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THE INVOLVEMENT OF A HIGH POTENTIAL ACCEPTOR IN THE ACID-BASE INDUCED REDUCTION OF THE ACCEPTOR Q IN CHLOROPLASTS *

HAIM HARDT

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 (U.S.A.)

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Summary

Study of the acid-base induced fluorescence transient, the so-called 'reverse electron flow', in chloroplasts revealed the following new properties:

(1) Experiments in which the acid-base transition was performed in the absence of the measuring beam showed that the high fluorescence state induced by a pH-jump was attained and decayed even in complete darkness. These results indicated that a non-photochemical electron transfer was affected by the pH transition. A pH-induced reverse electron transfer from some secondary electron acceptor to Q probably occurred during that process.

(2) This conclusion was supported by the effect of the Photosystem I electron acceptor methyl viologen. Methyl viologen accelerated the decay phase of the transient showing that this phase was controlled by the rate of electron flow to Photosystem I, but this acceptor did not diminish the size of the transient's initial rise, probably because this rise reflected a pH effect on a non-photochemical step located between Q and P-700.

(3) The size of the fluorescence transient was dependent upon the reduction state of both parts of the secondary pool of electron acceptors, A_2 and A_1.

(4) Redox potential measurements using ferricyanide-ferrocyanide mixtures showed that the size of the transient was directly dependent on a midpoint potential of +385 mV at pH 6.9 and with $n = 1$. This suggested the involvement of a high potential secondary electron acceptor in the acid-base induced reduction of Q.

* Dedicated to the memory of Dr. Bessel Kok (1918—1979).

(5) The restoration of the acid-base transient in long-time dark-adapted chloroplasts was demonstrated by using additional dark incubation with appropriate couples of reductants and lipophilic mediators. Most significant was the restoration of the full size of the transient by the couple ascorbate + diaminodurene which poised a potential of +100 mV, a potential at which the A_2 pool of 5 equivalents stayed completely oxidized in the dark. I conclude that the source of reducing equivalents for the 'reverse reduction of Q' was either in the high potential pool A_1 at the level of cytochrome *f* and plastocyanin or in a similarly high-potential side component.

Introduction

It is generally accepted that the fluorescence yield of chlorophyll *a* in isolated chloroplasts reflects the reduction state of Q, the primary electron acceptor of Photosystem II [1]. The dependence of the reduction level of Q on the oxidation state of A, the secondary pool of electron acceptors in-between the photosystems was analyzed in detail [2,3,4]. This pool has been assumed to be composed of a low potential part A_2 (about 5 equivalents) and of a high potential part A_1 (5—10 equivalents) [3]. In contrast with this assumption, the more recent data of Goldbeck and Kok [5] show both parts of the pool to titrate together as a single two-electron component with a midpoint potential of +106 mV. These latter results agree with the identification of A as a mostly plastoquinone pool, but do not account for the segment of cytochrome *f* and plastocyanin which has to be part of the high potential A_1 pool.

Shahak et al. found that by subjecting lettuce chloroplasts to an acid-base transition the fluorescence yield shows a temporary increase [6]. They suggested that the momentary pH difference across the thylakoid membrane induces a pulse of 'reverse electron flow' towards Q, causing its temporary reduction. This effect is different than the previous ATP driven 'reverse electron flow', which is completely dependent upon the ATPase activity of the chloroplasts [7].

The observation that far-red preillumination abolishes the acid-base induced transient indicates that the pool of electron carriers A may be the source of reducing equivalents for the 'reverse electron flow' [6]. In the present work, I investigated this proposed mechanism and some other factors which determined the capacity to produce an acid-base induced fluorescence transient. In addition, I describe the involvement of a high-potential component (+385 mV) in this process.

Materials and Methods

Spinach chloroplasts were prepared as described before [8]. The fluorescence measurements were carried out in the apparatus described earlier [9] in which the emission was collected from the front face of the irradiated sample, detected by an S-20 photomultiplier and recorded on a Brush recorder with a time resolution of 50 ms. The chloroplasts were used at concentrations

of 10—15 μg chlorophyll per ml. The acid stage medium of most experiments contained 30 mM KCl, 10 mM $MgCl_2$, 5 mM maleic acid (or succinic acid) at pH 6.0 in a volume of 2.25 ml. 0.4 M sorbital was added to this medium during the course of this study. The pH was raised to 9.0 by injection of 0.25 ml 1.0 M Tricine base. The base stage of a base to acid control experiment contained 30 mM KCl, 10 mM $MgCl_2$ and 10 mM Tricine buffer at pH 8.8 in 2.25 ml. The pH was lowered to 5.7 by injecting 0.25 ml of 1.0 M succinic acid at pH 4.0. Initial and final pH values of each experiment were routinely checked using a Corning combination electrode. The redox potential was measured with a pair of platinum and calomel electrodes. Their calibration was checked by a saturated solution of quinhydrone at pH 4.0 and 7.0.

