addition, the steady-state ΔpH has been simultaneously estimated from the fluorescence quenching of 9-aminoacridine. The following results were obtained. (1) The standard procedure for estimating K_m, by increasing the concentration of ADP, progressively lowered the steady-state ΔpH, thereby introducing an uncontrolled system variable into the K_m analysis. This has the effect of lowering the apparent K_m measured. (2) Lowering the light intensity lowered the observed K_m, and addition of uncouplers increased the observed K_m. The ability of uncouplers to increase K_m was enhanced at lowered light intensities. In contrast, the effect of lowered light intensity on the observed K_m was diminished and then reversed under progressively more uncoupled conditions. (3) The addition of energy-transfer inhibitors caused an increase in the observed K_m for ADP. (4) All of the observations are qualitatively predicted by a mathematical model based on simple delocalised chemiosmotic energy coupling and Michaelis-Menten kinetics for the chloroplast ATPase with respect to ADP. It is concluded that the complex behaviour of the apparent K_m for ADP under different conditions arises because ΔpH is an uncontrolled variable during the K_m analysis and that the results are entirely consistent with a model of delocalised chemiosmotic energy coupling.

Introduction

The observed affinity of the chloroplastic ATPase (CF_0-CF_1) for its substrate ADP in photophosphorylation has been shown to vary depending on the experimental conditions [1–3]. The apparent Michaelis-Menten constant (K_m) of CF_0-CF_1 for ADP was shown to decrease when the rate of ATP synthesis was lowered by reducing the light intensity [1–3] or by adding an electron-transport inhibitor [2]. When the rate of ATP synthesis was lowered by adding an uncoupler the apparent K_m was observed to increase [1,3]. The latter result has also been observed in submitochondrial particles [4,5].

If energy coupling occurs by a simple chemiosmotic mechanism [6], both uncouplers and a decrease in the rate of electron transport would be

Abbreviations: CF_0-CF_1, chloroplastic ATPase, $\Delta\mu_{H^+}$, difference in the electrochemical potential for protons between the intrathylakoid space and the outer aqueous medium; ΔpH, difference in pH between the intrathylakoid space and the outer aqueous medium; Mes, 4-morpholineethanesulphonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; P_i, inorganic phosphate.

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expected to decrease the maximum rate of photophosphorylation by lowering the difference in electrochemical potential for protons ($\Delta\mu_{H^+}$) across the thylakoid membrane. The opposing effects of these inhibitory treatments on the apparent K_m are not an obvious consequence of delocalised chemiosmosis and have been interpreted in more complex ways. Since the direction of the K_m changes appear to correlate with the overall rate of electron transport, two authors suggested that there existed more direct links than ΔpH between ATPase and electron-transport complexes [1,3]. Alternatively, Bickel-Sandkotter and Strotmann [2] suggested that the decrease in K_m associated with inhibition of electron transport could be explained by an activation of CF_0-CF_1 by $\Delta\mu_{H^+}$, though no explanation was offered for the effects of uncouplers. In contrast to this view, the effects of uncouplers in submitochondrial particles has also been used to support a $\Delta\mu_{H^+}$-dependent activation of ATP synthesis [4]. More recently and in contrast to other reports Davenport and McCarty [7] have measured an increase in the apparent K_m for ADP by CF_0-CF_1 at low uncoupler concentrations but this was followed by a decrease in the apparent K_m as the uncoupler concentration was increased further. It is difficult to reconcile these data with those reported from other laboratories.

The conflicting interpretation of results may be explained by the failure to measure or account for changes in $\Delta\mu_{H^+}$ that occur during the K_m analysis. We have repeated the analysis of the apparent K_m for ADP under various conditions but have incorporated simultaneous estimation of the ΔpH. Our results are in broad agreement with other reports but by taking into account the ΔpH changes that occurred during the K_m analysis the results can be explained by a delocalised chemiosmotic hypothesis. Data obtained from a simple mathematical model described previously [8–10] which allows for variations in ΔpH during the K_m determination show results in close agreement with those obtained experimentally.

Materials and Methods

Experimental

Intact chloroplasts were isolated from *Pisum sativum* (variety Feltham First) according to a method described previously [11] except the chloroplasts were lysed in a medium containing 5 mM $MgCl_2$, 5 mM ascorbic acid and 20 mM Mes-KOH buffer (pH 6.5) and then recentrifuged prior to the final resuspension. This facilitated the removal of any ADP present in the chloroplast stroma and any thioredoxin which could cause thiol reduction of CF_0-CF_1 during the experiment. Thylakoid membranes were prepared from dark-adapted plants and the experiments were generally complete within 3 h of preparation. A loss of ATPase activity was observed over the period of the experiment but this was typcially less than 20%. This was compensated for, where necessary, by averaging duplicate experiments carried out in reverse order.

