ACID–BASE DRIVEN REVERSE ELECTRON FLOW IN ISOLATED CHLOROPLASTS

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1. Introduction

ATP driven reverse electron flow in isolated lettuce chloroplasts has been recently demonstrated in our laboratory [1,2]. Upon addition of ATP, the light triggered Mg^{++} dependent ATPase activity of chloroplasts drives both the reduction of Q, the primary acceptor of photosystem II, and the oxidation of cytochrome *f*. The reduction of Q was measured as the rise in chlorophyll fluorescence yield at low intensity monitoring light.

The chemiosmotic theory [3] predicts that ATPase can affect the electron transport only through the formation of a transmembrane proton gradient. Rather large proton gradients were indeed demonstrated in chloroplasts to be induced by either electron transport [4] or light triggered ATPase [5]. ATP driven reverse electron flow [1,2] would thus be viewed as effected via the pH gradient formed by the ATPase activity. By this mechanism, it should be possible to drive reverse electron flow directly by an artificially induced pH gradient. Such a phenomenon was indeed observed and is reported on in the present communication.

2. Materials and methods

Chloroplasts were isolated from lettuce leaves [6] and chlorophyll concentration determined [7] as previously described. Acid–base transition was carried out in 1 cm square cuvettes. The standard acidic reaction mixture contained in 2.0 ml: maleic acid pH 5.3, 3 mM; $MgCl_2$, 10 mM; KCl, 30 mM; and chloroplasts containing about 40 μg chlorophyll. The transition to the base stage was effected by injecting 0.2 ml 1 M Tris–KOH of a predetermined pH so as to result in a final pH of about 9.5. The initial and final pHs were checked for each set of experiments. Fluorescence changes were measured in the apparatus constructed by Dr S. Malkin [8] as described [2].

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3. Results

The changes in chlorophyll fluorescence yield of isolated chloroplasts induced by an acid–base transition are illustrated in fig.1. After fluorescence

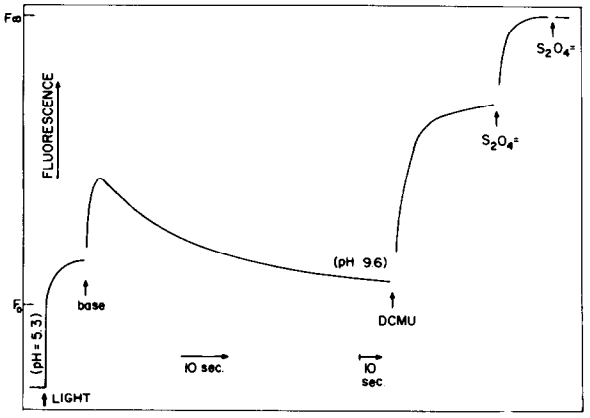

Fig.1. Acid–base driven reduction of Q. Conditions as described under Methods. Chlorophyll concentration, 20 μg/ml; DCMU added to a final concentration of 1.5 μM; Measuring light intensity, 950 ergs × cm^{-2} × sec^{-1}. $S_2O_4^{2-}$, indicates the addition of few grains of $Na_2S_2O_4$.

reached its steady state level, with low intensity measuring light [2] in the acid stage, injection of the base induced a rapid increase in the fluorescence yield followed by a slow decay down to a new steady state level. The rise in fluorescence was completed within 2–3 sec, but the decay required more than one minute for completion. The extent of the maximal rise corresponded to a reduction of 37% of Q, based on the premise that Fo represents a fully oxidized Q and F_∞ (induced by addition of sodium dithionite) a fully reduced Q [9]. Fo was measured at the acid stage, but as was previously reported Fo is insensitive to pH changes in the range of 5 to 10 [10].

Fig.2 shows that the fluorescence rise peak had an optimum pH in the acid stage of around 5.5 and in the base stage of around 9.3. Since the chloroplasts aggregated at pH's below 5 and above 10, the values beyond these limits may be artifactual.