The full light intensity of the measuring beam was 10—15 equivalents/s per trap. The calibration in equivalents/s per trap units was done by measuring the half-time of the exponential fluorescence rise after 1 min dark and by assuming: (a) the presence of 5 oxidizing equivalents in the A_2 pool and (b) validity of the Ixt relation for the photoreduction of this pool [2]. The exact number of equivalents was confirmed from the ratio of the area above the fluorescence curve after 1 min dark to the area after 2 min far-red preillumination and addition of 10 μM DCMU (the area presumably proportional to 1 equivalent). Lower light intensities were obtained by using neutral density filters made of fine metal screens. In most acid-base experiments the beam intensity was about 1 equivalent/s per trap. Far-red light was obtained from a 150 W lamp using a 720 nm interference filter with a bandwidth of 5 nm.

In the following text I denote ΔF_{AB} as the size of the fluorescence transient induced by an acid-base transition. ΔF_{BA} is denoted as the size of change induced by a base-acid transition. ΔF_{AB} is expressed as percentage of the variable fluorescence $F_v = F_{max} - F_0$ (see Fig. 1) and since F_0 may be defined as zero ΔF_{AB} is then expressed as percentage of F_{max}. ΔF_{AB} is expressed in some figures as percentage of the maximal transient (max ΔF_{AB}).

Results

Description of the acid-base fluorescence transient

The protocol of a standard acid-base experiment began by illuminating the chloroplasts 10 s with strong saturating light which reduced the pool A. This was followed by a dark period of 1.5 min during which Q and A_2 were reoxidized and the fluorescence yield returned to F_0. The weak measuring light of about 1 equivalent/s per trap was then turned on and the fluorescence was recorded (Fig. 1A). Such a weak beam could not photoreduce Q fast enough and therefore after about 30 s the fluorescence steady state (F_i) was only 10% above the F_0 level. At this point the base was injected and caused a rise of 32% in the fluorescence yield ($\Delta Q = 32\%$). The rise was completed within 1 s and was followed by a slow decay with a half-time of 8 s to a new steady state. The maximal level F_{max} (100%) was reached by addition of a few crystals of dithionite, which showed that Q was still reducible. The scale of variable fluorescence F_v was determined from F_0 in the acid to F_{max} in the base.

Fig. 1B shows a fluorescence transient on a more sensitive scale. The rise

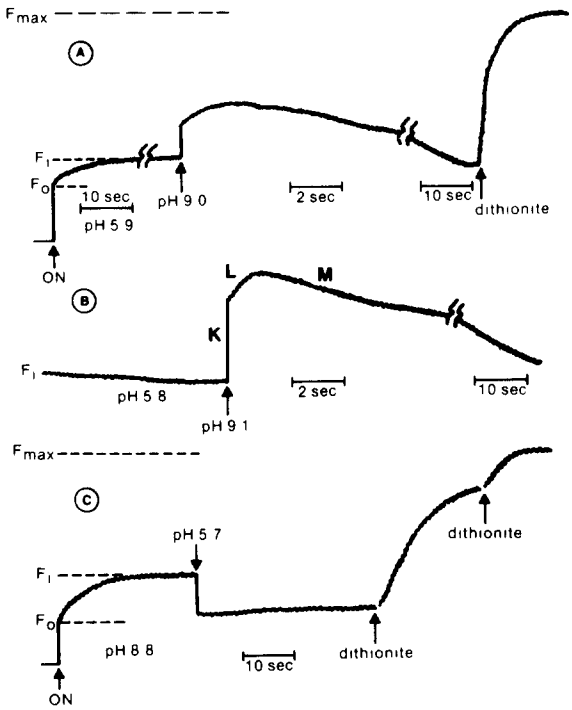

Fig. 1. Acid-base induced fluorescence transient and the control base-acid fluorescence change. Protocol and conditions for the acid-base and the base-acid experiments were as described in Materials and Methods. A. Display of the acid-base transient on the full scale of variable fluorescence from F_0 at pH 5.9 to F_{max} at pH 9.0. Q reduced = ΔF_{AB} = 32% of F_{max}. B. Display of the acid-base signal on a more sensitive recorded scale and the definition of its three kinetic phases K, L, M. C. Base-acid fluorescence drop on the scale of variable fluorescence from F_0 at pH 8.8 to F_{max} at pH 5.7. ΔF_{BA} = —25% of F_{max}. The illumination stages were the same as in acid-base experiment.

was composed of two phases, a fast phase K and a slow phase L. The ratio between these two phases was variable and was dependent upon factors such as the light intensity, the acid pH and the presence of electron acceptors in the medium. The decay phase M had a half-time which normally varied between 10 to 30 s. In several control experiments (done at different light intensities) such as that shown in Fig. 1C, I subjected the chloroplasts to a base-acid pH transition (pH 8.8 to 5.7) and observed an immediate drop in the fluorescence yield instead of a rise.

