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THE ENERGY-LINKED CONFORMATIONAL EQUILIBRIUM IN CHLOROPLAST ATPase

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SUMMARY

1 The capacity of chloroplast ATPase to drive energy-requiring processes increases with, but the maximal ΔpH maintained by ATPase is independent of the degree of modification by dithioerythritol

2 After modification by dithioerythritol, the ATPase remains subject to an energy-linked equilibrium between active and inactive ATPase conformations This results in an energetic threshold, the magnitude of which is independent of the degree of modification by dithioerythritol or the presence of P_i and/or ADP, but increases with medium pH

INTRODUCTION

In a previous paper [1], we presented a model for activation of chloroplast ATPase As shown again in Fig 1, dithioerythritol or trypsin can only attack the ATPase in high-energy conformation (B), from studies on purified coupling factor [2], it may be inferred that they destroy or interfere with the action of the so-called inhibitor, an ATPase-inhibiting subunit of the enzyme complex After this essentially irreversible modification (II), ATPase activity is regulated by a combination of an energy-linked equilibrium (III) between an ATPase active (C) and an ATPase-inactive (D) conformation and a non-energy linked, Mg^{2+}-catalyzed degradation (IV) of the latter

From a bioenergetic point of view, the ATPase reaction is interesting because of its capacity to drive energy-requiring processes, dissipation by uncoupler can be considered an artificial simulation of such a process For this reason, we wanted to investigate how variation of the degree of modification by dithioerythritol would affect this capacity Another question posed by the model was whether the position of equilibrium (III) is unequivocally determined by the chloroplast energy level

Abbreviation S_{13} 5-chloro-3-*tert*-butyl-2′-chloro-4 -nitrosalicylanilide

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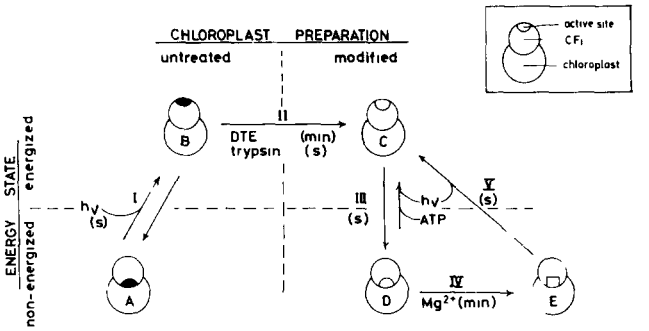


Fig 1 Model for activation of chloroplast ATPase Shading of active centre indicates presence of inhibitor [2] For further explanation, see text

METHODS AND MATERIALS

Type-C [3] spinach chloroplasts were prepared as described before [4], and suspended in a medium containing 100 mM KCl, 5 mM $MgCl_2$ and 5 mM sodium tricine (pH 8 0, unless indicated otherwise) Total chlorophyll was determined according to Whatley and Arnon [5] The reaction mixture was composed of the suspension medium supplemented with 10 μM pyocyanine and 50 μg chlorophyll Temperature was 20 °C, pH was 8 0 unless indicated otherwise Final volume was 2 5 ml, final concentration of ATP was 1 mM, that of 9-aminoacridine 4 μM The reaction vessel used was designed by Dr J W T Fiolet, and, as described before [4], offered the possibility of simultaneously monitoring medium pH and fluorescence The rate of decrease in the former was taken as a measure for ATPase rate [6], from the quenching of fluorescence of 9-aminoacridine relative to the level obtained after addition of a saturating amount of 5-chloro-3-*tert*-butyl-2'-chloro-4'-nitrosalicylanilide (S_{13}), a value for ΔpH was calculated [7] As before [4], the osmotic compartment of the chloroplasts was taken as 10 μl/mg chlorophyll, the relation between ΔpH and fluorescence quenching Q then becomes [7] $\Delta pH = \log [Q/(1-Q)] + \log 5000 = \log [Q/(1-Q)] + 3\,7$

9-Aminoacridine was obtained from British Drug Houses All other chemicals were analytical grade S_{13} was kindly donated by Dr P C Hamm, Monsanto Comp , St Louis, Mo (U S A)

