CONFORMATIONAL CHANGES IN COUPLING FACTOR 1 MAY CONTROL THE RATE OF ELECTRON FLOW IN SPINACH CHLOROPLASTS

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SUMMARY

The relationship between the rate of electron flow, internal H^+ concentration and the magnitude of the H^+ concentration gradient (ΔpH) in chloroplasts illuminated at various light intensities has been examined. At an external pH of 7.0, the internal H^+ concentration is a linear function of the rate of electron flow except at saturating light intensity. In contrast, at pH 8.1, this relationship between electron flow and internal H^+ concentration holds only at values of ΔpH below about 2.8 - 2.9 units. At higher ΔpH values, the rate of electron flow increases much more dramatically than the internal H^+ concentration. ATP (0.1 mM) prevents this increase. It is suggested that at pH 8.1 but not at pH 7.0, the conformation of coupling factor 1 is altered at high ΔpH values. Its altered conformation may result in an increased efflux of H^+ from the chloroplasts. This notion is supported by the effects of ATP on electron flow and ΔpH as well as the effect of external pH and light intensity on the reactivity of coupling factor 1 to N-ethylmaleimide.

Electron flow in chloroplasts is coupled to H^+ transport across thylakoid membranes. In the steady-state, the rate of electron flow must equal the rate of H^+ efflux from the internal thylakoid space for the H^+ concentration to remain constant [1]. Therefore, it has been proposed that the rate of electron flow is controlled by the internal pH [2,3]. Bamberger et al. [4], however, later suggested that the rate of electron flow was dependent in a more complex manner on not only the internal pH, but also the external pH and the magnitude of the

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Abbreviations used: NEM, N-ethylmaleimide; Tricine, N-tris(hydroxymethyl) methylglycine; CF_1, coupling factor 1 and MES, 2-[N-Morpholino]ethane sulfonic acid.

pH gradient across the membrane (ΔpH). Recently the validity of the technique used to estimate ΔpH in the investigation of Bamberger et al. [4], the fluorescence quenching of 9-aminoacridine, has been questioned [5].

We have investigated the relationship between electron flow and ΔpH using a microcentrifugation technique [6] involving the measurement of hexylamine uptake to estimate ΔpH. This method does not appear to have the limitations of the 9-aminoacridine fluorescence quenching technique[1].

MATERIALS AND METHODS

Chloroplasts were isolated from market spinach as described [7]. Chlorophyll [8] and photophosphorylation [9] were assayed by previously reported procedures. Hexylamine uptake and internal volumes were determined by rapidly centrifuging the chloroplasts through silicone fluid layers [6,10]. Electron flow was measured under identical conditions in the microcentrifuge as those used in the assay of amine uptake. The components of the reaction mixtures are given in the figure legends. Ferrocyanide was assayed by modification of the method of Punnet et al. [11]. Treatment of the chloroplasts with N-ethylmaleimide was performed as described [12]. Light intensity was varied by the use of photographic silver density filters obtained from Eastman Kodak.

RESULTS

The internal H^+ concentration ($[H^+]_{in}$) in chloroplasts illuminated to the steady state should be proportional to the rate of electron flow [1] when these processes are varied by changing the light intensity. This relationship holds true when the assays are performed at pH 7.0, except at the highest light intensity used (Fig. 1A). However at pH 8.1 , $[H^+]_{in}$ is a linear function of the rate of electron flow only at comparatively low light intensities (Fig. 1B). At light intensities sufficient to support a ΔpH of 2.8 or above, the rate of electron flow increases sharply with increasing light intensities whereas $[H^+]_{in}$ is little affected. Since an increase in the rate of electron flow at pH 8.0 at high ΔpH values does not proportionally increase $[H^+]_{in}$, the rate of H^+ efflux must also increase.

At pH 8.0 but not at pH 7.0, ATP or ADP reduces the rate of H^+ efflux [14,15]. As a consequence, the extent of H^+ uptake [14,15] and the magnitude

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[1] A.R. Portis, unpublished observations.