Effect of different electron acceptors

I measured the acid-base transient in the presence of two Photosystem I electron acceptors, potassium ferricyanide and methylviologen and found a marked difference between their effects on both its magnitude and shape. Whereas 40 μM ferricyanide added to the acid stage inhibited completely the transient, 50 μM methylviologen did not affect the magnitude of the rise but accelerated the decay of phase M. This effect became more pronounced at higher light intensities and the graphs in Fig. 2A show the results of a set of experiments where I, the intensity of the measuring beam, was varied from

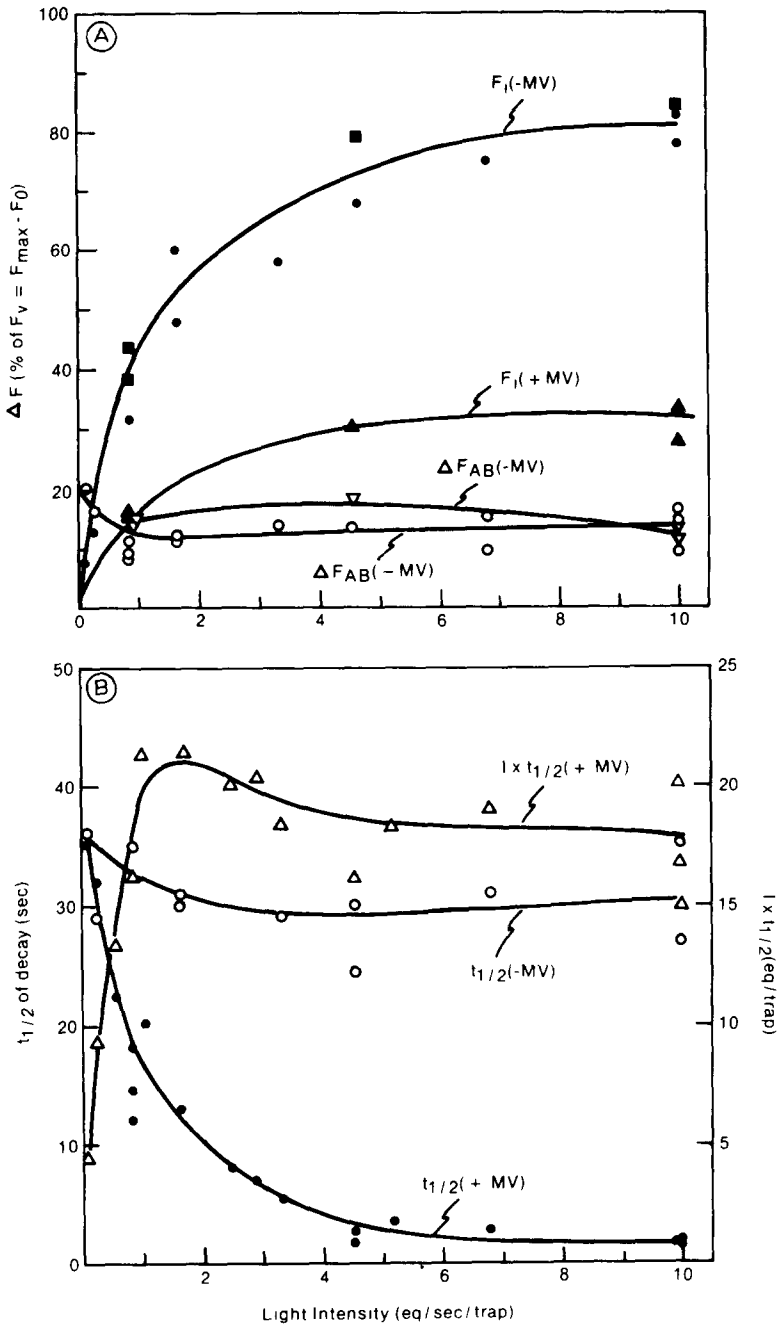

Fig. 2. The effect of methyl viologen on the parameters of the acid-base transient at different light intensities. The protocol of the experiments and the calibration of the measuring beam intensity in equivalents/s per trap were as described in Materials and Methods. 40 μM methyl viologen was added to the acid stage; the pH values of transition were 6.0 to 9.0. A. The dependence of F_i and ΔF_{AB} on the light intensity in the presence or absence of methyl viologen. The squares represent results from a different date. B. Dependence of the half-time of decay ($t_{1/2}$ of phase M) on the light intensity in the presence or absence of methyl viologen. The data is from the same experiments as in A. Also shown is the dependence of the product $Ixt_{1/2}$ on the light intensity for the experiments with methyl viologen.

less than 1 to more than 10 equivalents/s per trap. Over this range the steady state fluorescence F_i rose to 80% of F_{max} and the amount of Q^- at each intensity was roughly about 10% higher than F_i (assuming a probability of 0.5 for excitation transfer between different photosynthetic units) and approached 90% of Q_{max} at the highest intensity. Over this range, however, the magnitude of the acid-base signal ΔF_{AB}(−methyl viologen) remained practically unchanged (10—15% of F_v). This constancy of ΔF_{AB} was in marked contrast to the dependence on intensity seen in the photoreduction of Q. This reflected the non-photochemical nature of the acid-base induced process. The same figure shows the graphs from parallel experiments in which methyl viologen was added. F_i at each intensity was lower than in the absence of methyl viologen and approached 33% of F_{max} at the highest intensity. This lowering was expected because the rate limitation imposed on the electron transport by the Mehler reaction was removed by methyl viologen. Still, as observed in Fig. 2A, the magnitude of the acid-base signal ΔF_{AB}(+methyl viologen) remained unchanged and independent of the light intensity.

