REDOX POTENTIALS OF ELECTRON ACCEPTORS IN PHOTOSYSTEM II_α AND II_β

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1. Introduction

Redox titrations of the chlorophyll fluorescence yield usually showed two transitions which were ascribed to reduction of the primary acceptor of photosystem II [1–5]. The source of this heterogeneity remained obscure. The two components titrated near 0 mV and near −300 mV and were called Q_H and Q_L, respectively. In [6,7] we have shown that in chloroplasts two different types of system II antennae exist, connected to the α and β centers postulated in [8]. Different electron transport properties of the systems II_α and II_β have also been reported [9,10] and Q_β was found to have a much higher midpoint potential than Q_α [3,11,10]. However, in [12] Q_H was reported not to be related to Q_β, because the latter is reduced already at +120 mV.

We have reinvestigated the problem by measurement and analysis of the light induced fluorescence rise curves of tobacco chloroplasts over a wide redox potential range, in the presence and absence of DCMU. The results confirm that Q_β is reduced at +115 mV. Apart from a transition around +30 mV, due to some fluorescence quenching by the plastoquinone pool in its oxidized state, all other fluorescence changes were due to changes in the redox state of Q_α. During titration in the presence of DCMU, Q_α was reduced in a single transition around −300 mV. In the absence of DCMU part of Q_α may be reduced at much higher potentials, depending on the redox state of the plastoquinone pool and of the secondary acceptor, Q_2. A consistent interpretation of all these phenomena is presented, which also explains some apparent contradictions in the literature.

Although most, if not all, of the redox reactions described involve protons directly or indirectly, we did not study and do not discuss their pH-dependence. In the reaction schemes the protons are left out, but all thermodynamic data refer to the molecular structures actually present under the conditions of measurement, at pH 8.3. An extensive discussion on the protonation reactions involved in the analogous part of bacterial photosynthesis may be found in [13].

Abbreviations and symbols: chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Q, all plastoquinone molecules are denoted by the symbol Q, with a suffix indicating their functional position at a given moment as follows: Q_α, primary electron acceptor of system II_α; Q_β, primary electron acceptor of system II_β; Q_2, secondary electron acceptor, equivalent to B and R; Q_p, member of the plastoquinone pool; Q_Z, plastoquinone bound to the cytochrome *b*–*f* complex

2. Methods

Dark-adapted tobacco chloroplasts, isolated as in [14], were resuspended in 100 mM glycylglycine, 0.4 M sucrose, 10 mM KCl and 5 mM $MgCl_2$ (pH 8.3) at 50 μM chl, determined according to [15]. The potential of the medium was measured by means of a platinum electrode with an Ag/AgCl electrode as reference. The titrations were started at about +300 mV; the potential was lowered by the addition of small amounts of a dithionite solution, followed by an equilibration period of 10–15 min. Except for ferricyanide (5 μM) no redox mediators were added. The accumulated dithionite additions lowered the pH by at most 0.2 units. During the titration the chloroplasts were kept on ice, in complete darkness, under nitrogen. At each potential a small sample was taken for the measurement of a fluorescence induction curve. Chlorophyll fluorescence was excited by light transmitted by a heat reflecting filter and Corning CS 4-96 and CS 3-67 filters (550–600 nm), of sufficient intensity to reduce all Q_α and Q_β within 2 s, when DCMU (10 μM) was added. Fluorescence was detected at 685 nm (bandwidth 10 nm).