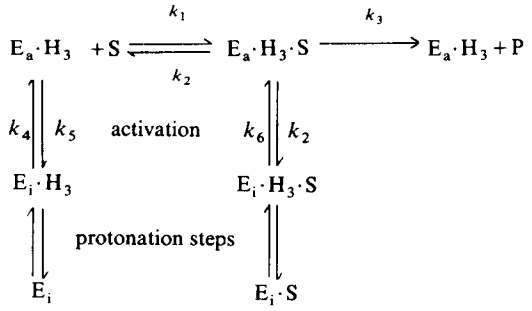
The rate of ATP synthesis and the magnitude of the transthylakoid ΔpH were measured simultaneously from thylakoids suspended in a medium containing 20 mM KCl, 5 mM $MgCl_2$, 1 mM P_i, 0.1 mM methyl viologen, 15 mM glucose, 10 units/ml hexokinase, 2.5 μg/ml diadenosine pentaphosphate, 20 μM 9 aminoacridine, 1000 units/ml catalase, 0.4 μCi $^{32}P_i$ (Amersham International) and 30 mM Tricine-KOH (pH 8.1) in an oxygen electrode maintained at 20 °C. The concentrations of ADP used to determine the apparent K_m were 3, 5, 8, 12, 18 and 25 μM, unless otherwise indicated. The ADP concentration was maintained during the assay by enzymic recycling; the concentration of ADP under steady-state phosphorylating conditions as determined by the luciferin-luciferase technique was always within 10% of the initial ADP concentration.

Illumination was provided by a halogen lamp filtered through an RG665 red cut-off filter. ΔpH was estimated using 9-aminoacridine fluorescence and was measured as described previously [11]. Photophosphorylation was initiated by illumination and terminated after 2 min by the addition of 200 μl of 20% trichloroacetic acid to 1 ml of assay medium containing 15 μg chlorophyll. The rate of ATP synthesis was determined by $^{32}P_i$ incorporation into glucose 6-phosphate as described previously [11]. The rate of ATP synthesis was varied by (a) reducing the light intensity with neutral density filters, (b) the addition of the uncoupler nigericin or (c) the addition of the energy-transfer

inhibitor tentoxin (Sigma chemical company); concentrations as indicated. Partial inhibition of the ATPase using tentoxin was difficult to achieve due to the slow inhibition kinetics especially at low tentoxin concentrations. Maximum inhibition required preincubation times exceeding 1 h. For tentoxin inhibition experiments the thylakoids were incubated in the assay medium for 20 min at 20 °C with the appropriate tentoxin concentration. Although inhibition may not have been saturated by this time there was a less than 3% change in rate of ATP synthesis over the 2 min assay period.

Theoretical

Theoretical data was calculated from a mathematical model designed to simulate photophosphorylation using simple pathways of proton flux. Michaelis-Menten type kinetics were assumed for CF_0-CF_1 with respect to ADP except that either the unbound enzyme ($E_a \cdot H_3$) or the enzyme-substrate complex ($E_a \cdot H_3 \cdot S$) could undergo $\Delta\mu_{H^+}$-dependent activation as shown in Scheme I and explained further in the discussion.



Scheme I. Kinetic scheme for CF_0-CF_1-catalysed ATP synthesis.

The external pH (H^+ out) was maintained as a constant and the internal pH was varied until the flux equations (Eqns. 1–3) satisfied Eqn. 4; this was solved by a computer iteration technique.

$$J_{H^+_{in}} = V_e \frac{K_a}{K_a + [H^+_{in}]} \tag{1}$$

$$J_{H^+_{out(P)}} = p \cdot [E_a \cdot H_3 \cdot S] \cdot k_3 \tag{2}$$

$$J_{H^+_{out(nP)}} = H^+_{in} \cdot C \tag{3}$$

$$J_{H^+_{in}} = J_{H^+_{out(P)}} + J_{H^+_{out(nP)}} \tag{4}$$

where

$$[E_a \cdot H_3 \cdot S] \cdot k_3 = v = \frac{V_P}{1 + \frac{k_4}{k_5}\left\{1 + \frac{K_{p1}}{[H^+_{in}]} \cdot \frac{[H^+_{out}]}{K_{n1}}\right\}^3 \cdot \frac{K_m}{[S]} + 1 + \frac{k_6}{k_7}\left\{1 + \frac{K_{p2}}{[H^+_{in}]} \cdot \frac{[H^+_{out}]}{K_{n2}}\right\}^3}$$

$K_m = \frac{k_1 + k_3}{k_2} =$ Michaelis-Menten constant, set at 12 μM

$KD_1 = \frac{k_4}{k_5} =$ equilibrium constant for activation of $E_i \cdot H_3$

$KD_2 = \frac{k_6}{k_7} =$ equilibrium constant for activation of $E_i \cdot H_3 \cdot S$

$[S] =$	concentration of substrate (ADP) in μM, value as indicated in text
$V_e =$	maximum rate of uncoupled electron-transport-drive proton translocation
$K_a =$	acid dissociation constant of an internal group controlling electron transport, set at $3 \cdot 10^{-5}$ M
$[H^+_{out}] =$	external proton concentration, set at $1 \cdot 10^{-8}$ M
$[H^+_{in}] =$	internal proton concentration calculated such that Eqn. 4 is satisfied
$J_{H^+_{in}} =$	the inward flux of protons due to electron transport
$J_{H^+_{out}(P)} =$	the outward flux of protons due to phosphorylation
$J_{H^+_{out}(nP)} =$	the outward flux of protons due to membrane leakage
K_{p1} and $K_{p2} =$	$1 \cdot 10^{-5}$ M, internal dissociation constant of CF_0-CF_1, see Ref. 10 for discussion
K_{n1} and $K_{n2} =$	$1 \cdot 10^{-8}$ M, external dissociation constant of CF_0-CF_1, see Ref. 10 for discussion
$C =$	passive permeability constant for diffusion of protons across thylakoid membrane; value set as indicated in text
$V_p =$	maximum rate of ATP synthesis; value set as indicated in text
$p =$	H^+/ATP ratio, set at 3

