Biochimica et Biophysica Acta, 635 (1981) 105—110

BBA 48019

THE EFFECT OF REDOX POTENTIAL ON THE KINETICS OF FLUORESCENCE INDUCTION IN PEA CHLOROPLASTS

I. REMOVAL OF THE SLOW PHASE

PETER HORTON

Department of Biochemistry, The University, Sheffield S10 2TN (U.K.)

(Received July 10th, 1980)

Key words: Fluorescence induction; Redox potential; Photosystem II; (Pea chloroplasts)

Summary

The effect of alteration of redox potential on the kinetics of fluorescence induction in pea chloroplasts has been investigated. Potentiometric titration of the initial (F_i) level of fluorescence recorded upon shutter opening gave a two component curve, with $E_{m(7)}$ at —20 mV and —275 mV, almost, identical to results obtained using continuous low intensity illumination (Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188—201). The slow or tail phase of induction observed in the presence of DCMU can be eliminated by poising the redox potential at approx. 0 to +50 mV. At this potential F_i was increased by less than 10% and the higher potential quencher described above was only marginally reduced. The disappearance of the slow phase titrated as an $n = 1$ component with an $E_{m(7)}$ of +120 mV. Therefore it seems unlikely that the slow phase of fluorescence induction is due to photoreduction of the —20 mV quencher. These results are discussed with reference to current ideas concerning heterogeneity on the acceptor side of Photosystem II.

Introduction

Fluorescence induction at room temperature in chloroplasts inhibited with DCMU reflects the light-induced reduction of Q, the primary electron acceptor of PS II [1]. It has long been observed that the shape of the induction curve

Abbreviations: PS II, Photosystem II; PS I, Photosystem I; Q_L, fluorescence quenching component with $E_{m(7)}$ around —250 mV; Q_H, fluorescence quenching component with $E_{m(7)}$ around —20 mV; F_0, fluorescence level when all PS II traps are open; F_i, initial level of fluorescence upon shutter opening; F_v, variable fluorescence; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

is not indicative of a single first order photochemical event [2—4]. In fact the curve deviates in two ways; first, the major part of the induction is distinctly sigmoidal; secondly, there follows an entended slow or tail phase. Several explanations have been put forward to explain these phenomena. The sigmoidicity has been suggested to be due to either a two-photon closure of PS II [2,4,5], or excitation energy transfer between PS II units [3,6,8]. The tail phase has been studied in detail by Melis and Homann [9—11] and has been suggested to represent the activity of a separate pool of PS II centres. This argument was strengthened recently by the observation that absorbance signals related to the primary acceptor of PS II, C-550 and X-320, also show a slow component analogous to that seen for fluorescence induction [12,13], although Joliot and Joliot [14], using flash excitation, do not observe a C-550 reduction accompanying the less efficient fluorescence rise. In recent years experiments on the redox titration of the fluorescence yield have indicated more than one Q component [15—19] and it seemed logical to consider whether the existence of these redox components was responsible for the complex kinetics of fluorescence induction. Reports by Horton and Croze [17] and Melis [20] have both indicated that reduction of the higher potential Q, Q_H, is responsible for the slow phase of fluorescence induction. Both these reports can be criticised in that induction curves were recorded at just two redox potentials. In the former, potentials at the start (Q fully oxidized) and plateau (Q_H fully reduced, Q_L fully oxidized) were used. In the latter, no indication of what point in the redox titration had been reached, was given. This report did indicate, however, that the tail phase was removed with only 8% of F_v being eliminated by chemical reduction. A direct relationship between Q_H and the slow phase would require that at least 50% and possibly as much as 70% would have to be removed by chemical reduction to remove the slow phase. The amplitude of the slow phase, however, is only approx. 15% of the F_v [11—13]. Even allowing for the well-known fact that chemical reduction always elevated the fluorescence above that obtainable by photoreduction [21], it is hard to accommodate the 50—70% of fluorescence seen in redox titration as being responsible for the slow phase of fluorescence induction. Supporting this conclusion is the fact that the flash-induced P-518 change due to PS II titrates as Q_L and Q_H [22], yet no P-518 change is associated with the inefficient phase of Q reduction [14]. Clearly a more detailed investigation of the relationship between Q_H and the slow phase is required.