3. Results and discussion

In the range from +300 to −400 mV the maximum fluorescence yield, F_{max}, obtained after complete photoreduction of Q_α and Q_β in the presence of DCMU showed a single increase around +30 mV (pH 8.3) of about 30% (fig.1A, ●). It coincides with the reduction of the plastoquinone pool (see below), which in the oxidized form is known to quench fluorescence to some extent [16]. The initial fluorescence yield, F_i, showed a broad transition around +65 mV and a further increase below −300 mV. The ratio F_{max}/F_i showed transitions around +115 mV and near −300 mV. Apparently quenching by the plastoquinone pool did not strongly affect this ratio. Analysis of the area over the fluorescence induction curve [9] showed that the two transitions in the F_{max}/F_i ratio corresponded to the reduction of Q_α and Q_β, respectively (fig.1B). Chemical reduction of Q_β took place in a one-electron reaction with a midpoint

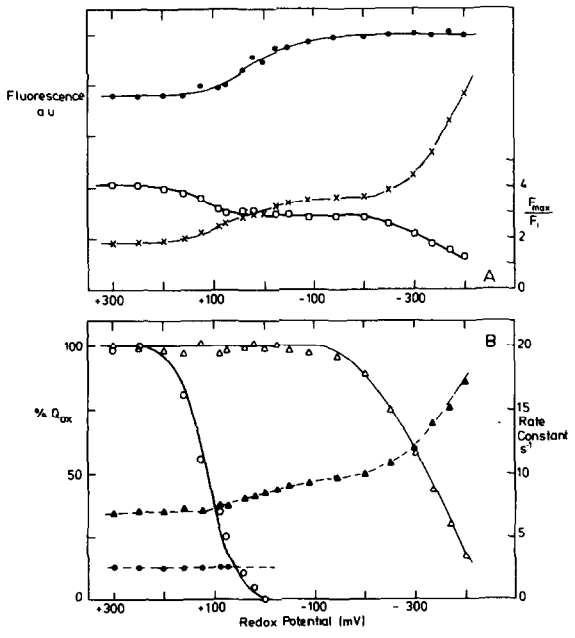


Fig.1. (A) Redox titration of initial (X−X) and maximal (○−○) fluorescence level of chloroplasts in the presence of 10 μM DCMU, at pH 8.3. The resulting F_{max}/F_i ratio is give by the squares (right scale). (B) Redox titration of Q_α and Q_β (△,▲ and ○,●, respectively) and of k_β and initial rate constant of Q_α photoreduction (● and ▲, respectively; right scale). The line accompanying the circles represents a one-electron transition with its midpoint at +115 mV. The circles below 75 mV were calculated assuming an invariant k_β.

potential of +115 mV at pH 8.3, in agreement with [12]. It may be noted that in the presence of hydroxylamine a lower midpoint potential was observed [10]. The photoreduction of Q_β had exponential kinetics with an invariant rate constant, k_β (●). All Q_α reduction took place in a broad transition around −300 mV (fig.1B, △). Due to energy transfer between the system II_α antennae, the absorption cross section and hence the observed rate constant of Q_α photoreduction increased with the fraction of Q_α reduced. Its initial value (k_α ▲) correspondingly increased upon chemical reduction of Q_α. Normal sigmoidal kinetics of fluorescence induction in system II_α were observed (in contrast to [17]), except that at negative potentials a small 'tail' occurred. Its kinetics were too fast to be due to Q_β and the amplitude and rate constant were apparently proportional to those of the main kinetic phase of Q_α photoreduction down to −350 mV. The change in $k_{\alpha,i}$ around +30 mV was not caused by a change in the amount of Q_α (fig.1B), but due to quenching by oxidized Q_p, which quenching lowered the quantum yields of Q_α photoreduction and fluorescence emission similarly (cf. fig.1A, ●).

Without DCMU, F_{max} did not change with the redox potential (fig.2A, ○,●) but above +100 mV F_{max} could only be reached by strong illumination. During the light-induced fluorescence rise the plastoquinone pool, too, was reduced as evidenced by the large area over the induction curve; this extra area titrated around +30 mV (not shown), in agreement with [5].