All proton fluxes are expressed as μmol per mg chlorophyll per h. Constants used in the model were manipulated in order to simulate experimental conditions; changing the rate of electron transport was achieved by altering the value of V_e, the extent of uncoupling was varied by altering the value of C and the extent of energy-transfer inhibition was simulated by varying the value of V_p.

Fig. 1 shows how the rate of photophosphorylation varied with the substrate (ADP) concentration calculated from the mathematical model. The data are shown as Lineweaver-Burk plots to permit easy determination of the apparent K_m and V values and to aid comparison of the data with previously published results [1–3]. The data approximate to straight lines for a large range of ADP concentrations. However, there is a slight non-linearity at high rates of photophosphorylation. The non-linearity was only generated when V_e and V_p were large and C was small. Data obtained experimentally were not sufficiently accurate to detect such a proposed deviation from Michaelis-menten kinetics. In order to counteract the effect of this deviation on the comparison of theoretical and experimental results, the values for K_m and V obtained from the model were calculated from substrate concentrations used experimentally, and a straight line was fitted to the data by a linear-regression technique.

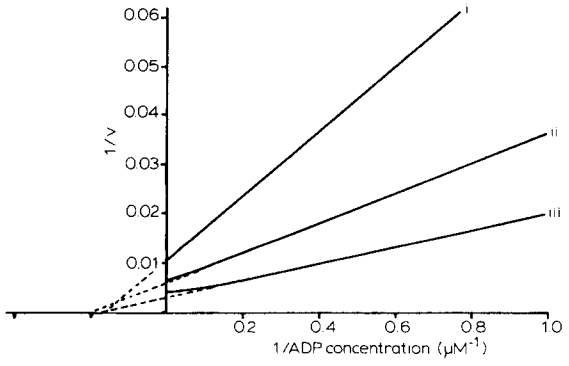


Fig. 1. Lineweaver-Burk plots of the rate of phosphorylation vs. ADP concentration calculated from a computer model for three values of C: $2 \cdot 10^7$, $1 \cdot 10^8$ and $2 \cdot 10^8$. V_p and V_e were constant at 2500 and 1000, respectively. Other model parameters are given in Materials and Methods. $v = \mu$mol ATP produced per mg Chl per h.

Results

Fig. 2 shows how the measured rate of photophosphorylation varied with substrate (ADP) concentration for a variety of experimental conditions. For all the experimental conditions examined the data are linear on a Lineweaver-Burk plot in agreement with other reports [1–3,5]. The straight line was fitted using linear regression and the correlation coefficient was generally better than 0.985. Fig. 3 shows a similar series of graphs to Fig. 2, except that the data were calculated from a mathematical model where the values of C, V_e and V_p were manipulated to simulate the experimental conditions used in Fig. 2. The model data also show a good approximation to a straight line;

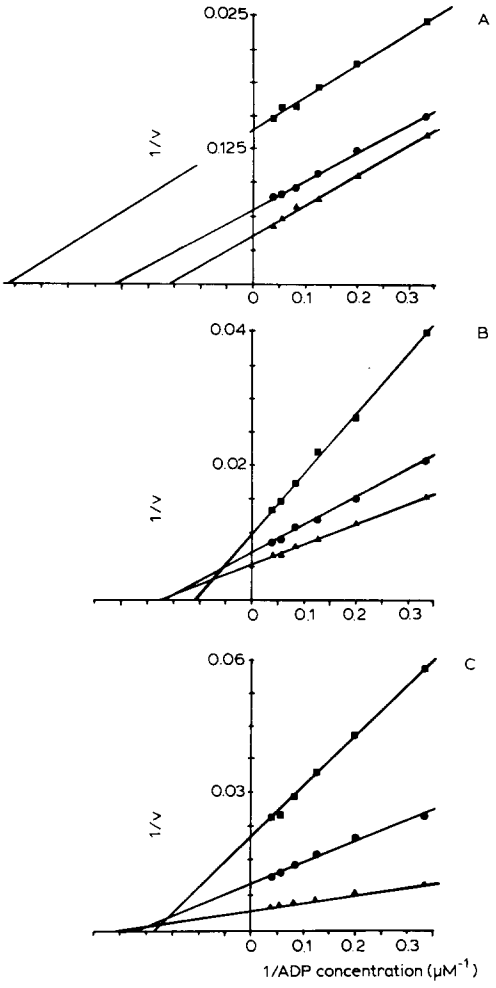


Fig. 2. Lineweaver-Burk plots of the rate of photophosphorylation vs. the ADP concentration obtained experimentally. The rate of photophosphorylation was reduced by: (A) lowering the light intensity to (▲) 240 $W \cdot m^{-2}$, (●) 53 $W \cdot m^{-2}$ or (■) 29 $W \cdot m^{-2}$; (B) increasing the uncoupler concentration to (▲) 0 nM nigericin, (●) 60 nM nigericin or (■) 90 nM nigericin; (C) increasing the energy-transfer inhibitor concentration to (▲) 0 nM tentoxin, (●) 96 nM tentoxin or (■) 240 nM tentoxin. v = μmol ATP produced per mg Chl per h.

