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Modes of action of uncouplers in thylakoids

External and internal pH profiles of electron transport

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The pH dependence of electron transport in isolated lettuce chloroplasts can be simulated by a model involving four ionizable groups: two facing the inside and two facing the outside of the thylakoids. The pH dependence can be resolved into separate pH profiles for the outside and the inside of the thylakoids, with optima at 7.1 and 5.6, respectively. A ΔpH of 3 units across the thylakoid membrane allows only partial overlapping of the two profiles and relatively low rates of electron transport. Uncouplers stimulate electron transport by reducing the ΔpH, thereby improving the overlapping of the two pH profiles.

Chloroplast; Thylakoid membrane; Electron transport; ΔpH; pH dependence; Uncoupler

1. INTRODUCTION

The light-saturated rate of electron transport in isolated chloroplasts varies with the pH of the medium, reaching a maximum at pH_o 8–9 [1]. Uncouplers, which reduce the ΔpH, shift the pH_o optimum to lower levels indicating a dependence of ET not only on pH_o but also on pH_i [1–3]. In the present study we reevaluate the rules governing the pH dependence of photosynthetic ET.

In analyses of pH dependence of enzymatic activity, the enzyme is often represented as a dibasic acid – EH_2 with two non-identical ionizable groups [4]. The dissociation reactions are written as:

$$EH_2 \overset{K_1}{\rightleftharpoons} H^+ + EH^- \text{ and } EH^- \overset{K_2}{\rightleftharpoons} H^+ + E^{2-} \quad (1)$$

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Abbreviations: ET, electron transport; ET_m, maximal rate of electron transport; pH_i and pH_o, the pH inside and outside the thylakoids respectively; ΔpH, $pH_o - pH_i$

If EH^- is the only active form of an enzyme catalyzing ET, the reaction rate should be proportional to the fraction of EH^- of the total of all forms of the enzyme. These different forms can be readily obtained in terms of the pH and the two pK values [4]. Let ET_m denote the maximal rate obtained in a case where all enzyme molecules are in the active (EH^-) form. The pH dependence of ET can accordingly be described by

$$ET = ET_m \times \frac{1}{10^{(pK_1 - pH)} + 1 + 10^{(pH - pK_2)}} \quad (2)$$

The first model used here to analyze the pH dependence of ET in chloroplast thylakoids involves, indeed, an enzymatic complex with two ionizable groups. This complex, however, is further assumed to span the membrane in such a manner that one of the groups faces the inside of the thylakoids and the other faces the outside. Because of the ΔpH across the membrane the ET rate is expected to be a function of both pH_i and pH_o.

$$ET = ET_m \times \frac{1}{10^{(pK_1 - pH_i)} + 1 + 10^{(pH_o - pK_2)}} \quad (3)$$

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2. MATERIALS AND METHODS

Envelope-free chloroplasts were isolated from lettuce leaves and photoreactions were assayed essentially as described [5,6]. A magnetically stirred glass cuvette, equipped with a pH combination electrode and an oxygen electrode, was installed in the sample compartment of a Jasco FP-550 spectrofluorometer. This arrangement permitted simultaneous measurements of pH_i via fluorescence changes of 9-aminoacridine [7] and of ET. The reaction mixture contained in a final volume of 3 ml: 50 mM KCl, 2 mM $MgCl_2$, 2 mM Na_2HPO_4, 0.1 mM methyl viologen, 2 mM NaN_3, 2 μM 9-aminoacridine and chloroplasts equivalent to 24 μg Chl/ml.

3. RESULTS AND DISCUSSION

3.1. *The 2-pK model*

Fig.1 shows a comparison of the experimentally determined pH dependence of ET, with predictions based on the model summarized in eqn 3. Parallel measurements of light saturated ET rates and of ΔpH were conducted at different pH_o values between 6 and 9, in the presence of various concentrations of gramicidin. All ET measurements from the same experiment were regrouped according to the measured ΔpH values and plotted vs pH_o. Data points denoted by the same symbol in fig.1, accordingly represent rates of ET obtained at the same ΔpH.

The curves shown in fig.1 were drawn according to eqn 3, using $pH_o - \Delta pH$ instead of pH_i. Values substituted for pK_1, pK_2 and ET_m were those providing the best fit for the data. Note that eqn 3 was solved so as to fit the results of the entire experiment. Rather than describe a single curve, eqn 3 represents a family of curves, one for each ΔpH value.