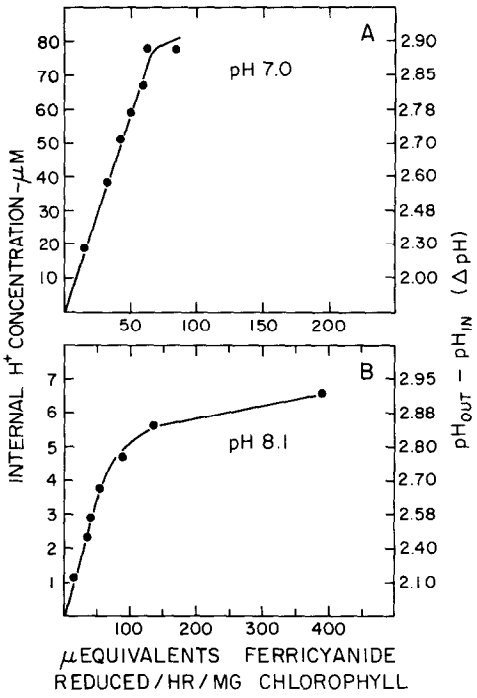

Figure 1. The relationship between internal H^+ concentration and the rate of electron flow obtained by varying the incident light intensity at pH 8.1 and 7.0. The reaction mixtures contained in a total volume of 0.5 ml, 50 mM NaCl, 5 mM $MgCl_2$, 40 mM Tricine-NaOH, 1 mM (pH 8.1) or 0.5 mM (pH 7.0) $K_3Fe(CN)_6$, 0.025 mM [^{14}C]hexylamine (1.30 μCi per μmole), [^{3}H]sorbitol (0.5 μCi per 0.5 ml of reaction mixture), and chloroplasts equivalent to 50 μg of chlorophyll. Aliquots of 0.1 ml were illuminated or kept dark in the microcentrifuge and assayed for amine uptake and ferrocyanide. The maximum incident light intensity used was 6.2×10^5 ergs per cm per s and was reduced by neutral density filters with an absorbance from 1.1 to 2.3.

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of ΔpH [10] are enhanced and the rate of electron flow is reduced [13]. Therefore, the relationship between electron flow and $[H^+]_{in}$ was examined at pH 8.0 with and without 0.1 mM ATP. As expected, ATP diminished the rate of electron flow and enhanced $[H^+]_{in}$ at high light intensities (Fig. 2). More significantly however, $[H^+]_{in}$ was a linear function of the rate of electron flow at all light intensities when ATP was present. In the absence of ATP, the linear relationship between electron flow and $[H^+]_{in}$ held only at ΔpH values below about 2.9, similar to the results in Fig. 1b.

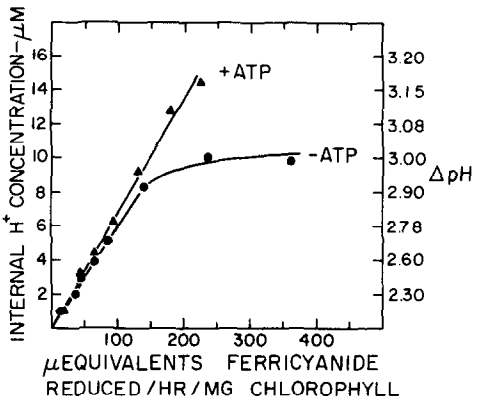

Figure 2. The relationship between internal H^+ concentration and electron flow obtained by varying the light intensity at pH 8.0 in the absence and presence of ATP.
The reaction mixtures and conditions were similar to those given in Figure 1. The ATP concentration was 0.1 mM.