RESULTS

As shown in Fig 2, after addition of uncoupler to the ATPase mixture during the dark stage, 9-aminoacridine fluorescence almost instantaneously adjusts to a higher level Above a certain uncoupler concentration the ATPase rate shows an initial stimulation followed by relaxation towards a lower steady-state velocity, as found before [8, 9] From then on, the initial stimulation increases and the final steady-state rate decreases with the amount of uncoupler added

In Fig 3, the initial ATPase rate after addition of uncoupler is plotted as a function of the accompanying ΔpH for three different preillumination times in the presence of dithioerythritol, i e three different degrees of modification (solid lines)

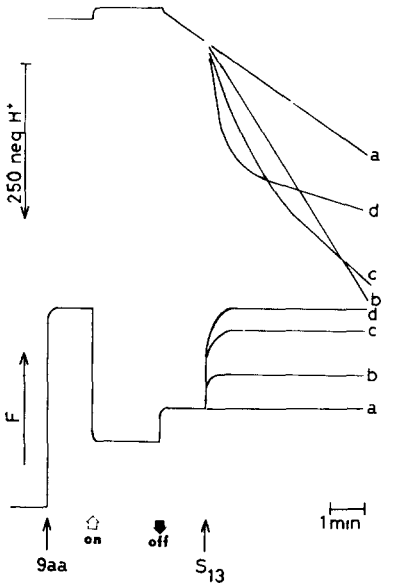


Fig 2 Relaxation behaviour of uncoupler-stimulated ATPase The reaction mixture was as described under Methods and Materials 9aa, 9-aminoacridine Final concentrations of S_{13} added a 0 nM, b, 20 nM, c, 100 nM, d, 200 nM

At high values of ΔpH (high degree of coupling) the curves converge The dotted lines connecting points of equal uncoupler concentration give a picture comparable to Fig 5 of ref 4

In terms of Fig 1, it seemed reasonable to assume that before addition of uncoupler all the modified ATPase would be preserved in active form C by dark ATP

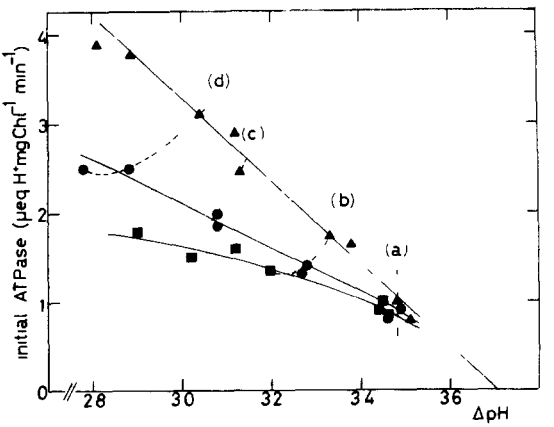


Fig 3 Initial rate of uncoupler-stimulated ATPase as a function of concomitant ΔpH Different degrees of modification were obtained by varying the preillumination time in the presence of dithioerythritol ■—■, 1 min, ●—●, 2 min, ▲—▲, 5 min The dotted lines connect points of equal uncoupler concentration for (a), (b), (c) and (d) concentration S_{13} 0 20, 40 and 80 nM, respectively

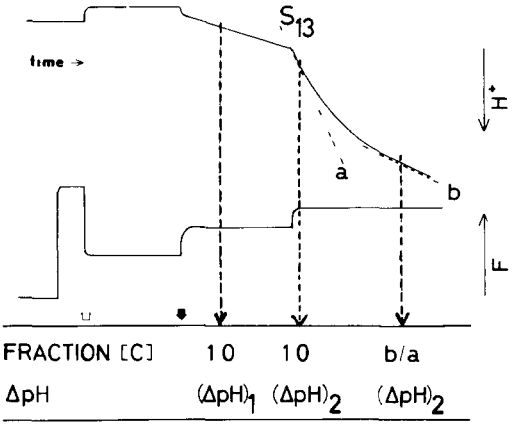


Fig 4 Calculation of the ratio [C]/([C]+[D]) in uncoupler-induced steady state, for explanation, see text