The components required for the acid–base driven Q reduction are indicated in table 1. It can be seen that the activity was not dependent upon the nature of the buffer used in the acid stage, with succinate maleate, or aspartate (or none at all) being about equally effective. There was a requirement for $MgCl_2$ and salt, with high (10 mM) $MgCl_2$ being able to fulfill both requirements. The acid–base induced reduction of Q was sensitive to electron transfer inhibitors like DCMU (fig.3). In order to avoid the rapid rise in fluorescence yield caused by DCMU under the normal experimental conditions ([9], fig.1) a considerably weaker measuring light was employed in this experiment. A DCMU induced slow rise in fluorescence yield can still be observed in fig.3, plus the DCMU induced instantaneous 'jump' in fluorescence

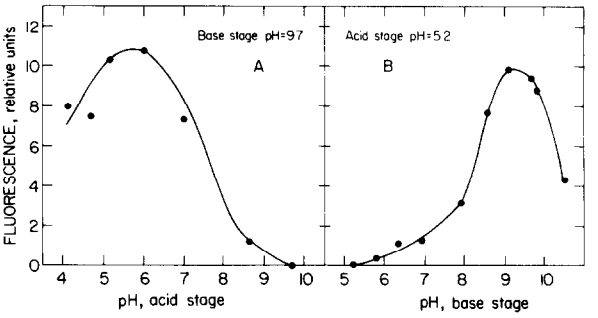

Fig.2. pH optima of the acid and the base stages. The reaction mixture of the acid stage contained in 2 ml: succinate, 2 mM; $MgCl_2$, 5 mM; KCl, 50 mM; sorbitol, 400 mM; and chloroplasts containing 40 μg chlorophyll. pH was preadjusted by the addition of HCl or KOH to the acid reaction mixture or to the Tris, as indicated. Light intensity, 950 ergs × cm $^{-2}$ × sec $^{-1}$. The ordinate expresses the difference between the peak of the fluorescence rise and the steady state level after complete decay in the base stage.

Abbreviations: DCMU = 3-(3,4-dichlorophenyl)-1,1 dimethylurea; NQNO = 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide; DTT = Dithiothreitol; PMS = Phenazine methosulfate.

Table 1
Component requirement of acid–base induced reverse electron flow

Components added (mM)	Maximal Fluorescence Increase (rel. units)	ΔQ (%)
KCl (30), $MgCl_2$ (10), maleate (5)	280	32
KCl (30), $MgCl_2$ (10), maleate (1)	275	34
KCl (30), $MgCl_2$ (10), succinate (5)	373	38
KCl (30), $MgCl_2$ (10), aspartate (5)	360	37
KCl (30), – maleate (5)	60	11
KCl (30), $MgCl_2$ (1), maleate (5)	138	22
– $MgCl_2$ (10), maleate (5)	350	35
– – maleate (5)	23	3

The components of the acid stage varied as indicated, keeping pH at 5.3. Base pH was 9.5. Maximal fluorescence increase was calculated as in fig.2. 100% Q was measured for each sample after 1 min far red light (details as under fig.4) in order to obtain the real Fo. Exciting light intensity, 950 erg × cm $^{-2}$ × sec $^{-1}$; chlorophyll, 26 μg/ml.

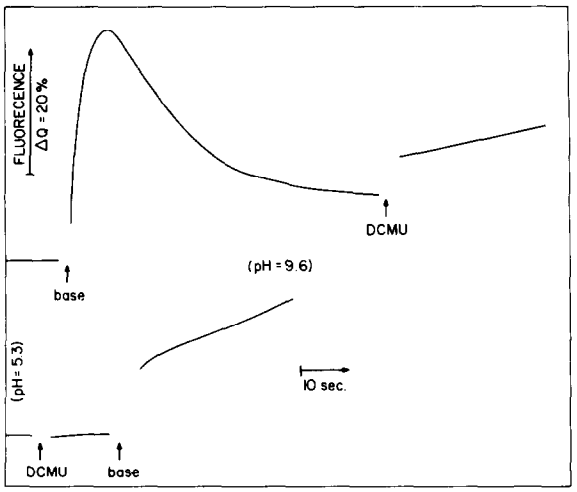

Fig.3. DCMU inhibition of acid–base induced Q reduction. Conditions as described under Methods. Chlorophyll concentration, 22 μg/ml; DCMU concentration 10 μM; light intensity 48 erg × cm $^{-2}$ × sec $^{-1}$. 100% Q determined as in table 1.