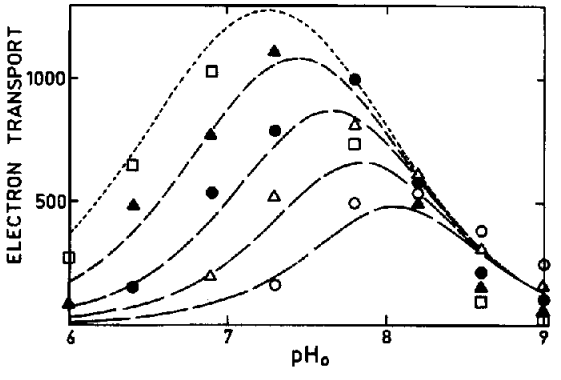

Fig.1. Dependence of ET on pH_o at fixed ΔpH levels. Electron transport is in μ-equivalents·mg $Chl^{-1}\cdot h^{-1}$. Data points denoted by the same symbol represent ET rates measured at the same ΔpH: ○, 3.2; △, 2.8; ●, 2.4; ▲, 2.0; □, 1.6. Dashed curves were drawn by substituting the same ΔpH values into eqn 3, after replacing pH_i by $pH_o - \Delta pH$; with $pK_1 = 5.0$, $pK_2 = 7.9$ and $ET_m = 2000$ μ-equivalents·mg $Chl^{-1}\cdot h^{-1}$. Decreasing dash length represents decreasing ΔpH values.

Although the 2-pK model seems to provide a good fit for the experimental data over considerable parts of the tested pH_o and ΔpH ranges, it fails to do so at both high and low levels of these parameters. For example, eqn 3 predicts the convergence of the different curves at alkaline pH_o values, while the data indicate considerable divergence in this range (fig.1).

3.2. *The 4-pK model*

A better fit for the experimental data was obtained using a more elaborate model. This model portrays the rate limiting step in ET as depending on the dissociation state of four different groups, two facing the inside and two facing the outside of the thylakoids. One internal group and one external group are assumed to be protonated in the active state, while the other two are non-protonated.

The use of this model is significantly simplified by resolving the pH dependence into separate external and internal pH profiles. Eqn 1, after substituting the appropriate constants, can be used to describe the dissociation steps which take place either inside or outside the thylakoids. If all external parts of the ET complexes are in the active form, then the pH_i profile of electron transport is as follows:

$$ET = ET_m \times \frac{1}{10^{(pK_1 - pH_i)} + 1 + 10^{(pH_i - pK_2)}} \qquad (4)$$

The pH_o dependence of ET is similarly defined for a case where the internal parts of all ET complexes are in the active form, and is given by:

$$ET = ET_m \times \frac{1}{10^{(pK_3 - pH_o)} + 1 + 10^{(pH_o - pK_4)}} \qquad (5)$$

The overall pH dependence of ET is accordingly described by:

$$ET = ET_m \times \frac{1}{10^{(pK_1 - pH_i)} + 1 + 10^{(pH_i - pK_2)}} \times \frac{1}{10^{(pK_3 - pH_o)} + 1 + 10^{(pH_o - pK_4)}} \qquad (6)$$

In order to check the adequacy of this model, the results of each set of parallel measurements of ET, pH_o and pH_i were substituted into eqn 6. Groups of equations from the same experiment were solved simultaneously for pK_1–pK_4, and for ET_m, and the results were averaged. The pK values of the internal groups, thus determined, were 4.9 for pK_1 and 6.3 for pK_2. The corresponding values for the external groups were 6.1 for pK_3 and 8.1 for pK_4. ET_m was 2200 μequivalents·mg $Chl^{-1}\cdot h^{-1}$.

Fig.2A shows the dependence of ET on pH_o as obtained from eqn 6, after replacing pH_i by pH_o–ΔpH and using the values determined for pK_1–pK_4 and ET_m. Different curves are given for different ΔpH values so that each curve can be compared with experimental data obtained at the same ΔpH. A complementary picture is shown in fig.2B, where ET rates measured at constant ΔpH values are plotted vs pH_i. Here also the experimental results are compared with curves calculated using the 4-pK model. Note again that the curves in fig.2 are not independent of each other. By setting values for the four pK values and for ET_m, one describes simultaneously all the curves illustrated in fig.2A and B. The good correlation between experimental results and theoretical predictions seems to support this model.