Fig. 2B shows the dependence of the half-time of decay (phase M) on I in the same set of experiments. In the absence of methyl viologen there was almost no change in the half-time of decay over this range of intensities (except for very weak light where it was expected to drop) whereas in its presence, the decay became faster at higher light intensities. For the experiments done in presence of methyl viologen the plot of $1/t_{1/2}$ of the decay against the intensity revealed a linear dependence (not shown) and consequently the product $I \cdot t_{1/2}$, shown in Fig. 2B, was constant over the range of about 1 to 10 equivalents/s per trap. This indicated that the decay of the transient in presence of methyl viologen was affected by the photooxidation of Q via Photosystem I, a proposition which was supported by the observation that the linear plot of $1/t_{1/2}$ against I crossed the origin of the coordinates as is expected in a photochemical effect. The value of the product $I \cdot t_{1/2}$ was equal to 19 equivalents/trap, which showed probably the number of equivalents which were removed via Photosystem I before a new steady state of Q was approached. The ability to observe the acid-base induced transient in the presence of methyl viologen implied that the transient did not originate from a momentary inhibition of the Mehler reaction or of another reaction on the acceptor side of Photosystem I. Similarly, the ability to observe the signal in the presence of 10 mM hydroxylamine argued against an pH effect on the donor side (Z) of Photosystem II. Rather, an effect on the electron transport in between the two photosystems seemed to be more likely. The independence of the transient on the intensity of the measuring light shown in Fig. 2A also implied that the acid-base transition affected a dark reaction and not a photochemical event.

Correlation with the oxidation state of pool A

To estimate the relation between the oxidation state of the pool A and the size of the acid-base transient I varied the dark period between the preillumination and the fluorescence measurement. Since no electron acceptor was present, the preillumination raised the fluorescence to the F_{max} level. During the following dark period the capacity to induce a signal (by the injection

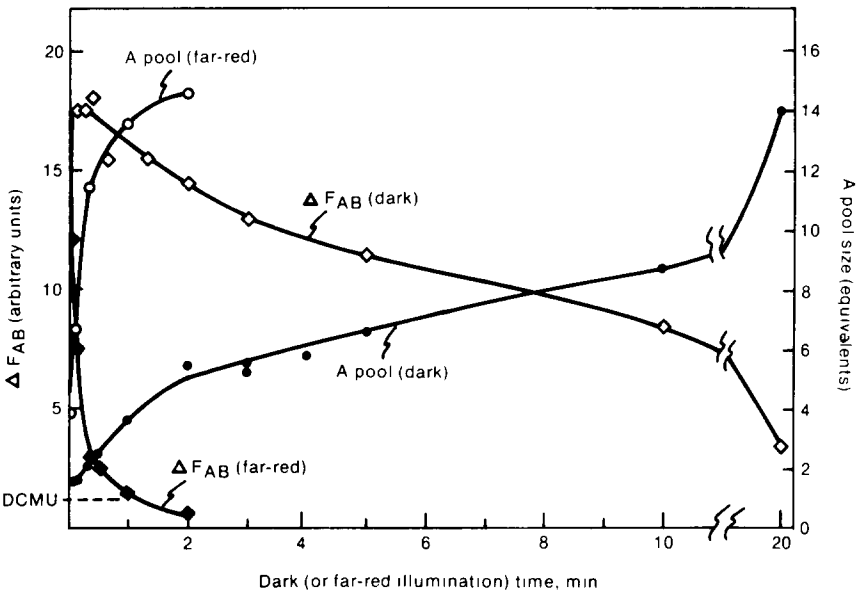

Fig. 3. The decay of the capacity of the acid-base induced transient (ΔF_{AB}) and the restoration of the oxidized pool A during the dark period after the preillumination. The effect of intervals of far-red light introduced during the dark time is also shown. The pH values of transition were 6.2 to 9.0.

of base) diminished with a time course as shown in Fig. 3. In a parallel experiment, using the same batch of chloroplasts, I measured the area above the fluorescence induction curve as a function of the dark period. The increase in the area reflects the reoxidation of the pool A. The dark reoxidation was biphasic, one part was oxidized in 1—2 min (A_2) and the other required 10—20 min (A_1). As seen in Fig. 3 the reoxidation of the pool in the dark was parallel to the decrease in the capacity of acid-base signal and the phases corresponding to A_2 and A_1 were also apparent in the decrease of the acid-base signal. The rate of the oxidation of the pool is accelerated by introducing during the dark a short period of far-red illumination which activates Photosystem I [2]. As seen in Fig. 3 the far-red light was similarly effective in decreasing the capacity of the acid-base induced transient. The number of equivalents in the pool which was oxidized parallel to the disappearance of the capacity for the acid-base transient was about 15.