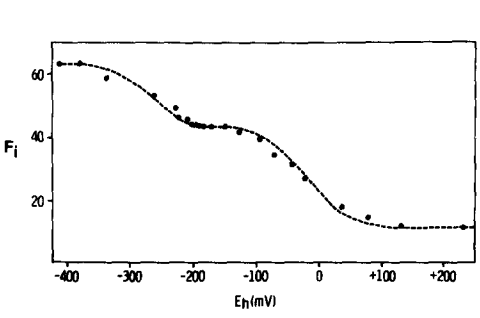
Materials and Methods

Pea chloroplasts were isolated as described previously [23]. Redox potentiometry followed procedures published earlier [17] except that extra care was given to choice and concentration of redox mediators, some of which can act as electron acceptors or quenchers [17—19]. Fluorescence induction curves were recorded as previously described [17] but with data being stored in a Data lab 905 Transient recorder. For measurement of area above the induction curve, data was subsequently transferred and manipulated by a Datalab DL4000 Microprocessor.

Results

The level of fluorescence recorded using a low intensity measuring beam shows two transitions during a potentiometric redox titration, termed Q_L and Q_H [17]. In the experiments described in this paper redox equilibration takes place in darkness and fluorescence is recorded during brief illumination in the form of induction curves. Fig. 1 shows the changing value of the F_i or initial fluorescence level as the redox potential is altered. The data, recorded at pH 7.0 show two redox transitions with $E_{m(7)}$ values of −20 mV and −275 mV comparing favourably in value and proportions to previous data [17]. An exactly similar titration was obtained when DCMU was added prior to induction. It should be added here that this data was obtained during oxidative titrations; it has proved difficult to show clearly the existence of two Q components in reductive titrations performed in complete darkness. No such problem is observed when a low intensity measuring beam is used [17] so that it is concluded that in some way a slow rate of excitation aids reductive equilibration of Q_H.

Having shown that F_i can be titrated, the effect of redox potential on the tail phase was tested. Fig. 2 shows two induction curves recorded in the presence of DCMU. At +190 mV a slow phase was observed, as seen in chloroplasts under normal aerobic conditions [4,9—13]. However, at +50 mV the slow phase was abolished. These curves were recorded in the complete absence of redox mediators to avoid any distortion by electron acceptance or quenching. The redox potential values are therefore not completely accurate but are within ±20 mV of the values given. However, it is clear that the slow phase is removed with only a small increase in F_i and clearly, at the most, only about 20% of Q_H has been reduced at this stage. Melis and co-workers [9—13] have analysed induction curves recorded in the presence of DCMU by the use of logarithmic plots of area accumulation above the induction curve. This analysis



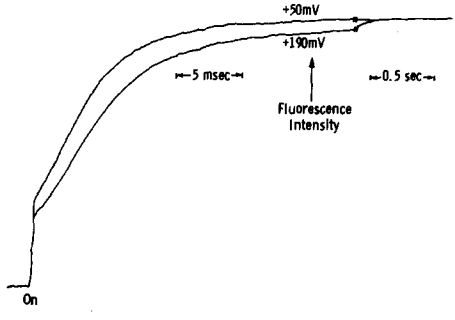


Fig. 1. The redox titration of the initial level of fluorescence (F_i). Induction curves were recorded in the absence of diuron and in the presence of 1,4-naphthoquinone, indigotetrasulfonic acid, 2-hydroxy-1,4-naphthoquinone, anthroquinone disulfonate and anthraquinone sulfonate as redox mediators at pH 7.0. Titration was oxidative. Dotted line is a composite of two Nernst equations (n = 1) with $E_{m(7)}$ −20 mV (62%) and −275 mV (38%).

Fig. 2. Induction curves at different redox potential. Fluorescence was measured with the transient recorder on dual time base mode as shown in the presence of 5 μm DCMU added one minute prior to induction and in the absence of added redox mediators at pH 7.0. Chlorophyll concentration, 10 μg/ml.

