# OXIDATION–REDUCTION POTENTIAL DEPENDENCE OF THE TWO KINETIC COMPONENTS IN CHLOROPLAST SYSTEM II PRIMARY PHOTOCHEMISTRY

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Received 31 August 1978

## 1. Introduction

Potentiometric titrations of the primary electron acceptor Q of photosystem II have been performed in the past from measurements of the fluorescence yield [1] and of the amplitude of the electrochromic band shift at 518 nm associated with the primary charge separation of photosystem II [2]. The results obtained indicated the existence of two primary photosystem II components the midpoint redox potential of which differed by ~250 mV.

The kinetics of the system II primary photochemistry occurring upon the onset of continuous illumination, are biphasic [3–6]. The biphasic energy conversion kinetics at photosystem II have been interpreted in the past as a reflection of the function of two different types of system II reaction centers, the $\alpha$-centers and the $\beta$-centers, the photochemical activity of which can be monitored kinetically [4,6]. Spectrophotometric measurements in the ultraviolet showed that the primary electron acceptor of both types of system II reaction centers is a special plastoquinone molecule which is reduced to its plastosemiquinone anion upon continuous illumination [6].

An attempt is made here to combine the findings from the potentiometric titration experiments with the kinetic measurements of the system II primary photochemistry. It will be shown that at a relatively high redox potential of the chloroplast medium (+380 mV) the kinetics of energy conversion at system II are biphasic. At a lower redox potential value (−80 mV), however, the slow phase is selectively eliminated from the overall kinetics. Thus, the functional heterogeneity of photosystem II, as detected in the kinetic measurements, is well correlated with the heterogeneity revealed by potentiometric titration measurements. The evidence indicates that the primary electron acceptor plastoquinone molecule of the $\beta$-centers has, under equilibrium conditions, a midpoint redox potential considerably higher than that of the $\alpha$-centers. Two possible explanations are discussed.

## 2. Materials and methods

The chloroplast isolation procedure and the apparatus for the fluorescence measurements have been described [6]. The method for the kinetic treatment of the fluorescence data has been reported [3]. Potentiometric titrations of the chloroplast suspension were performed under anaerobic conditions in a cuvette described [7]. The redox mediators and the method of obtaining anaerobic conditions were similar to those in [8,9]. Potassium ferricyanide and sodium dithionite were the added oxidant and reductant, respectively.

## 3. Results

In this work the kinetics of the fluorescence induction from DCMU-poisoned chloroplasts have been studied at two different redox potentials of the suspending medium. The fluorescence induction curve shows a typical time course which, in isolated and DCMU-poisoned chloroplasts, is a measure of the rate of photoreduction of the primary electron

acceptor Q of system II [6,10]. We observed that the kinetics of the variable part of the fluorescence yield of chloroplasts depend, among other parameters, on the redox potential of the suspending medium. In the following, the phenomenology and detailed quantitative analysis of the fluorescence induction curve will be presented for two characteristic redox potential values. The value of the high redox potential chosen (+380 mV) is not critical for the results to be presented. It was found, however, that a low redox potential of −80 mV was the most appropriate one for the clear elimination of one of the two kinetic components to be described below.

Figure 1 shows the variable fluorescence yield changes of DCMU-poisoned chloroplasts for the two different redox potential values of the suspending medium. For the sake of comparison the two curves have been normalized to the same maximum and $F_o$ values. Table 1 presents the absolute values of $F_{max}$, $F_o$ and their ratios at the two redox potentials. It is obvious from fig.1 that at −80 mV the variable fluorescence yield rises to the $F_{max}$ level considerably faster than at +380 mV. The quantitative