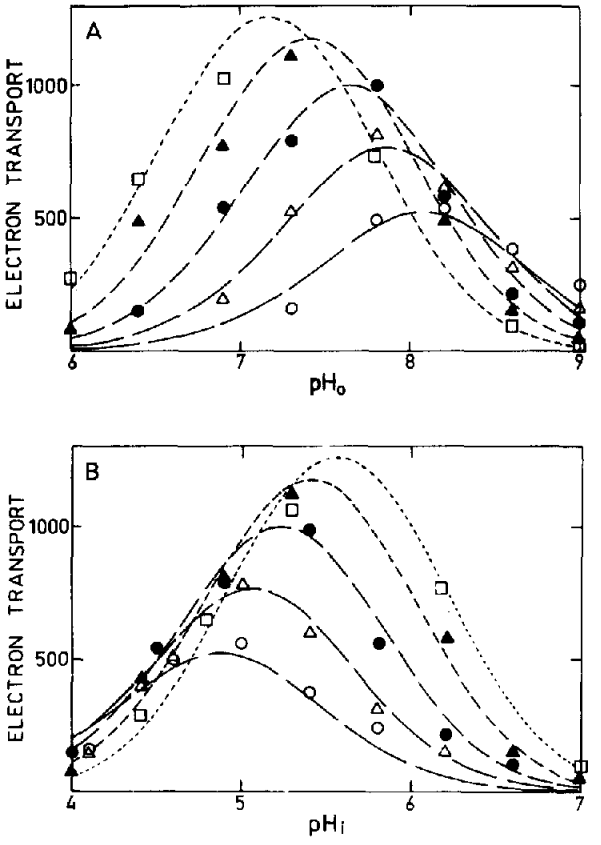

Fig.2. Dependence of ET on pH_o (A) and on pH_i (B) at fixed ΔpH levels. ΔpH levels are denoted by the following symbols: ○, 3.2; △, 2.8; ●, 2.4; ▲, 2.0; □, 1.6. Dashed curves were obtained by substituting the same ΔpH values into eqn 6. Decreasing dash length represents decreasing ΔpH. The pK_1–pK_4 values used were 4.9, 6.3, 6.1 and 8.1, respectively. ET_m was 2200 μ-equivalents·mg $Chl^{-1}\cdot h^{-1}$. In calculations for (A), pH_i was replaced by $pH_o - \Delta pH$; in (B), pH_o was replaced by $pH_i + \Delta pH$. Electron transport is in μ-equivalents·mg $Chl^{-1}\cdot h^{-1}$.

The resolved pH_i and pH_o profiles, and the resultant overall pH dependence of ET are illustrated in fig.3, for different ΔpH values. The pH_i profiles (dotted curves) and pH_o profiles (dashed curves) were drawn using eqns 4 and 5, respectively. The resultant pH dependence (solid curves) was obtained using eqn 6. The pH_o profile, being determined by two acidic groups with pK values at 6.1 and 8.1, reaches a maximum at pH_o 7.1. The pH_i profile is similarly characterized by acidic groups with pK values at 4.9 and 6.3, and an optimum at pH_i 5.6. Due to the closer proximity of these two pK values, the pH_i profile is somewhat narrower and lower than the pH_o profile.

The solid curves in fig.3 show the combined limitations of ET by pH_i and pH_o. The pH_i and pH_o profiles are offset with respect to each other by the size of the ΔpH across the thylakoid membrane (fig.3). The ΔpH determines the degree of overlapping between the two profiles and thereby the resultant ET rate. A ΔpH of 3 units, which is commonly obtained under physiological conditions, permits only partial overlapping of the two pH profiles and relatively low ET rates. Decreasing the ΔpH by an uncoupler shifts the pH_i profile with respect to the pH_o profile allowing better overlapping of the two profiles and higher ET rates. Maximal overlapping is obtained with a ΔpH of 1.5 units. Further decrease of the ΔpH results, however, in decrease of overlapping and in ET inhibition, which is especially evident at alkaline pH_o values.

Fig.3 indicates that with ΔpH levels of 2–3 units, it is mainly the (internal) pK-4.9 and (external) pK-8.1 groups which determine the ET rate.