ATP (or ADP) exerts its effects on H^+ uptake and ΔpH through an interaction with coupling factor 1 (CF_1) [14]. CF_1 undergoes conformational changes upon illumination [12,16,17,18] and these changes may influence the permeability of chloroplasts to H^+. The light-dependent reaction of CF_1 in chloroplasts with N-ethylmaleimide [12,16] results in inhibition of photophosphorylation and is a convenient way to monitor conformational changes in CF_1. In order to correlate conformational changes in CF_1 to the breakdown of the proportionality between $[H^+]_{in}$ and the rate of electron flow, the effects of pH and of light intensity on the reaction of CF_1 in chloroplasts with N-ethylmaleimide were examined. The ability of N-ethylmaleimide to inhibit phosphorylation was markedly reduced as the pH was lowered below 8.0 (Fig. 3). Only 9% inhibition was observed at pH 7.0, whereas at pH 8.0, the inhibition was 70%. The poor reactivity of CF_1 with N-ethylmaleimide at pH 7 is not due to an energy deficiency since ΔpH is the same at this pH as it is at pH 8.0 [19]. Thus, the conformational change in CF_1 as monitored by NEM inhibition is probably pH-dependent.

The reaction of CF_1 in chloroplasts with N-ethylmaleimide at pH 8.0 was quite sensitive to the magnitude of ΔpH when it was varied by reducing the light

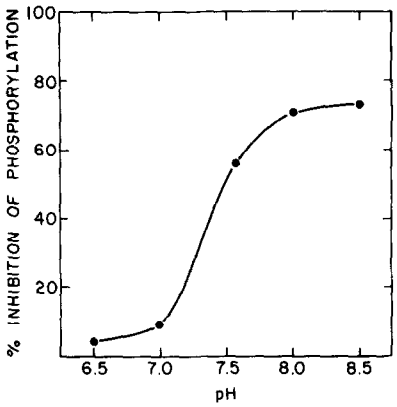

Figure 3. The pH dependence of the reaction of CF_1 with NEM. The chloroplasts were incubated for 90 s in the light with and without 1 mM NEM in a mixture that contained 50 mM NaCl, 5 mM $MgCl_2$, 0.05 mM pyocyanine, 20 mM Tricine-NaOH (pH 7.6, 8.0 and 8.5) or MES-NaOH (pH 6.5 and 7.0) and chloroplasts equivalent to 10 µg chlorophyll in a total volume of 0.1 ml. Subsequent phosphorylation activity was assayed by dilution to 1.0 ml with a reaction mixture that contained 50 mM NaCl, 5 mM $MgCl_2$, 3 mM ADP, 2 mM phosphate, 0.05 mM pyocyanine, 50 mM Tricine-NaOH (pH 8.0) and 1 mM dithiothreitol. Control phosphorylation rates were 310, 525, 587, 553 and 572 µmoles ATP formed/hr/mg chlorophyll for chloroplasts preilluminated at pH 6.5, 7.0, 7.6, 8.0 and 8.5 respectively.

intensity (Fig. 4). Little subsequent inhibition of phosphorylation was found when chloroplasts were illuminated in the presence of N-ethylmaleimide at light intensities which gave ΔpH values below 2.8. At higher light intensities, small increases in ΔpH were accompanied by dramatic increases in the inhibition. Therefore conditions which result in high rates of electron flow also result in maximal reactivity of CF_1 in chloroplasts with N-ethylmaleimide.

DISCUSSION

The correlations between the rates of electron flow and the light-dependent inhibition of phosphorylation by N-ethylmaleimide suggest that conformational changes in CF_1 may control the rate of electron flow. At alkaline pH values, CF_1 in chloroplasts illuminated in the absence of added ATP or ADP undergoes conformational changes which may allow the leakage of H^+ from inside the chloroplasts, possibly through CF_1 itself. These conformational changes are sensitive