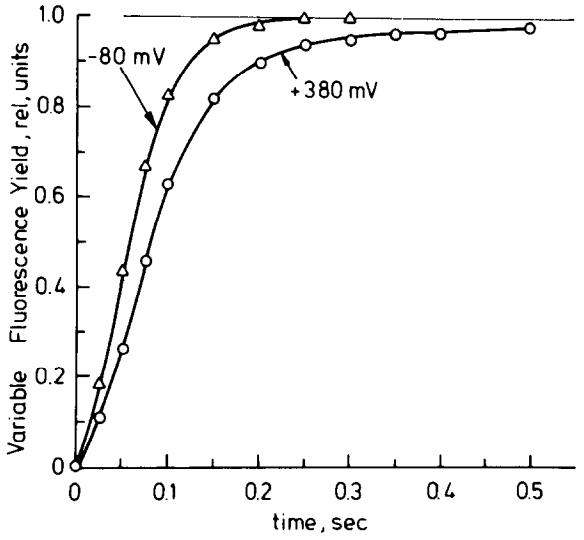


Fig.1. The time course of variable fluorescence yield ($F_v$) of isolated chloroplasts at two different redox potential values of their suspending medium. The amplitudes of $F_v$ have been arbitrarily normalized to the same relative value. The sample contained 10 μg chl./ml in a 1 cm optical pathlength cuvette and 15 μM DCMU, pH 7.8.

Table 1
Fluorescence and kinetic parameters of the system II primary photochemistry at different oxidation–reduction potential values of chloroplast suspending medium

| | Oxidation–reduction potential | |
|---|---|---|
| | +380 mV | −80 mV |
| $F_{max}$ | 6.0 (1.00) | 7.9 (1.32) |
| $F_o$ | 1.7 (0.28) | 2.7 (0.45) |
| $F_{max}/F_o$ | 3.53 | 2.93 |
| $a_{max}$ | 33.0 (1.00) | 23.5 |
| $\alpha_{max}$ | 21.5 (0.65) | ~23.5 |
| $\beta_{max}$ | 11.5 (0.35) | ~ 0.0 |

The maximum size of the complementary area $a_{max}$ is the sum of two independent components ($a_{max} = \alpha_{max} + \beta_{max}$) each of which represents a kinetically different pool of primary electron acceptor molecules of photosystem II. The values in parentheses represent fluorescence and area data normalized to their respective maximum values at +380 mV

kinetic evaluation of the above fluorescence data is possible only through the study of the complementary area over the fluorescence induction curve since this parameter is directly proportional to the amount of Q reduced [3,6,10]. The experimentally determined absolute size $a_{max}$ of the complementary area at −80 mV and +380 mV is shown in table 1. A direct comparison of the $a_{max}$ values at +380 mV and −80 mV may not be valid since the proportionality constant between area growth and $Q^-$ accumulation may depend on the redox potential of the medium. Nevertheless, we have estimated that at −80 mV, ~30–40% of the primary acceptor pool of system II is already in the reduced state.

Figure 2 shows the kinetics of Q reduction at −80 mV and +380 mV as measured by the growth of the complementary area. Although the absolute values of $a_{max}$ are not the same for the two cases (see table 1), the results of fig.2 have been normalized to the same relative value in order to facilitate a comparison of their kinetics. It is observed that the photoconversion of the primary electron acceptor pool is faster at −80 mV than at +380 mV. Furthermore, the kinetic pattern appears to be different for the two cases. Exact quantitative information about the kinetics shown in fig.2 is obtained by plotting in fig.3 the natural logarithm of the quantity (1−AREA)

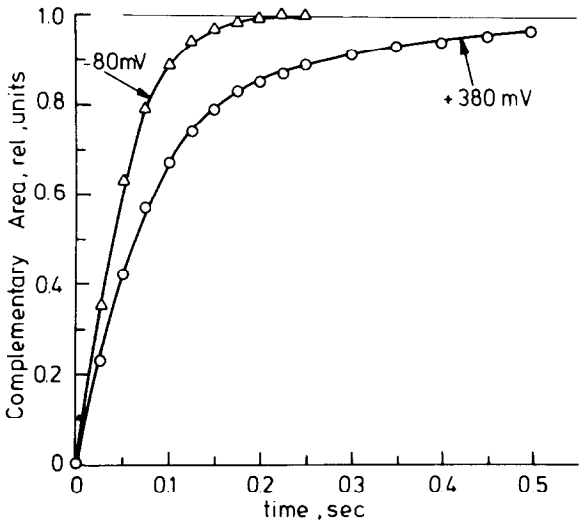


Fig.2. Kinetics of the area growth over the variable fluorescence curves measured at +380 mV and −80 mV of the chloroplast suspending medium. The results are normalized to the same relative maximal change.