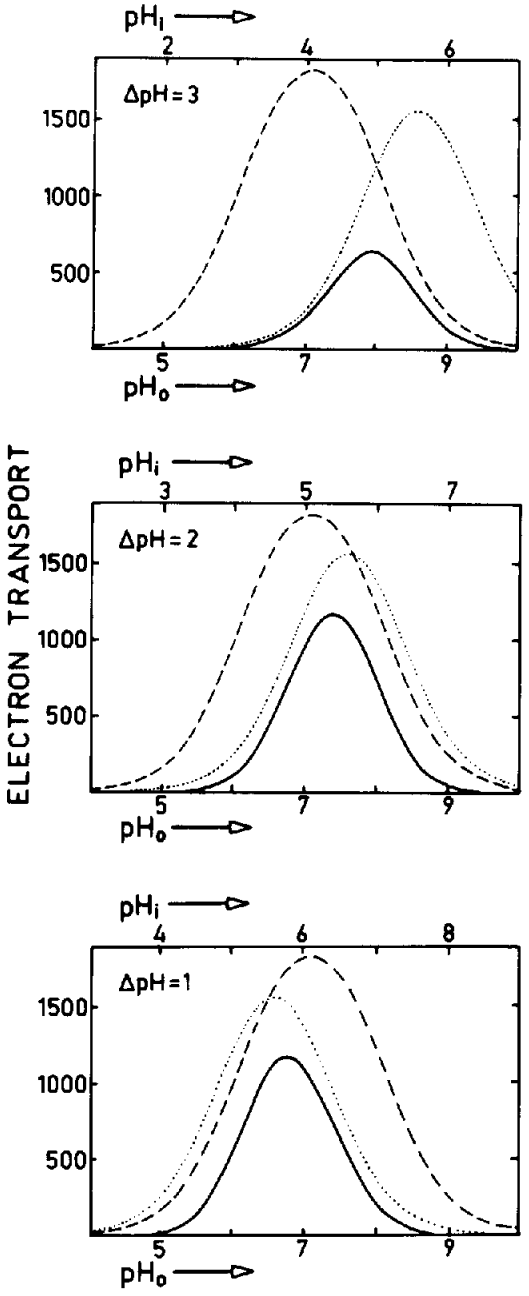

Fig.3. Simulation of interaction of the pH_i and pH_o profiles in determining the overall pH dependence of ET. The pH_i scale is shifted in each frame with respect to the pH_o scale in proportion to the indicated ΔpH. The pH_i profiles (dotted curves), pH_o profiles (dashed curves) and the resultant pH dependence (solid curves) were constructed by using eqns 4, 5 and 6, respectively. The parameters used are the same as in fig.2. Electron transport is in μ-equivalents · mg $Chl^{-1} \cdot h^{-1}$.

This is the reason why it was possible to obtain a fairly good simulation of the pH dependence with the 2-pK model (fig.1). At lower ΔpH levels, all four groups are evidently involved in determining the rate.

In a previous study of the pH dependence of ET [4], it was concluded from largely similar experimental results that ET is a function of the average between pH_i and pH_o and that the rate controlling site is embedded in the thylakoid membrane. Since the results presented here could be fully explained in terms of a dependence of ET on pH_i and pH_o, there was no reason to invoke a dependence on the proton concentration in any other compartment except that sensed by the 9-aminoacridine.

The results presented indicate a dependence of ET on pH_i and pH_o, but not on ΔpH as an energetic entity. If ΔpH were limiting the ET rate due to energetic constraints one could expect this rate to be independent of pH_i and pH_o as long as ΔpH is constant. Fig.2 shows that this was not the case (see also [4]). At any constant level of ΔpH, ET was still found to vary with either pH_i or pH_o. The ΔpH probably never reaches the level necessary to impose energetic limitations on ET, as while growing it drives pH_i and/or pH_o to kinetically-inhibitory levels.

In view of arguments that ΔpH levels measured by the 9-aminoacridine method are overestimated (see discussion in [8]), the pK values determined in this work may be regarded as approximations. This should not, however, affect the basic results and conclusions. The results and model presented here indicate that the pH dependence of ET can be resolved into separate pH_o and pH_i profiles and that uncouplers change the degree of overlapping between the two profiles.

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