hydrolysis, and that this would still be the case immediately after addition of uncoupler Neglecting Reaction IV, the ratio of the final steady-state rate (b) over the initial uncoupler-stimulated rate (a) would thus provide a value for the fraction of modified ATPase molecules preserved in active conformation C (ratio [C]/([C]+[D])) during the new steady state reached after relaxation (Fig 4) In Fig 5a, this fraction is plotted against ΔpH maintained during different steady states reached by uncoupler titration As expected, it increased with ΔpH Variation of the degree of modification did not have a significant effect on this relationship The dotted line shows the fraction of active ATPase as a function of accompanying ΔpH for one particular uncoupler concentration during the course of relaxation As could be inferred already from Fig 2, and schematically indicated in Fig 4, after addition of uncoupler ΔpH almost instantaneously drops to its new steady-state level, only slowly followed by a decline in ATPase activity Clearly, the fraction C shows hysteresis with respect to ΔpH if compared with the steady-state relationship expressed by the solid line in Fig 5a

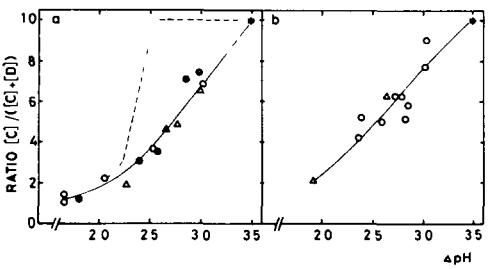


Fig 5 a Ratio [C]/([C]+[D]) as a function of ΔpH in uncoupler-induced steady state, calculated as indicated in Fig 4 ○, ● 2 min preilluminated, △, ▲ 5 min preilluminated in the presence of dithioerythritol Open and closed symbols denote two different experiments Dotted line dependence of the ratio on ΔpH for one uncoupler concentration during the course of relaxation *, ATPase before addition of uncoupler (assumed starting point) b Ratio [C]/([C]+[D]) as a function of ΔpH in the steady state obtained after different periods of dark decay calculated as indicated in Fig 6 ○, △ denote different experiments (△, uncoupler added)

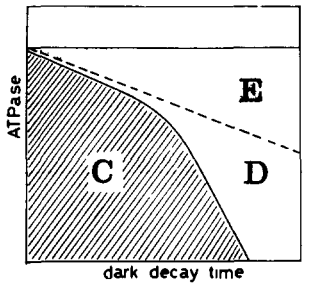
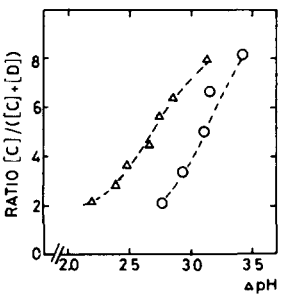


Fig 6 Interpretation of dark decay of ATPase in terms of the mechanism depicted in Fig 1 For explanation, see text

This relationship, if unequivocal, should be independent of the way in which the steady state is reached Thus, it should also apply to the decline in ATPase activity after increasing dark periods in the absence of ATP Dark decay curves like those in Figs 7 and 8 of ref 1 can be interpreted as outlined in Fig 6 It is assumed then that during dark decay (i) degradation IV (formation of E) proceeds linearly in time, and (ii) initially, the ATP-induced autocatalytic activation process [1] will reconvert to active form C virtually all modified ATPase not yet degraded to E, after some dark decay time, however, the potential supply of energy by C will drop so far that equilibrium III will cause an appreciable amount of ATPase molecules to remain in inactive form D in the steady state From ref 1, Figs 7 and 8, the initial decline in ATPase rate (i e formation of E) can be estimated to be about 10 % per min (in the presence of Mg^{2+}) Based on these assumptions, the ratio [C]/([C]+[D]) has been calculated and plotted against ΔpH in the steady-state situations reached after different dark decay times (Fig 5b) the picture obtained is indeed quite comparable to that in Fig 5a