versus time. Figure 3 shows that at +380 mV the kinetic pattern of Q reduction is biphasic. The fast phase lasts for ~0.15 s under our experimental conditions and is followed by a slow phase which lasts

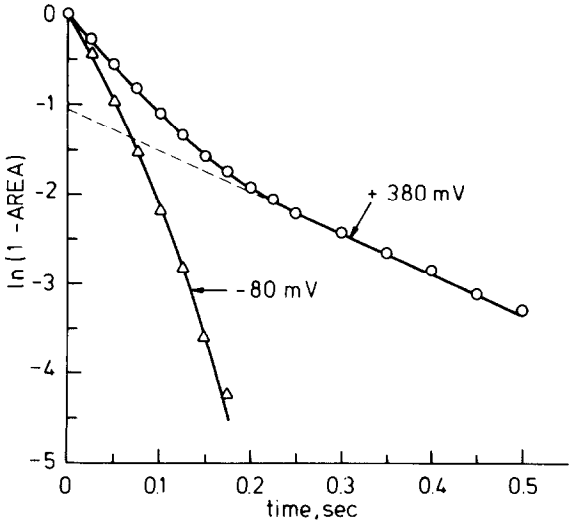


Fig.3. A first order reaction kinetic treatment of the results of fig.2. AREA refers to the fraction of the corresponding area size increase with time.

for >0.5 s. By the end of the slow phase the photoconversion of open system II centers has been completed. It has been discussed earlier [4,6] that the fast phase is a composite kinetic phenomenon and as such it could be resolved into two components: the α-component, which monitors the photochemical activity of the α-centers, reaches a steady-state in ~0.15 s and the early part of the β-component which monitors the activity of the β-centers. The remainder of the β-component lasts considerably longer in time and is expressed during the slow phase (0.2–0.5 s) in the logarithmic plot of fig.3 (+380 mV curve).

The logarithmic plot of the kinetic data at −80 mV (see fig.3) reveals that, at this redox potential, only the pure form of the α-component of the system II photochemistry is detected. Presumably, at −80 mV the primary electron acceptor molecules of the β-centers no longer quench the excitation energy because they are in the reduced state. From fig.3 (+380 mV) it is possible to determine the relative contribution of the β-component to the overall kinetics. Such a value is obtained from the intercept of the extrapolated slope of the β-component in the logarithmic plot with the ordinate at zero time (dashed line in fig.3). The results of such a calculation are shown in table 1. At +380 mV the β-component monitors the reduction of ~35% of the overall Q ($\beta_{max}$ = 35%) while the α-component accounts for the rest or ~65% ($\alpha_{max}$ = 65%).

The relationship between the reduced fraction of the primary electron acceptor Q and the relative variable fluorescence yield change is usually non-linear [11]. Such a relationship is shown in fig.4 where the complementary area is plotted as a function of the corresponding variable fluorescence yield level. At the redox potential value of +380 mV the function $Q^- = f(F_v)$ initially shows a large positive deviation from linearity which then is followed by a relatively small negative deviation. Based on the kinetic data of fig.1,2,3, one can easily relate the positive and negative deviations of the function $Q^- = f(F_v)$ from linearity to the kinetic properties of the α- and β-centers, respectively. The positive deviation from linearity, as shown in a pure form for the curve measured at −80 mV, has been interpreted [6,11] as resulting from excitation energy transfers between the neighbouring photosystem II units of the α-centers. Obviously, the negative devia-

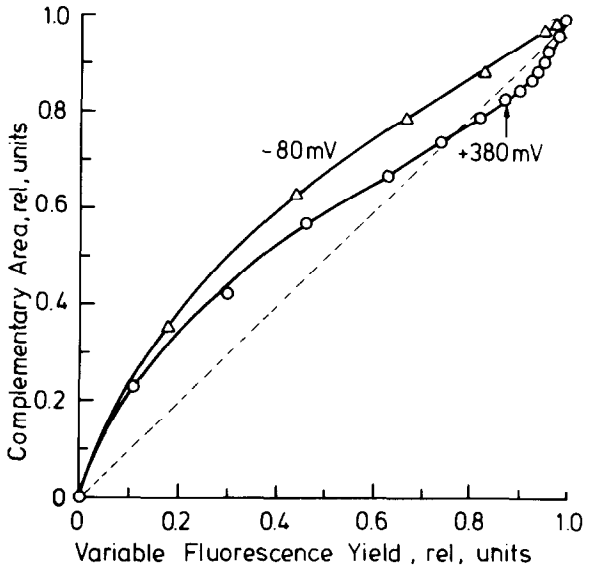


Fig.4. The redox potential dependence of the kinetic relationship between the amount of reduced Q (complementary area) and the corresponding variable fluorescence yield change.