The stability of the acid-base state in the dark

Did the acid-base transition create a new state when carried out in complete dark? To test this possibility I altered the experimental procedure: initially the standard protocol was followed (10 s preillumination; 1.5 min dark) however, the measuring beam was applied only about 3 s after the injection of the base in the dark (Fig. 4B). The fluorescence transient in this case was still composed of a fast rise and a slow rise to a maximum followed by a slow decay to a steady state. By comparing this transient to the transient of a normal experiment such as the one shown in Fig. 4A one can see that the rise contained the fast initial components of a fluorescence induction, $F_0 + F_i$,

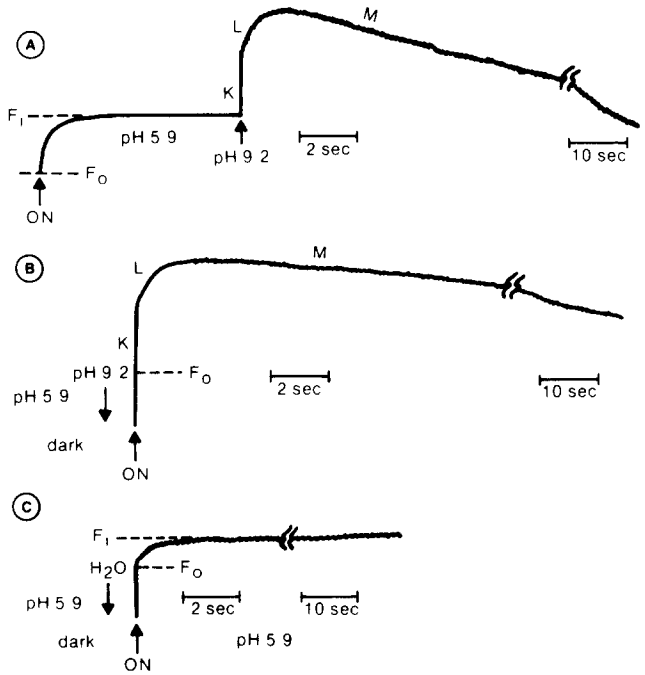

Fig. 4. Demonstration of the attainment of an acid-base induced state in complete dark. The pH values of transition are shown in the figures. The sensitivity was the same in A, B and C. A. A standard acid-base induced fluorescence transient. B. Fluorescence rise following the injection of the base in the dark. F_0 level was estimated from the control experiment shown in C. The protocol: 15 s preillumination, 1.5 min dark, injection of base during the dark, application of the measuring light. C. Fluorescence rise following the injection of water (instead of base) in the dark. The protocol was the same as in B.

and the contribution of the acid-base signal, i.e., phases K and L which were described in Fig. 1. The magnitude of the additional fluorescence components which originated from the dark pH transition (i.e., the size of the components K + L) was estimated by measuring the difference, ΔF, between the signal maximum and the following steady-state level attained at the end of phase M. A different way to demonstrate the contribution of a dark acid-base component was via a control experiment in which instead of base an equal volume of water was injected in the dark (Fig. 4C). The fluorescence rise curve was composed in this case simply of F_0 and F_i (at pH 6.0). Using this control for comparison, one can estimate the acid-base component above the F_i level in Fig. 4B to be about 20% of F_{max}.

By allowing progressively longer dark periods to elapse between the injection of the base and the application of the weak measuring beam I measured the decay of the 'acid-base induced state' in the dark at the high pH. In practice I followed in a set of such experiments the disappearance of the estimated acid-base component. As shown in Fig. 5 the half-time of decay was about 20 s (at pH 9.0). I compared this decay to the more characterized time course of dark reoxidation of Q and of A following their reduction by a light saturating period of 10 s. The values of the reduced Q and A were computed in the same way as described by Malkin [4]. The decay in the dark of the 'pH-

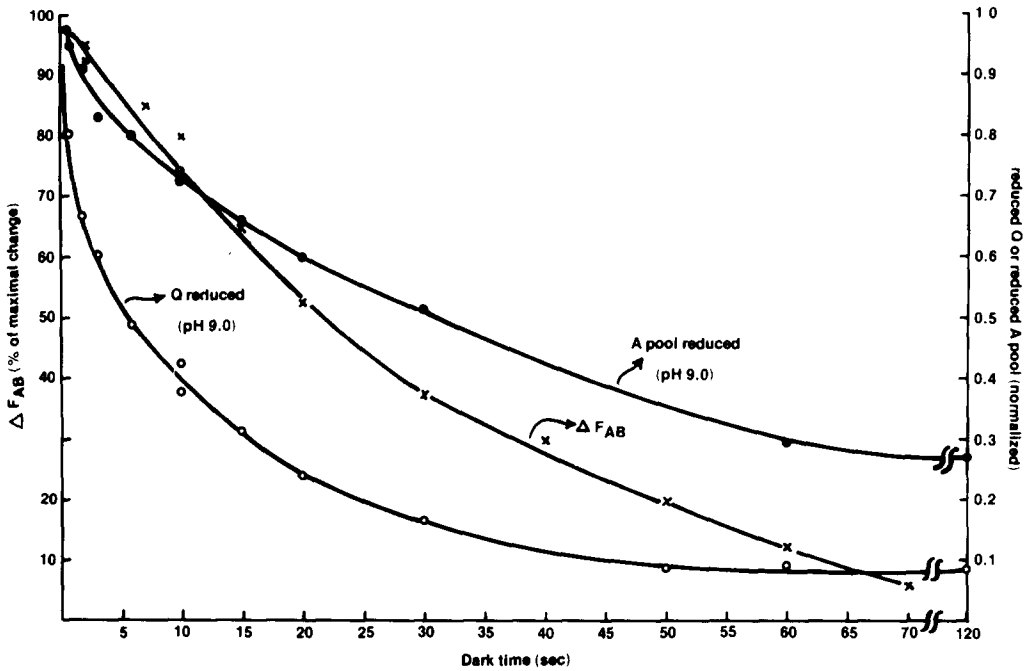

Fig. 5. Dark decay of the acid-base induced state built in complete dark at pH 9.0. Acid-base transition was performed as descrined in Fig. 4B; plotted on the abcissa is the dark time between the injection of the base and the application of the measuring beam. The size of ΔF_{AB} was estimated as explained in Fig. 4 by using the water injection control. The pH values of transition were 6.0 to 9.0. The dark reoxidation of Q and of the pool A at pH 9.0 is shown for comparison.