In the absence of DCMU the high potential transition in the initial fluorescence yield (fig.2A, □) was not only due to the reduction of Q_β and Q_p, as in the titration with DCMU (■), but also contained about 20% of the variable fluorescence of system II_α, corresponding to about 50% reduction of Q_α. The value of 50% was obtained from analysis of the induction curve in the presence of DCMU, and can also be estimated from the data in fig.1. If DCMU was added after setting the redox potential to values between −100 and −200 mV, it caused a dark oxidation of all Q_α^- (X−X). The addition of DCMU not only led to a lower F_i but also to a faster light-induced fluorescence rise and in fact the area over the induction curve was essentially unaffected (fig.2B). This shows that in the absence of DCMU, although half of the α centers were closed, the total amount of electrons that could be stored was equal to the total amount of α centers. The similar shape of the kinetics suggests that the

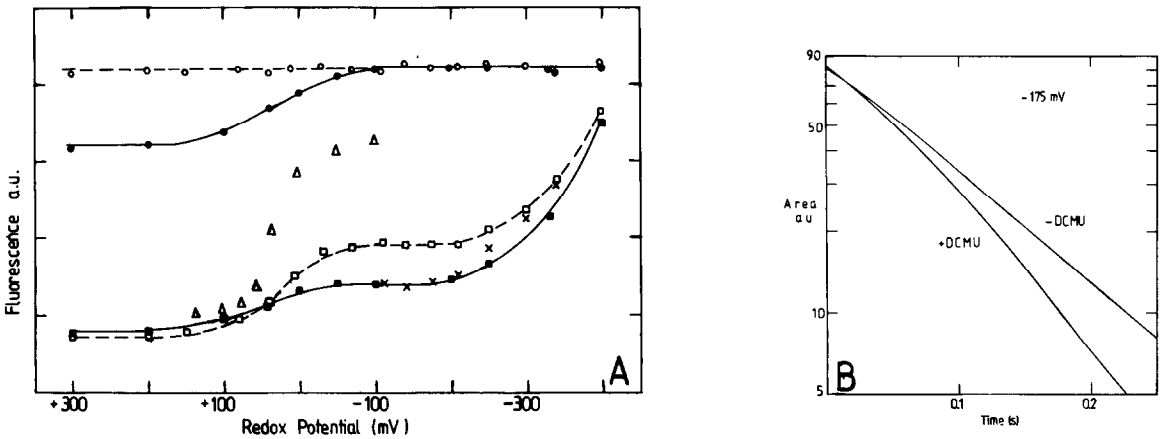


Fig.2. (A) Redox titration of intitial (□,■) and maximal (○,●) fluorescence yield of chloroplasts in presence (closed symbols) or absence (open symbols) of DCMU, at pH 8.3; (X–X) addition of DCMU after poising the potential of the medium resulted in the initial fluorescence yields; (△) titration of chloroplasts with most PS II_α centers in the state $Q_\alpha Q_2^-$. (B) Kinetics of the area over the fluorescence induction curve at −175 mV, before and after addition of DCMU. In both cases the areas and hence also the electron storage capacities of the reducing side of system II_α are of equal size.

fluorescence rise did not require two successive photoreactions but only one, proceeding with a 20% decreased quantum efficiency. Thus we conclude that each system II_α center accepted one electron and that initially Q_α in each center was in the reduced state half of the time, in equilibrium with the reduced secondary acceptor:

$$Q_\alpha^- Q_2^- \rightleftarrows Q_\alpha Q_2^{2-} \qquad (K = 1)$$

Apparently DCMU strongly shifts the equilibrium to the right. Comparison of the two titrations of F_i in fig.2A shows that reduction of Q_2 takes place at or somewhat below the midpoint potential of Q_p, in agreement with [5,18].

At higher potentials, where Q_p is oxidized, DCMU addition may lead to reduction of Q_α. This effect depends on preillumination, being larger after an uneven than after an even number of short saturating flashes, as first described in [19]. Subsequent fluorescence induction revealed no oscillations in the amplitude of the β phase, but a fixed amount of 20% reduced Q_β after one or more flashes preillumination (fig.3). The fluorescence oscillation with flash number was totally due to Q_α reduction and $k_{\alpha,i}$ (inset) oscillated correspondingly. The reduction of Q_α is ascribed to a DCMU-induced return of the electron which is stored every other photoreaction on a secondary acceptor, Q_2, which is required for the two-electron transport via Q_p [19].