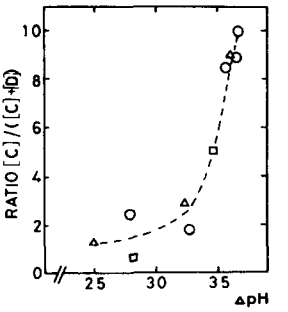


Fig 7 The influence of the pH of the medium on the relation between ratio [C]/([C]+[D]) and ΔpH in the steady state The experiment was performed as that depicted in Fig 5a △—△ pH 7 4, ○—○, pH 8 4

Fig 8 The influence of P_i and/or ADP on the relation between ratio [C]/([C]+[D]) and ΔpH in the steady state The experiment was performed as that depicted in Fig 5b Medium pH was 8 5 ○—○, steady-state ratio after dark decay without additions, △—△, 2 mM P_i present in the reaction mixture and 50 μM ADP added at the beginning of the dark stage, □—□, 50 μM ADP added at the beginning of the dark stage

Thus, the position of equilibrium III is unequivocally determined by the energy level in the steady state However, the relation between those two parameters does show a dependency on the pH of the medium, as shown in Fig 7

From Fig 8, it appears that steady-state situations obtained after addition of P_i and/or ADP to the dark decay stage fit the same curve as those obtained in the absence of those effectors

DISCUSSION

Since the initial rate of uncoupler-stimulated ATPase is assumed to correspond with maximal activity of the modified ATPase (Fig 4), the ATPase turnover capacity is constant along each of the solid lines in Fig 3, it increases with modification time At low degree of coupling (left half of Fig 3), the dotted lines connecting points of equal uncoupler concentration run more and more horizontally this shows that the resistance against uncoupling (and thus the capacity to drive energy-requiring processes) increases with ATPase turnover capacity, as expected On the other hand, the solid lines in Fig 3 all seem to converge to $\Delta pH \approx 3\,7$, this 'static head' ΔpH, that would be maintained by ATPase under conditions of ideal coupling, thus is independent of the ATPase-turnover capacity It is significantly lower than that supplied by saturating light (for which case, in the absence of uncoupler, $\Delta pH \approx 4\,2$) However, the difference is of the same magnitude as that between the values calculated for 'State 4' and 'State 3' [10] This means that ATPase can maintain a ΔpH equal to that supplied by electron transport under phosphorylating conditions

As set out in Introduction, there does indeed exist a straightforward relationship between the position of energy-linked equilibrium III in the steady state and the energy level associated with it, independent of the way in which the steady state is reached (Figs 5a and b) Effectively, this relation shows that there is an energetic threshold for ATPase activity, for instance, in Fig 5 only above $\Delta pH \approx 2\,5$ significant steady-state ATPase activity occurs The degree of modification appears to have no influence on the magnitude of this threshold (Fig 5a) This is in line with the assumption [1] that unmodified chloroplasts are subject to the same energy-linked conformational equilibrium It may be speculated that this equilibrium exerts a regulating effect on photophosphorylation as well (compare also ref 11), in this respect it is relevant that a similar 'ΔpH threshold' has been found for post-illumination phosphorylation This threshold showed a dependency on medium pH quite comparable to that for ATPase (Fig 7), it increased from $\Delta pH = 2\,4$ at a medium pH of 7 5 to $\Delta pH = 3\,0$ at a medium pH of 8 5 [12]

ADP is known to promote the dark decay [13] Antagonistically, P_i retards the dark decay [13], and generally has a stabilizing influence on ATPase [1] This might be thought to be a consequence of some effect on equilibrium III One possibility is that ADP and P_i shift the ΔpH threshold towards a higher or lower value, respectively, i e influence the free-energy change of the reaction $D \rightarrow C$ As shown in Fig 8, that is not the case In fact, this could be inferred already from the observation that obviously the energy threshold is independent of the degree of progression of the ATPase reaction (that is different for all points in Fig 5), and thus of the amount of ADP and P_i formed This, together with the fact that ADP and P_i have to be present during the whole decay period to exert their action (ref 1, compare also ref 13), indicates that those compounds affect non-energy linked degradation IV

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