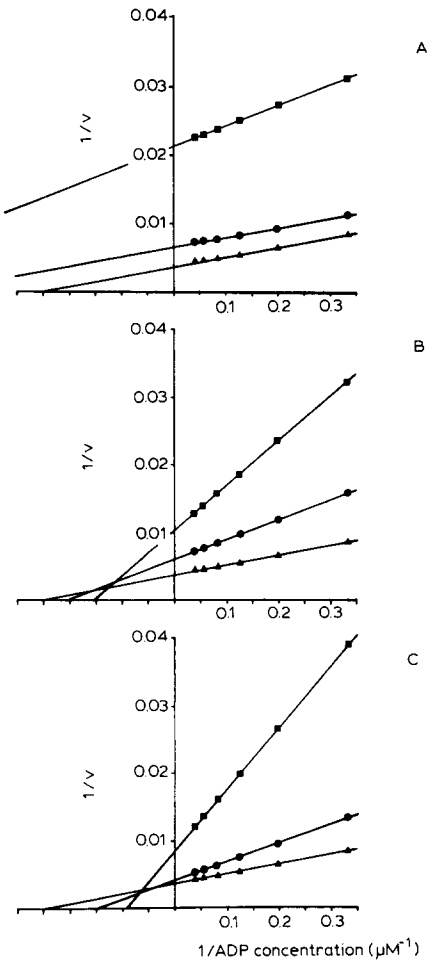


Fig. 3. Lineweaver-Burk plots calculated from a computer model for an identical range of substrate concentrations as those used experimentally in Fig. 2. (A) Lowering light intensity was simulated by reducing V_e to 1000 (▲), 600 (●) or 200 (■); (B) increasing uncoupler concentration was simulated by increasing C to $2 \cdot 10^7$ (▲), $1 \cdot 10^8$ (●) or $2 \cdot 10^8$ (■); (C) increasing energy-transfer inhibitor concentration was simulated by decreasing V_p to 2500 (▲), 1500 (●) or 500 (■), v = μmol ATP per mg Chl per h.

correlation coefficients were greater than 0.99. The data in Fig. 3 show close similarity to the data in Fig. 2; changes in light intensity (Fig. 2A) or changes in V_e (Fig. 3A) lead to a series of roughly parallel lines, indicating that both the apparent K_m and V values were changing in a similar direction. In Fig 2B and C, where the concentrations of nigericin and tentoxin were varied, and in Fig. 3B and C, where the values of C and V_p were varied, a change in slope was observed for both experimental and theoretical data.

The magnitude of the ΔpH component of $\Delta\mu_{H^+}$ maintained across the thylakoid during these experiments was estimated simultaneously by the 9-aminoacridine technique. This method has been questioned as a quantitative measurement and is known to overestimate ΔpH [12,13]. However, the data will only be interpreted in qualitative terms

and no attempt has been made to correct the estimates in any way.

Fig. 4 shows that in general the magnitude of ΔpH was observed to decrease as the ADP concentration increased. However, the extent of this decrease was dependent on the prevailing conditions. When the rate of photophosphorylation was reduced by lowering the light intensity (Fig. 4A) the average ΔpH was reduced but the decrease in ΔpH observed as the ADP concentration increased was largely unaffected. Lowering the rate of photophosphorylation by increasing the nigericin concentration (Fig. 4B) not only reduced the overall ΔpH value, but also the extent of change in ΔpH due to increasing the ADP concentration. Increasing the tentoxin concentration (Fig. 4C) to reduce the rate of photophosphorylation increased the average magnitude of the ΔpH and also decreased the change in the ΔpH due to an increase in the ADP concentration. In Fig. 5 ΔpH values were calculated from a mathematical model for an identical range of ADP concentrations to those