tion does not fit into this pattern and can be explained only by the assumption that the β-centers have different excitation energy transfer properties and, therefore, a different $Q^-$ vs $F_v$ relationship than the α-centers. Figure 4 also shows that at −80 mV the function $Q^- = f(F_v)$ is 'symmetrical' with a positive deviation from linearity while the negative deviation has been eliminated. This is in agreement with the results of fig.3 which show that at −80 mV the β-component is selectively eliminated.

We observed that the complete elimination of the α-component required the further lowering of the redox potential to values in the vicinity of −300 mV (results not shown) which is in agreement with the earlier potentiometric titrations [1,2].

## 4. Discussion.

The conclusion to be drawn from this work is that the apparent midpoint redox potential of the primary electron acceptor of the α-centers is different from that of the β-centers. Recent work in this laboratory [6] has shown that both types of system II reaction centers involve as a primary electron transfer step the photoreduction of a special plastoquinone molecule to its plastoquinone anion (a 1 $e^-$ transition). It therefore appears that the chemical identity of the electron acceptors corresponding to the two types of system II reaction centers is basically the same. In addition, the pH dependence of the oxidation–reduction midpoint potential of the two plastoquinone primary electron acceptors (~−58 mV/pH unit [2], see also [12]) indicates that, under equilibrium conditions, the reduction of the primary acceptors involves both an electron and a proton. Therefore, an attempt at explaining our results could involve the following two possibilities which are not mutually exclusive:

1. The operational midpoint potential of the primary acceptor of the α- and β-centers is the same (~−300 mV) for the 1 $e^-$ light-induced transition but the p$K$ values of the two plastoquinone–protein complexes are different. Different p$K$ values for the plastoquinone acceptor complexes imply that the redox couples $Q^- + H^+ = QH$ of the α- and β-centers are not at the same protonation equilibrium at a given pH value, thus the detected difference in their apparent midpoint redox potential. We have estimated that a p$K$ difference $\Delta pK$ ($pK_\beta - pK_\alpha$) of 4–4.5 pH units could explain the observed results. In this case, it must be noted that the apparent difference in the midpoint redox potential under equilibrium conditions is not necessarily expressed during the fast turnover of electrons (~600 μs) normally occurring under steady-state electron flow conditions.

2. The p$K$ values of the two primary acceptor plastoquinone complexes are similar which would imply that their true operational midpoint potentials are different as detected. Such a possibility would involve drastic structural differences between the primary acceptor complexes of the α- and β-centers which, although possible [13], are not spectrophotometrically detected [6].

At present it is not clear why there are two types of system II reaction centers which differ, among other things, in their photoconversion kinetic proper-

ties and the midpoint redox potential of their primary electron acceptor. In this context it may be important that a similar heterogeneity concerning another electron carrier of photosystem II (the cyt. $b_{559}$) has been extensively discussed in the literature (reviewed [14]). Thus, one may combine the observation of a high and low potential cyt. $b_{559}$ with the high and low potential primary electron acceptor of the $\beta$- and $\alpha$-centers, respectively. An analogous heterogeneity is not observed among the photosystem I reaction centers or the system I-associated electron transfer compounds. This is indicated by the single midpoint redox potential of the P-700 pool in chloroplasts (+385 mV, independent of pH [2]) and by the kinetically monophasic photooxidation of P-700 (A.M., unpublished). The physiological significance of the functional heterogeneity in photosystem II has not yet been elucidated and remains an important (and open) field for future research.

## Acknowledgements

The advise of Professor L. N. M. Duysens and Dr R. van Grondelle during the preparation of this work is gratefully acknowledged. The author was supported by a long term fellowship from the European Molecular Biology Organization (EMBO). This investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Pure Research.

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