Since the midpoint potential of Q_β seems too high for electron transport via Q_p, the absence of a two-electron gate in system II_β is not surprising. In support of earlier indications [20,21], we have recently shown [22] that the ultraviolet aborption difference spectrum of the reduction of Q_2 to Q_2^- is nearly identical to that of Q_1 ($\alpha + \beta$) and shows the reduction of a plastoquinone to the semiquinone anion. The absorbance measurements also showed that the amount of Q_2 corresponds only to the amount of Q_α and, in combination with fluorescence measurements, indicated a quantitative oxidation of Q_2^- by Q_α upon addition of DCMU.

Most of the reaction centers of system II_α can be brought in the state $Q_\alpha Q_2^-$ by illuminating the chloroplasts in the presence of *o*-phenanthroline and NH_2OH and subsequently adding Zn^{2+}, to relieve the inhibitory effect of *o*-phenanthroline on Q_α^- oxidation [23]. Titration of chloroplasts pretreated this way (fig.2A, △) showed a large increase of F_i around a midpoint potential of 30 mV. Of the about 90% Q_α^- produced in this transition only a small part could be reoxidized by adding DCMU, indicating that the fluorescence rise was caused by reduction of $Q_\alpha Q_2^-$ to $Q_\alpha^- Q_2^{2-}$.

The reduction of Q_α and oxidation of Q_α^- by Q_2^- upon addition of DCMU suggest that the semiquinone form of Q_2 is unstable in the presence of the inhibitor. This unstability is most easily explained in terms of a

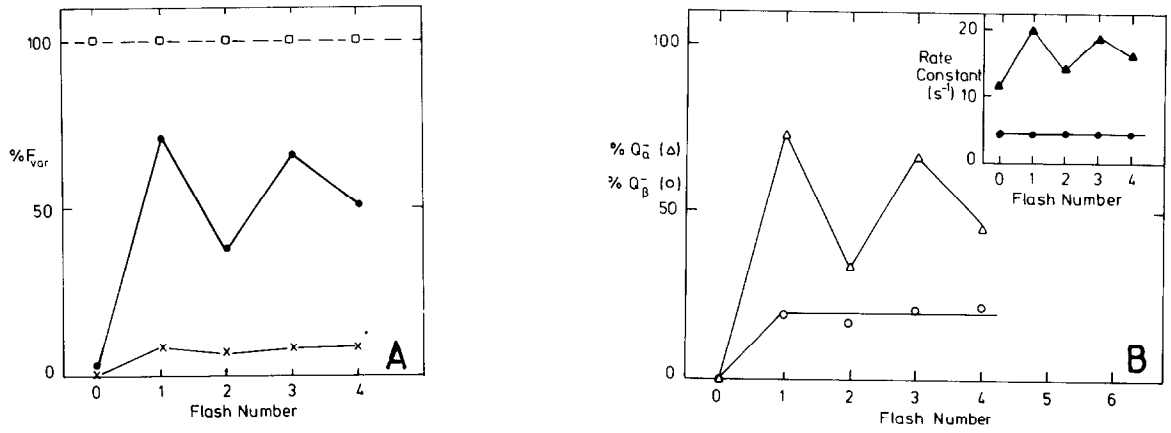


Fig.3. (A) The addition of 10 μM DCMU to chloroplasts, which, with NH_2OH added to prevent back reaction, had been illuminated with a number of saturating flashes ($t_{1/2}$ = 3 μs), caused an increase of fluorescence from the (×–×) level to (○–○) level. Thereafter a fluorescence rise curve was recorded; (□–□) the maximum fluorescence level reached. Without preillumination the F_{max}/F_i ratio was 4.8. The redox potential was +150 mV. (B) Fractions Q_α and Q_β (△,▲ and ○,●, respectively) found in the reduced state, plotted against the number of flashes given before addition of DCMU. The behavior of k_β (dots) and the initial rate constant of Q_α photoreduction (triangles) are shown in the inset.