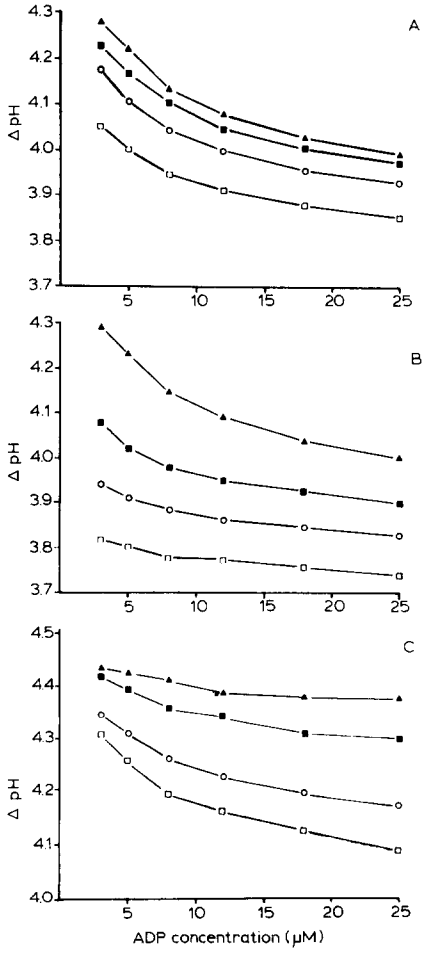


Fig. 4. The magnitude of the transthylakoid ΔpH, measured during steady-state rates of photophosphorylation for a range of ADP concentrations, varied under; (A) different light intensities of (▲) 240 $W \cdot m^{-2}$, (■) 110 $W \cdot m^{-2}$, (○) 53 $W \cdot m^{-2}$ or (□) 29 $W \cdot m^{-2}$; (B) different uncoupler concentrations of (▲) 0 nM, (■) 30 nM, (○) 60 nM or (□) 90 nM nigericin; (C) different energy-transfer inhibitor concentrations of (□) 0 nM, (○) 48 nM, (■) 144 nM or (▲) 240 nM tentoxin.

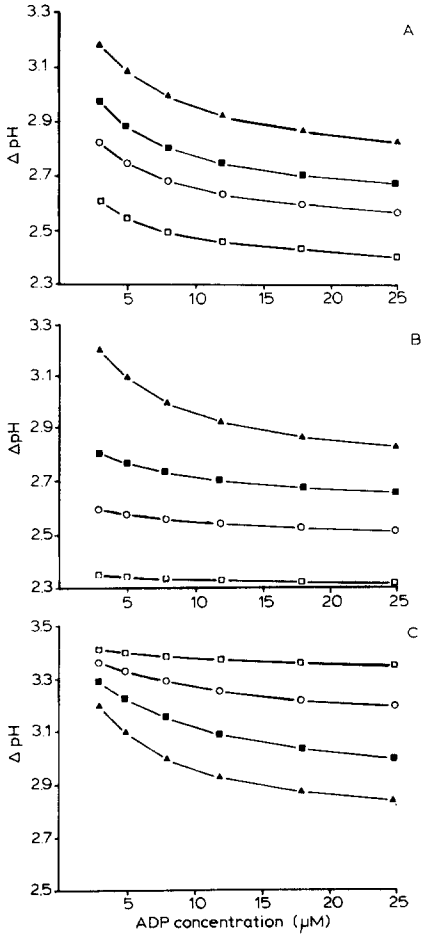


Fig. 5. The calculated ΔpH values ($\log([H^+_{out}]/[H^+_{in}])$) from the computer model for a range of ADP concentrations under various conditions: (A) $V_p = 2500$, $C = 2 \cdot 10^7$, $V_e = 200$ (▲), 400 (■), 600 (○) or 1000 (□). (B) $V_e = 1000$, $V_p = 2500$, $C = 2 \cdot 10^7$ (▲), $1 \cdot 10^8$ (■), $2 \cdot 10^8$ (○) or $4 \cdot 10^8$ (□). (C) $V_e = 1000$, $C = 2 \cdot 10^7$, $V_p = 250$ (□), 750 (○), 1500 (■) or 2500 (▲).

eused in Fig. 4. Only the value of V_e (Fig. 5A), C (Fig. 5B) or V_p (Fig. 5C) was manipulated in order to simulate the experimental conditions used in Fig. 4. All other values remained as given earlier. The theoretical data show similar trends to those observed experimentally; lowering the V_e value lowered the average ΔpH value but the change in ΔpH due to ADP was largely unaffected; increasing the value of C also reduced the overall ΔpH but the effect of increasing the ADP concentration on the ΔpH was reduced; decreasing the value of V_p increased the average ΔpH value and also decreased the effect of increasing the ADP concentration on ΔpH.

The value of the apparent K_m and V determined either experimentally (A) or calculated from a mathematical model (B) are shown in Fig. 6 for a variety of experimental or simulated experimental conditions. In general the data show that reducing the light intensity (Fig. 6A) or V_e (Fig. 6B) leads to a decrease in the apparent K_m for ADP by CF_0-CF_1 and increasing the uncoupler (nigericin) concentration (Fig. 6A) or the value of C (Fig. 6B) leads to an increase in the apparent K_m for ADP by CF_0-CF_1. These relationships are not strictly linear and this is emphasised when combinations of intermediate light intensity or V_e and intermediate uncoupler concentrations or C are used. As shown in Fig. 6A, when thylakoids are partially uncoupled, lowering the light intensity no longer causes the K_m to be decreased at low light intensities, and indeed, when the uncoupler concentration is high, lowering the light intensity increases the K_m over the entire light-intensity range. Conversely, at constant but non-saturating light intensities the ability of uncouplers to increase the apparent K_m is enhanced. A similar pattern of trends is shown in the simulated data obtained by modelling (Fig. 6B).

The data shown so far are broadly in agreement with other published results. However, modeling shows that the results are entirely consistent with a simple delocalized coupling mechanism once it is appreciated that ΔpH is a system variable that changes during the K_m analysis. In order to test these ideas further we investigated the prediction of the model that energy-transfer inhibitors should lower the observed V but increase the K_m (ADP).