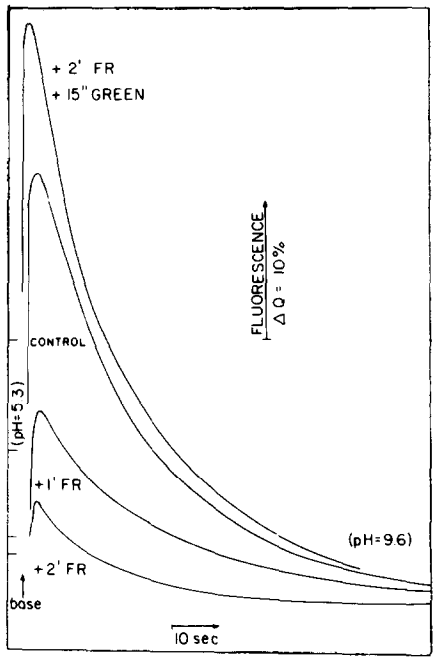

Fig.4. Effect of pre-illumination with far red and green light on acid–base driven reverse electron flow. Reaction mixture as described under Methods. Chlorophyll concentration, 19.5 μg/ml; Far red light obtained from a Xenon lamp filtered through a 724 nm interference filter. Green light obtained from the same lamp but filtered through 545 nm interference filter Light intensities on the surface of the cuvette in erg × cm $^{-2}$ × sec $^{-1}$ were around 3 × 10^3 for the far red light, 6 × 10^4 for the green light and 950 for the measuring light.

previously observed [2], but the acid–base induced signal was completely abolished. Similar inhibition but at higher inhibitor concentration was obtained with NQNO and *O*-phenanthroline.

As can be expected, the acid–base driven reverse electron flow, unlike the ATP driven reaction [2], did not require light triggering, DTT or PMS. However, the lack of requirement for an added electron donor was not expected and suggested that the endogenous reduced intermediates provided sufficient electrons for the reverse electron flow pulse. This interpretation is supported by the data of fig.4 which show that pre-illumination with far red light, which should result in the oxidation of the electron carriers between the two photosystems, severely inhibited the acid–base driven reverse electron flow, and the effect was fully reversible by a following short green light preillumination.

4. Discussion

The data presented provides a new demonstration of the coupling between electron transport and the ΔpH across the thylakoid membrane. Acid–base transition in isolated chloroplasts, induces a DCMU sensitive reduction of Q, as indicated by the increase in chlorophyll fluorescence yield. This increase is rapidly induced, and decays slowly. The decay does not seem to reflect the dissipation of ΔpH but rather the reoxidation of Q^- since it was considerably slower than the decay of ΔpH, relatively insensitive to uncouplers and very sensitive to oxidants (Shahak, Pick and Avron, data not shown). The lack of a requirement for an acidic buffer indicates that no significant internal buffer capacity to maintain the ΔpH across the membrane for a relatively long time was necessary, as is the case in acid–base induced ATP formation [11]. This again supports the interpretation that the reverse electron flow requires only a very short timed ΔpH across the membrane, and the decay of the Q^--produced is no longer dependent upon the maintainance of the ΔpH.

There are several differences between the ATP driven [1,2] and the acid–base driven reverse electron flow. In addition to requiring light triggering, the former seems to slowly increase the fluorescence yield to a high steady state which is maintained for as long as the ATPase is active and an electron donor is available. In the latter, the driving force seems to be of a very short duration, inducing a pulse of electrons to flow backwards. For that pulse the reduced intermediates which have been shown to exist in chloroplasts [12] seem to provide sufficient electrons, and therefore no requirement for an external electron donor was observed unless the electron carrier pool had been pre-oxidized (fig.4).

In summary, a DCMU sensitive reverse electron flow driven by artificially formed ΔpH was demonstrated. This ΔpH driven reverse electron flow reaction provides a new tool for further studies of the localization and mechanism of the coupling reaction along the electron flow chain of isolated chloroplasts.

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