competition of DCMU with Q_2 for a common binding site on the reaction center. In [24] evidence is presented for a rapid exchange between Q_p and Q_2 or Q_2^{2-}, whereas Q_2^- is firmly bound to the reaction center (see also [22]). The reduction of Q_α by Q_2^- upon addition of DCMU can then be written as:

$$Q_\alpha Q_2^- \rightleftharpoons Q_\alpha^- Q_2 \qquad (K_1 \simeq 0.1, \text{fig.3A and [25]})$$

$$Q_\alpha^- Q_2 \rightleftharpoons Q_\alpha^- + Q_p$$

$$\frac{Q_\alpha^- + DCMU \rightleftharpoons Q_\alpha^-\,DCMU}{Q_\alpha Q_2^- + DCMU \rightleftharpoons Q_\alpha^-\,DCMU + Q_p} \qquad (K>1, \text{fig.3B})$$

and the DCMU-induced oxidation of Q_α^- by Q_2^- as:

$$Q_\alpha^- Q_2^- \rightleftharpoons Q_\alpha Q_2^{2-} \qquad (K_2 \simeq 1, \text{fig.2A})$$

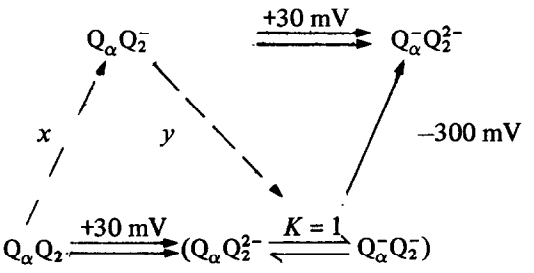
$$Q_\alpha Q_2^{2-} \rightleftharpoons Q_\alpha + Q_p^{2-}$$

$$\frac{Q_\alpha + DCMU \rightleftharpoons Q_\alpha\,DCMU}{Q_\alpha^- Q_2^- + DCMU \rightleftharpoons Q_\alpha\,DCMU + Q_p^{2-}} \qquad (K>1, \text{fig.2A})$$

Unless Q_2^- is present, deactivation of the S-states is not accelerated by reduction of Q_p [25] and probably does not significantly proceed via back reaction. This finding was ascribed to a large value of K_2, but we believe that it is not incompatible with the low value of K_2 found in [5] and by us. Our data show that the semiquinone pair $Q_\alpha^- Q_2^-$ is as stable as $Q_\alpha Q_2^{2-}$ and the activation energy for its oxidation to $Q_\alpha Q_2^-$ by back reaction with the S-states may be prohibitively high.

4. Conclusions

At pH 8.3 in the presence of DCMU all Q_α titrates in a single transition around –300 mV. In the absence of DCMU Q_α is reduced together with Q_2 near the midpoint potential of the plastoquinone pool, +30 mV, yielding an equilibrium with equal amounts of $Q_\alpha Q_2^{2-}$ and $Q_\alpha^- Q_2^-$. This mixed state was further reduced to $Q_\alpha^- Q_2^{2-}$ at –300 mV. The reduction of $Q_\alpha Q_2^-$ to $Q_\alpha^- Q_2^{2-}$ was found to occur also near +30 mV. The reactions at the acceptor side of system II_α upon reductive titration can be summarized as follows (leaving out the protons):



The reductions indicated by the dashed arrows did not occur during titration, but their midpoint potentials follow from $x + y = 2 \times 30$ mV and $y - 300$ mV = 2×30 mV, so that $x = -300$ mV and $y = +360$ mV. Q_α and Q_2 should not be considered as independent components in redox titrations.

In the presence, and presumably also in the absence of DCMU the midpoint potential of Q_β/Q_β^- is +115 mV (pH 8.3). In agreement with the high potential of its primary acceptor PS II_β electron transport does not proceed via the two electron accumulating plastoquinone Q_2. Possibly Q_β is in close contact with Q_z, which has a similar midpoint potential [26], implying that oxidation of Q_β^- does not drive proton translocation across the membrane. The absence of a transition at +115 mV in the flash-induced electrochromic change [27] supports this tentative conclusion.

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