The effect of energy-transfer inhibitors on the

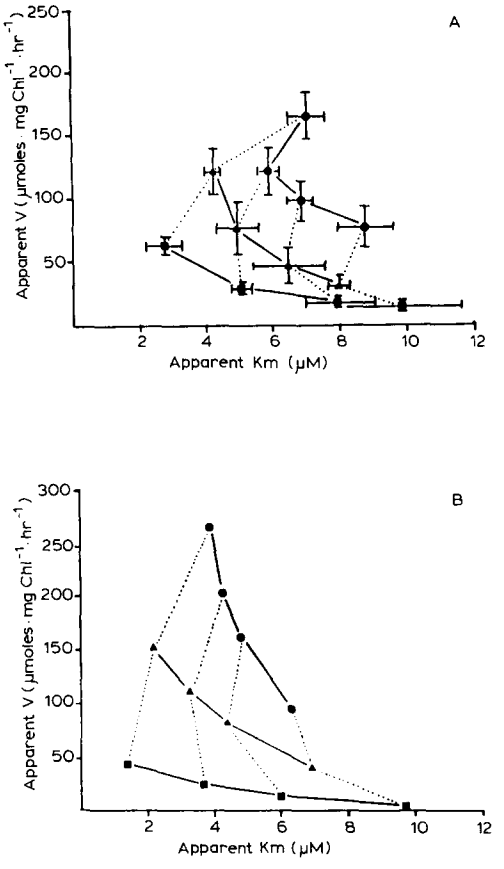


Fig. 6. Relationship between V and K_m at different light intensities and uncoupler concentrations experiments (A) and calculated from a mathematical model (B). Solid lines connect points of equal light intensity (A) or V_e (B). Dotted lines connect point of equal uncoupler (nigericin) concentration (A) or C (B). In (A) the light intensities were 240 $W \cdot m^{-2}$ (●), 53 $W \cdot m^{-2}$ (▲), 29 $W \cdot m^{-2}$ (■) and the uncoupler concentrations were 0, 30, 60, 90 nM nigericin from left to right, respectively. Standard error bars are shown and were calculated from three or more replicates. In (B) the values of V_e were 1000 (●), 400 (▲), 200 (■) and the values of C were $2 \cdot 10^7$, $6 \cdot 10^7$, $1 \cdot 10^8$ and $2 \cdot 10^8$ respectively, from left to right. V_p was kept constant at 2500.

apparent K_m and V is shown experimentally in Fig. 7A and theoretically in Fig. 7B. In Fig. 7A the apparent K_m for ADP is observed to increase as the concentration of tentoxin increased and this increase is more marked at a lower light intensity. For both light intensities used the data tends to a common point of intercept on the x-axis. In Fig. 7B the data calculated from a mathematical model shows similar trends. Lowering the value of V_p increased the apparent K_m for a high and a low

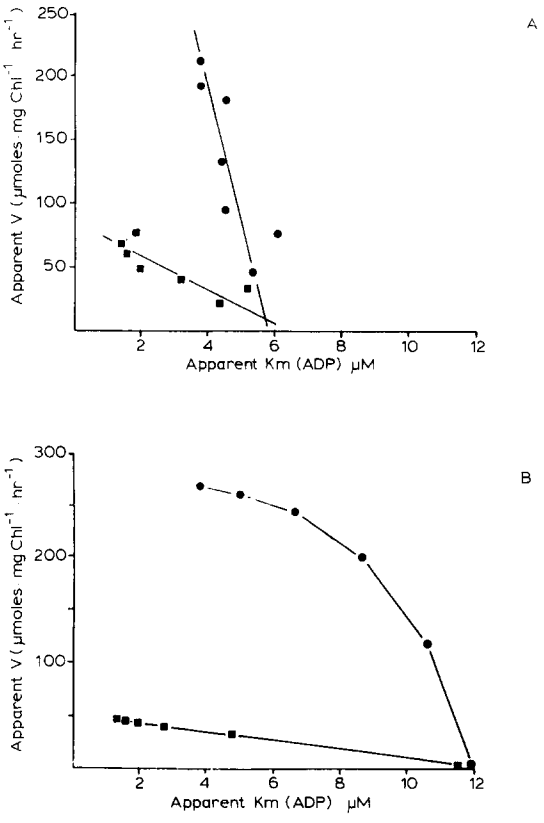


Fig. 7. Relationship between V and K_m at two light intensities and different energy-transfer inhibitor concentrations experimental (A) and calculated from a mathematical model (B). In (A) the light intensity was 53 $W \cdot m^{-2}$ (■) or 240 $\cdot m^{-2}$ (●), and the concentration of tentoxin was 0, 24, 48, 96, 144, 192 and 240 nM, respectively, from the top to the bottom of the graph. In (B) $V_e = 200$ (■) or 1000 (●), $C = 2 \cdot 10^7$, $V_p = 2500$, 2000, 1500, 1000, 500 and 20, respectively, from the top to the bottom of the graph.