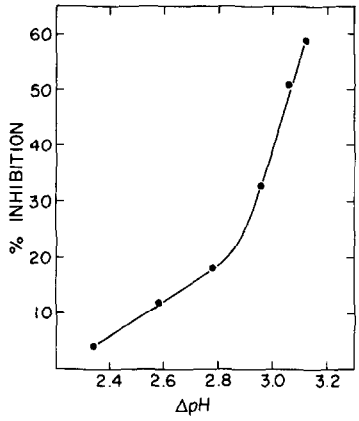

Figure 4. The dependence of the reaction of CF_1 with NEM on the magnitude of the pH gradient. The chloroplasts were illuminated at various light intensities in the microcentrifuge with and without 1 mM NEM for 2 minutes in reaction mixtures similar to those in Fig. 3 except that 40 mM Tricine-NaOH (pH 8.0) was used and 0.025 mM [^{14}C] hexylamine (1.30 µCi per µmole) and [^{3}H]sorbitol (0.5 µCi per 0.5 ml of reaction mixture) were also present. Aliquots (0.1 ml) were assayed for amine uptake during the illumination and for subsequent phosphorylation activity after dilution to 2.0 ml with a reaction mixture similar to that used in Fig. 3. There was no effect of NEM on ΔpH. Control phosphorylation rates varied from 485 after illumination at the highest light intensity used to 690 at lower light intensities.

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to the pH of the suspending medium, the magnitude of the pH gradient, and to low concentrations of ATP or ADP [14]. The pH dependence of basal electron transport in chloroplasts may be rationalized in terms of the changes in CF_1. At pH 7.0 where the rate of electron flow is low, CF_1 cannot assume its altered conformation. As the pH is elevated, CF_1 alters its conformation at values of ΔpH of 2.8-2.9 or above, resulting in an increased rate of H^+ efflux from the chloroplasts. However, ΔpH is essentially independent of the external pH over the range of 7.0 to 8.5 [19]. To compenstate for the increased rate of H^+ efflux at more alkaline pH values, the rate of electron flow must also rise. Thus, CF_1 may be considered as a gated translocator of H^+.

The induction of Mg^{++}-dependent ATPase activity in chloroplasts by light and sulfhydryl compounds probably involves a conformational change in CF_1 [20].

The pH dependence of the activation of the ATPase[2] is similar to that of basal electron flow and the ability of N-ethylmaleimide to inhibit phosphorylation.

The magnitude of ΔpH generated by non-cyclic electron flow from water to methylviologen is lower than that generated by pyocyanine-dependent cyclic electron flow [10]. Consequently, the maximal inhibition of phosphorylation by N-ethylmaleimide observed with methylviologen-supported electron flow is lower than that obtained with pyocyanine (44 vs. 67%)[3] in agreement with the finding that the reaction of CF_1 with N-ethylmaleimide is very sensitive to the magnitude of ΔpH. The sensitivity of the inhibition to uncouplers [12] also suggests that conformational changes in CF_1 occur maximally at high ΔpH values.

Although we have emphasized the role of energy-dependent conformational changes in CF_1 in regulating electron flow, it is quite possible that these changes are involved in phosphorylation. The pH dependence of phosphorylation is similar to that of basal electron flow and the reaction of CF_1 with N-ethylmaleimide. Even though the magnitude of ΔpH at pH 7.0 is the same as that at pH 8.0, the rate of phosphorylation is much reduced at the lower pH. Therefore, it is the terminal step of phosphorylation itself, rather than generation of the high energy state which is sensitive to the pH of the medium. Since CF_1 is exposed to the medium and catalyzes the terminal step in ATP synthesis [21], it could be the pH-sensitive component.

Phosphorylation, like the reaction of CF_1 in chloroplasts with N-ethylmaleimide, is very dependent on the magnitude of ΔpH [10]. Thus, conformational changes in CF_1 may be obligatorily involved in phosphorylation. Boyer *et al.* [22] and Cross and Boyer [23] postulated that conformational changes in the coupling factors of mitochondria, chloroplasts, and bacteria are the major energy-requiring steps in phosphorylation. It was proposed that these changes reduce the affinity of the coupling factors for newly synthesized ATP. Although the correlations be-

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[2] R.E. McCarty, unpublished observations.

[3] R.P. Magnusson, unpublished observations.

tween conformational changes in CF_1 and phosphorylation obtained both in this laboratory and in Jagendorf's [17,18], are consistent with Boyer's concept, it is possible that the conformational changes have a function secondary to the mechanism of phosphorylation. For example, CF_1 may have to assume a certain conformation before it can actively catalyze photophosphorylation.

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