induced state' was slower than the reoxidation of Q^- and faster than that of A^- at pH 9.0. Its half-time value of 20 s resembled the half-time of decay of phase M which varied between 10 to 30 s in very weak measuring light. The attainment of a pH-induced fluorescence state in complete dark and its further decay in the dark indicated also that Q was temporarily reduced through an electron transfer that involved a non-photochemical step, i.e. via a secondary dark reaction.

Redox potential measurements of the acid-base transient

The observation that in addition to far-red light and to dark adaption, low concentrations of ferricyanide were able also to diminish the acid-base transient prompted me to measure the magnitude of the signal as function of different ratios of ferrocyanide/ferricyanide. Varying this ratio changes the redox potential of the medium without affecting the pH values of the transition. Fig. 6 shows a plot of dependence of the acid-base signal size on the redox potential in experiments in which the pH transition was from 6.9 to 9.2. The results fitted a theoretical titration curve of one electron with a midpoint potential of +397 mV at pH 6.9. Similarly, the results from a set of pH 4.9 to 9.1 transition experiments and a set of pH 5.9 to 9.1 transition experiments fitted theoretical curves with midpoint potentials of +360 mV and +408 mV, respectively (data not shown). These results indicated the involvement of a high-potential com-

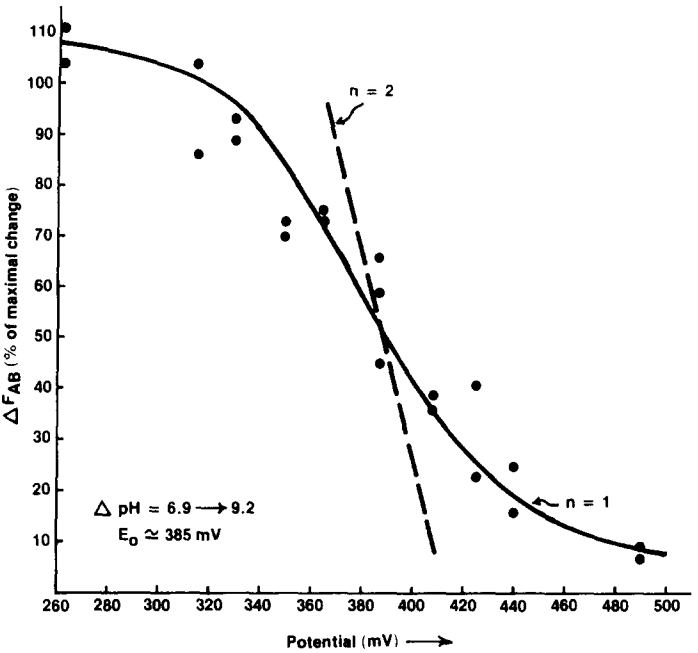

Fig. 6. Effect of the redox potential on ΔF_{AB}. The redox potential was poised by varying the ratio of ferrocyanide/fericyanide, keeping the combined concentration constant at 2.5 mM. The standard protocol was followed except that 50 µl of ferrocyanide/ferricyanide was added about 20 s after the F_i level was attained and the base was injected 20 s later. The full line represents the theoretical titration curve of a one-electron component, the broken line represents that of a two-electron component. E_0 is the midpoint potential of the theoretical curve.

ponent and were surprising in view of the low midpoint potential of Q, which is around 0 mV [10].

The possibility to affect the magnitude of the transient by setting the appropriate redox potential led me to check whether it was possible to produce a signal in long-term dark-adapted chloroplasts by additional incubation at a reducing potential, thereby excluding the step of preillumination. Table I shows the effects of additional dark incubation (of 30 min dark-adapted chloroplasts) in the presence of reducing agents such as dithiothreitol or ascorbate with or without a low concentration of a lipophilic mediator. It is obvious that incubation either in dithiothreitol or in ascorbate alone was not sufficient to induce the capacity for an acid-base transient in spite of the low potential of the suspension (—200 and +80 mV, respectively). This implied that the electron carriers which were responsible for the acid-base signal were not accessible to a direct reduction by dithiothreitol or by ascorbate probably because they were embedded in the thylakoid membranes. I tried, therefore, to couple these reductants with low concentrations of lipophilic mediators which penetrate into thylakoid membranes. With dithiothreitol as a reductant I found that addition of low concentrations of menadion (2-methyl-1,4-napthoquinone), 1,4-napthoquinone, or 2-hydroxy-1,4-naptholquinone enhanced the capacity for the acid-base signal following a 4 min dark incubation. Other mediators such as 1,2-napthoquinone or methylene blue were not effective, not even when I used longer times of incubation such as 10 min or more. With

TABLE I

INDUCTION OF THE ACID-BASE SIGNAL IN LONG-TERM DARK-ADAPTED CHLOROPLASTS BY ADDITIONAL DARK INCUBATION WITH REDUCING AGENTS

Chloroplasts were dark-adapted for more than 30 min; the preillumination stage was excluded. The pH values of transition were 5.8 to 9.1. The potential was measured in the acid stage.