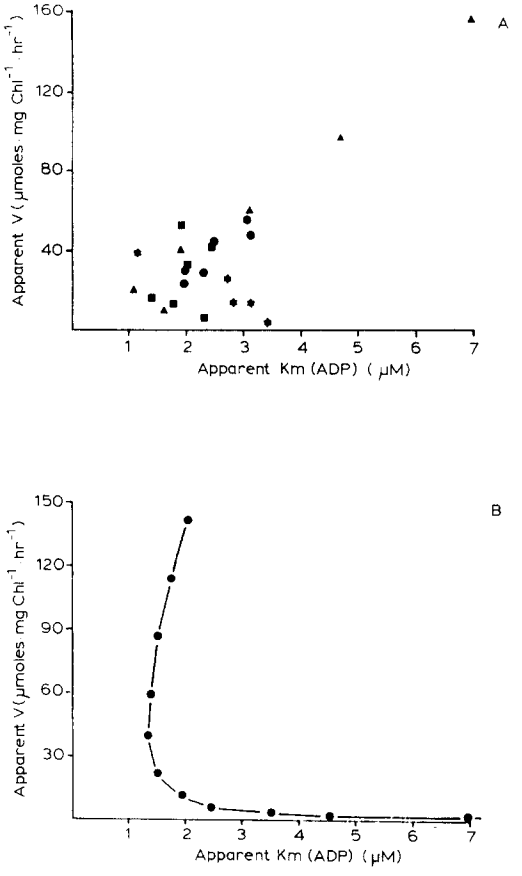


Fig. 8. Relationship between V and K_m at very low light intensities experimental (A) and calculated from a mathematical model (B). In (A) similar symbols indicate data points obtained from a single batch of chloroplasts. The rate of photosphorylation was varied by altering the light intensity in the range of 8–60 $W \cdot m^{-2}$. In (B) the value of V_e was varied between 10 and 200. K_m (ADP) was calculated from data obtained using 3, 4, 5, 7, 10 and 16 μM ADP concentrations.

value of V_e. When $V_e = 200$ the data were approximately linear, similar to the low light data on Fig. 7A. When $V_e = 1000$ the data are more curved, which is not apparent for the high light data of Fig. 7A. For both values of V_e the data had a common intercept on the x-axis at very low V_p values. The experimental and theoretical intercept values are different although this can be altered by manipulation of the model parameters (see Discussion).

The results obtained using an energy-transfer inhibitor can be rationalized with the other data in terms of the ratio of the phosphorylating and non-phosphorylating proton effluxes during steady-state photophosphorylation (see Discussion). A specific prediction of this explanation is that as the light is lowered to very low levels, the apparent K_m should begin to increase again, since at very limiting ΔpH, very little H^+ efflux occurs via the phosphorylating pathway. Fig. 8 shows how the apparent K_m varies for low light intensities (A) or values of V_e (B). The theoretical data predict that the K_m should decrease first with decreasing values of V_e but for very low V_e values the K_m should start to increase and intercept the x-axis at a value of 12. The experimental data show that this is the case although the precise

position of the intercept is not resolved, due to large errors in measuring the K_m at such low rates of ATP synthesis.

Discussion

The experimental data presented here confirm and extend earlier observations on the apparent K_m for ADP during photophosphorylation. Using coupled chloroplasts under high-intensity illumination as the reference point, we confirm that the general effect of lowering V by reducing light intensity is to also decrease the K_m, but that uncouplers cause the K_m to increase. In addition, we have shown that (i) the effect of lowering light intensity on the K_m is non-linear; (ii) the ability of uncouplers to increase K_m is enhanced by lowering the light intensity; (iii) energy-transfer inhibitors also cause the K_m to increase. Since the latter effect will be associated with decreased rates of electron flow, it is clear that an increase in K_m is not always associated with increased rates of electron transport, ruling out direct links between the ATPase and electron-transport complexes as the cause of the phenomenon.

The most important extension to the observations is provided by the simultaneous estimation of ΔpH during the experiments. The magnitude of ΔpH will also affect the rate of ATP synthesis and we have shown that under most conditions, the value of ΔpH falls as the ADP concentration is increased. The ΔpH is thus an uncontrolled system variable during the K_m analysis. The main effect of this is to cause the apparent K_m to be underestimated. The effect of inhibitory treatments on the apparent K_m will depend on the ratio of phosphorylating and non-phosphorylating proton effluxes from the thylakoid. When the phosphorylating H^+ efflux is limited solely by the availability of ADP, increasing this substrate will stimulate proton efflux and lower ΔpH. This occurs over most of the light-intensity range, and the effect of lowering the light intensity is to lower the K_m progresively. However, when H^+ efflux is dominated by the non-phosphorylating pathway, as is the case in the presence of energy-transfer inhibitor or uncoupler, then the ΔpH will not change in response to ADP, and the K_m will tend to increase, particularly if the light intensity is low and the starting point of the K_m estimations is therefore low.

It is not intuitively obvious that a simple chemiosmotic coupling mechanism predicts these complex effects and we have therefore carried out extensive mathematical modelling to show that this is indeed the case. The model is remarkably simple, and represents a minimal scheme that nevertheless is able to predict all of the experimentally observed trends. Certain assumptions in the model require some comment. The assumption that the ATPase displays simple Michaelis-Menten kinetics is almost certainly an oversimplification, since the enzyme probably has multiple catalytic sites [14]. To model the effect of ΔpH on ATP synthesis, we have assumed that the rate is controlled kinetically by the activation of CF_0-CF_1. For simplicity, we have also assumed that activation of both the free enzyme and the enzyme-substrate complex by ΔpH is equally effective. We should point out that neither of these assumptions are necessary in order to model the results. The formalism of Eqn. 3, though derived for an activation model is almost identical to that derived assuming a Michaelis-Menten two-substrate mechanism, where ΔpH is the second substrate. Thus the analysis is valid of ATP synthesis is controlled thermodynamically by ΔpH as would be the case if the enzyme were thiol reduced, and similar results can be seen for this situation in an earlier report [9].

In the model it is necessary to assign some value for the 'true' K_m, though this may have little meaning in reality. One consequence of this, and of the assumption of equivalent activation of the free enzyme and enzyme-substrate complexes, is that the measured value can never exceed this 'true' K_m value. In modelling the data presented here, a true K_m value of 12 μM was chosen. Results from other laboratories have shown that the apaprent K_m can reach much higher values, around 50 μM, particularly when V is high when phenazine methosulphate-driven cyclic photophosphorylation was measured [1,2]. These data can be accommodated in the model by slight changes in the assumptions. Fig. 9A shows calculated data where value for K_m was set at 50 and the KD_1/KD_2 ratio at 3. With these conditions the high K_m values observed at high V values are

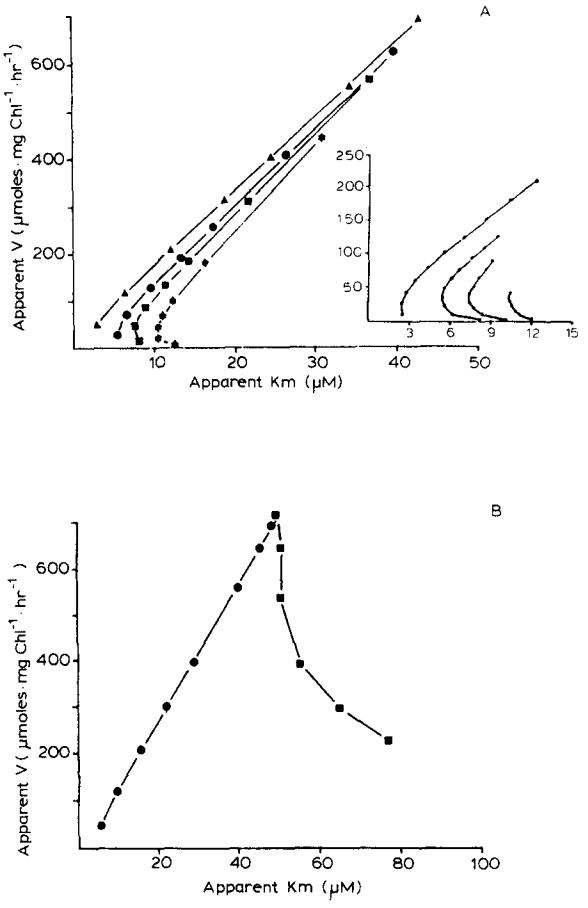


Fig. 9. Theoretical relationship between V and K_m at very high rates of electron transport (V_e). In (A) $KD_1 = 0.1$, $KD_2 = 0.03$, $K_m = 50$ and $C = 2 \cdot 10^7$ (▲), $1 \cdot 10^8$ (●), $2 \cdot 10^8$ (■) and $4 \cdot 10^8$ (*). The inset in (A) shows an expanded region of the main figure at low V values. In (B) $KD_1 = 0.03$, $KD_2 = 0.1$, $K_m = 50$. The value of V_e was varied up to 3500 and $C = 2 \cdot 10^7$ (■). The value of C was varied between $2 \cdot 10^7$ and $4 \cdot 10^8$ and the value of $V_e = 3500$ (■).

simulated. The expansion of the low V region of the graph (see inset Fig. 9A) shows data in good agreement with the experimental data of Fig. 5A. It is interesting to note that a decrease in the apparent K_m with an increasing uncoupler concentration observed by Davenport and McCarty [7] is also seen here for higher V_e values. However, the increase in apparent K_m (ADP) with uncoupler observed by other authors even at high rates of electron transport [1,3] is not satisfied by these model parameters. When KD_1 is much greater than KD_2, the model is similar to that of Bickel-Sandkotter and Strotmann [2]. Their model can explain the tight binding of ADP and the effect of light intensity on the apparent K_m for ADP, but does not account for the observed increase in the apparent K_m for ADP asosciated with partial uncoupling.

Reversing the value of KD_1 and KD_2 so that the KD_1/KD_2 ratio is 0.33 (Fig. 9A) shows data in good agreement with Vinkler [1] and Loehr et al. [3]. Thus by minor alterations apparent in the KD_1 and KD_2 values all the published data on the K_m(ADP) can be simulated. However, as yet, we cannot simulate all the data using one set of parameters. This may reflect inadequacies of the model, contradictions in the reported data or a reflection on the natural variation of the KD values. Nevertheless, it is clear that the overall complex behaviour of apparent K_m for ADP in energy coupling ATPase systems is well accommodated by, and indeed is a necessary consequence of, a delocalised chemiosmotic coupling system.

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