Reducing agent	Concn.	Incubation time (min)	Redox potential (mV)	ΔF_{AB} (% of F_{max})
No addition	—	4	+300	0
Dithiothreitol	8 mM	4	—200	12
Dithiothreitol + 1,4-napthoquinone	8 mM 20 μM	2	+100	44
Dithiothretol + 1,4-napthoquinone	8 mM 20 μM	4	+100	50
Ascorbate	16 mM	4	+80	8
Ascorbate + DAD	0.4 mM 0.4 mM	4	+200	26
Ascorbate + DAD	16 mM 0.4 mM	4	+160	42
Ascorbate + DAD + DCMU	16 mM 0.4 mM 6 μM	4	+160	5

ascorbate as a reductant I tried to couple mediators which have higher redox potentials and found that DAD (diaminodurene) was very effective. DCIP (dichlorophenol indophenol) was less and dimethylquinone, phenylenediamine and benzoquinone were least effective even at high concentrations (0.4 mM). Addition of DMCU during the dark incubation inhibited the acid-base signal as expected. As observed in Table I, the incubation in either dithiothreitol + 1,4-napthoquinone or in ascorbate + DAD induced the capacity for quite a high acid-base signal — of the order of 40—50% of F_{max}. The redox potential measured in these chloroplast suspensions was relatively high, +100 mV and +160 mV, respectively. Like the above redox titration results, these experiments indicated that the carrier, or pool of carriers which had to be reduced prior to the production of the transient were at a relatively high potential.

To determine the reduction state of the pool A at these potentials I carried fluorescence inducation measurements in presence of ascorbate + DAD which are summerized in Table II. In the presence of this redox couple alone (potential +170 mV) a pool of about 13 equivalents was photooxidized by far-red illumination via Photosystem I. Only part of this pool was rereduced in the following 10 min dark incubation. The remaining 5 equivalents of A_2 were still oxidized. As was shown above (Table I, line 7) dark incubation in the ascorbate + DAD system under aerobic conditions led to a high acid-base signal. Since A_2 stayed under these conditions in an oxidized state it could not have served as a source of reductants for the reduction of Q. The source of must had therefore been in another part of the pool and at a higher potential (A_1 ?).

The other lines in Table II summerize my attempts to find proper conditions under which the A_2 pool could be reduced too by dark incubation with

TABLE II

EFFECT OF REDUCING CONDITIONS ON THE SIZE OF THE ELECTRON ACCEPTOR POOL A IN CHLOROPLASTS

The size of pool A was calculated from the half-time of the fluorescence induction curve and normalized by the half-time in presence of DCMU (see Materials and Methods). The medium pH was 5.8; 1% glucose was present in the medium where shown; catalase and glucose oxidase were added by stirring a few crystals.

Reducing conditions	Redox potential (mV)	Dark or far-red condition	Dark or far-red time (min)	Pool size (equivalents)
Ascorbate, DAD	+170	far-red	2	13
Ascorbate, DAD	+170	dark	10	5
Ascorbate, DAD, glucose, glucose oxidase, catalase	+100	far-red	2	6
Ascorbate, DAD, glucose, glucose oxidase, catalase	+100	dark	10	1
Ascorbate, glucose, glucose oxidase, catalase	+90	dark	10	13
DAD glucose, glucose oxidase, catalase	+190	dark	10	15
Glucose, glucose oxidase, catalase	+260	dark	10	15

ascorbate + DAD. I succeeded in finding them by switching to anaerobic conditions. Anaerobiosis was attained by adding a mixture of glucose, glucose oxidase and catalase which removed the oxygen from the chloroplast suspension. Under anaerobic conditions plus the ascorbate + DAD reductant (potential of +100 mV) far-red light oxidized only a pool of 6 equivalents, as shown in Table II, but this pool was rereduced during a following 10 min dark incubation leaving only one equivalent not reduced, i.e. Q. In the three control experiments in which I omitted either ascorbate or DAD or both from the medium the complete A pool remained oxidized. These results showed that A_2 pool could be reduced by ascorbate under anaerobic conditions at a potential of +100 mV provided that a suitable lipophilic mediator was present.

Discussion

The profile of reduction of Q by an acid-base transition was found to be composed of three kinetic phases: K and L seen during the rise of a maximum and M the slow decay from that level. Preliminary experiments suggested that the slow rise phase, L, reflected a photochemical reaction involving probably Photosystem II. This was deduced from the linear dependence of 1/rise-time on the light intensity (data not shown). The decay phase M was affected by the photo-oxidation of Q via Photosystem I as deduced from the effect of methyl viologen (Fig. 2B). Unlike methyl viologen, which is a pure Photosytem I electron acceptor, ferricyanide is known to interact with the electron trans-

port segment in between the two photosystems. This interaction was probably responsible for the complete inhibition of the transient by 40 M ferricyanide, in contrast to methyl viologen which had no effect on the size of the acid-base signal.

In the absence of artificial electron acceptors the capacity for an acid-base signal diminished in the dark with a time course similar to that of the reoxidation of the pool A (Fig. 3). It appeared that both parts of this pool had to be reoxidized prior to the complete loss of the capacity for the signal. The dependence of the capacity on the reduction state of A_2 pool (plastoquinone) which has a redox potential of about +80 to 100 mV [5,11] may have been expected because the potential difference between Q and A_2 is only 40 or 100 mV (depending on the exact midpoint potential of Q which is discussed below). Not expected was the dependence on the reduction state of A_1 which presumably has a more positive potential. These results suggested therefore that reductants from A_1 participated in the acid-base induced reduction of Q.

The redox potential measurements (Fig. 6) indicated the probable involvement of a high potential component in the acid-base-induced process. The measured potential range of +360 to +408 mV of this component must be either in the segment of cytochrome *f* and plastocyanin (+360 to +370 mV range) or alternatively in a side chain. There was a resemblence in the potential of this component and that of the high-potential electron acceptor Q2 (E_0 = +400 mV at pH 7.0) described by Bowes et al. [12]. However, their Q2 is found presumably before the DCMU block in Photosystem II, like the 360 mV component described earlier by Ikegami and Katoh [13]. Further comparison was limited also by the fact that the pH dependence of the midpoint potential could be only roughly estimated in our study. From the few data points presented there seemed to be no dependence on the pH between 6.9 and 5.9 and a roughly −60 mV/pH unit dependence between pH 5.9 and 4.9.

Additional information on the redox state of the components involved in the acid-base induced reduction of Q was drawn from the dark 'restoration' of the acid-base signal by incubation in proper reducing systems (Table I). Of more interest was the couple with the more positive potential ascorbate + DAD (+160 to 170 mV). The acid-base induced reduction of Q was completely restored after 10 min incubation with this couple whereas the A_2 pool was maintained fully oxidized as seen from the second line in Table II. The source of reducing equivalents for Q was therefore either in a part of A_1 (cytochrome *f*-plastocyanin segment) or in some other component which was kept reduced at +170 mV by ascorbate + DAD. To enable us to explain the decay of ΔF_{AB} in the dark (Fig. 3) such a component would have to be reoxidized in the dark with a time course similar to the reoxidation of both parts of the pool A_2 and A_1.

The mechanism proposed by Shahak et al. [6] for the acid-base induced reduction of Q envisioned a momentary pH created across the thylakoid membrane as the driving force for this 'reverse reduction'. However the intensitivity to uncouplers (unpublished observations) does not support such a mechanism. In a tentative model if is possible that the acid-base transition affected pH-dependent reactions which are located on the opposite sides of the thylakoid membrane. Such reactions may involve plastoquinone and cyrochrome *f* on the

inner face and another part of the plastoquinone pool and B or R on the outer face of the membrane. An acid-base transition could have led to an instantaneous de-protonation of those carriers which are on the outer face and thereby may have shifted the redox equilibrium between Q and such carriers to a new state, forcing Q to undergo a temporary reduction which was viewed by the fluorescence transient.

Q is presently suggested to have a redox potential around 0.0 V at pH 7.0 and a pH dependence of −60 mV/pH unit [10]. More recently Golbeck and Kok titrated Q at a midpoint potential of +68 mV at pH 7.3 and with the same pH dependence [5]. I used these latter results and the midpoint potential values shown in Fig. 6 and mentioned above to calculate the potential difference between Q and the high potential component described in this study. This difference is about +299 mV at pH 6.9, +262 mV at pH 5.9 and +159 mV at pH 4.9 which shows that the potential gap decreased at lower pH values. This agrees with the tendency of the transient to increase at lower pH values of the acid stage (see Fig. 2 in Shahak et al. [6]). However, in spite of this favorable tendency it is still difficult to account for the energy required for a 'reverse reduction of Q'. A pH difference of 3 units could supply only 180 mV for a one-electron transfer which means that an acid-base transition from pH 6.0 to 9.0 was not able to provide energy to raise an electron in the reverse direction over a potential gap of 262 mV.

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References

1 Duysens, L.N.M. and Sweers, H.E. (1963) Studies on Microalgae and Photosynthetic Bacteria, pp. 353—372, University of Tokyo Press, Tokyo
2 Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413—432
3 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243—253
4 Malkin, S. (1971) Biochim. Biophys. Acta 234, 415—427
5 Golbeck, J.H. and Kok, B. (1979) Biochim. Biophys. Acta 547, 347—360
6 Shahak, Y., Hardt, H. and Avron, M. (1975) FEBS Lett. 54, 151—154
7 Rienits, K.G., Hardt, H. and Avron, M. (1974) Eur. J. Biochem. 43, 291—298
8 Schwartz, M. (1968) Biochim. Biophys. Acta 112, 915—919
9 Doschek, W.W. and Kok, B. (1972) Biophys. J. 12, 832—838
10 Knaff, D.B. and Malkin, R. (1978) Curr. Topics Bioenerg. 7, 139—172
11 Okayama, S. (1976) Biochim. Biophys. Acta 440, 331—336
12 Bowes, J.M., Crofts, A.R. and Itoh, S. (1979) Biochim. Biophys. Acta 547, 320—335
13 Ikegami, I. and Katoh, S. (1973) Plant Cell Physiol. 14, 829—836