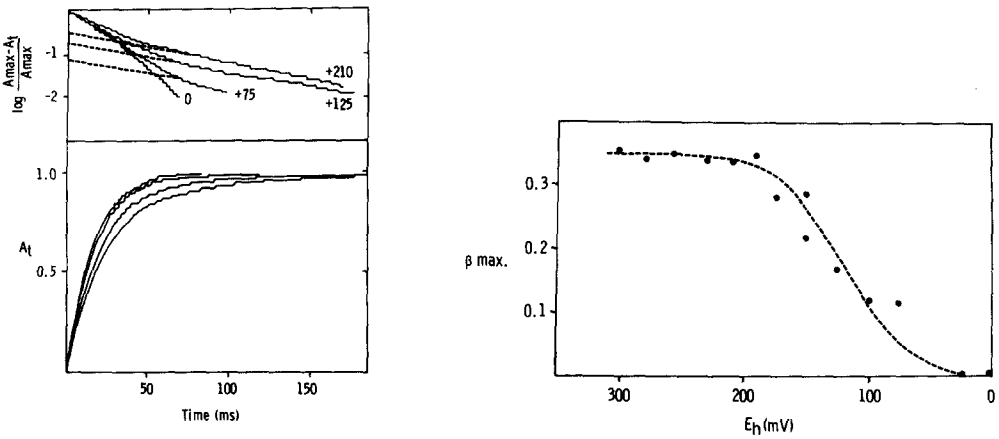


Fig. 3. Analysis of fluorescence induction curves with different amounts of slow phase. Induction curves were recorded as in Fig. 2, except that redox mediators (10 μM each of 1,4-naphthoquinone, indigotetrasulfonic acid, and 2-hydroxy-1,4-naphthoquinone) were included for redox stabilization. Bottom: normalized plots of area accumulation over the induction curve. Top: logarithmic plots of area accumulation to show the slow (β) phase. Dotted lines show intercept on Y axis and reveal the proportion of β phase (β max), see Ref. 11.

Fig. 4. Redox titration of the slow phase. Data was extracted from induction curves at different E_h and manipulated as in Fig. 3. The dotted line represents a Nernst equation (n = 1) with an $E_{m(7)}$ of +120 mV.

produces a value for the proportion of area involved in the slow phase, β_{max} [10—13]. Fig. 3 shows normalised area accumulation at 4 different redox potentials (bottom) and first order analysis (top). As the potential is decreased from +210 mV the proportion of the slow phase seems to decrease. The slope of the slow phase is approximately constant throughout, indicating that a change in proportion of a process with a constant rate is occurring. At 0 mV a slow phase is undetectable and the logarithmic plot shows the typical deviation from first order character of the 'pure' fast component of the induction curve [10—13]. The intercept on the Y axis of the upper graph in Fig. 3 provides values for β_{max}. Fig. 4 is a plot of β_{max} recorded at several different redox potentials. The data fit a standard n = 1 Nernst plot with an $E_{m(7)}$ of approx. +120 mV. The maximum value of 0.35 for β_{max} compares well with data of Melis and co-workers [10—13].

Discussion

The data presented in this paper argue against the notion, previously proposed, that Q_H and the slow phase of fluorescence induction are different manifestations of the same phenomenon [17,20]. Essentially, the crucial observation is that the slow phase can be removed by poising at a redox potential shown to reduce only a small (approx. 10%) amount of Q_H. A similar observation can be found in Table I of a recent paper by Melis [20] although at —80 mV larger amounts of Q_H would have been expected to have been reduced.

Based on the constancy of the intensity X time product for the slow phase it has been concluded to represent a photochemically limited process and not one

linked by a secondary dark reaction [9—13,24]. It has been shown that reduction of C-550 and X-320 also shows a slow phase with the same rate constant as the change in area above the induction curve [12,13]. It has been argued, based on the first order kinetics of the slow phase (compared to the non-first order fast phase) and on the selective effect of Mg^{2+} on the kinetics of the fast phase, that these phases represent two physically separate types of PS II centres, α and β [10—13]. The less efficient β centres were proposed to exist in unstacked stromal membranes [10]. Based on the absence of Q_L in agranal chloroplast it was recently proposed that Q_H may be predominant in stromal membranes, in agreement with an earlier observation concerning preferential energy transfer from chlorophyll *b* to Q_L [25]. Thus a logical explanation of the data presented in this paper is that there is indeed a separate pool of PS II centres responsible for the slow phase of fluorescence induction but having a Q with an E_m more positive than the major pool of Q_H. This would leave the remaining proportion of Q_H and all Q_L to be found together in the stacked membrane regions.

Other explanations are possible, however; Joliot et al. [14] have studied the slow phase under repetitive flash conditions and it is clear that photoreduction associated with this phase is inefficient. The +120 mV redox transition could then be interpreted in terms of a component involved in preventing efficient stabilization of Q reduction. Hence it could be an auxilliary electron donor to the β centres that is normally oxidized and is ineffective. Alternatively it could represent a component involved in re-oxidation of Q in the presence of DCMU. The $E_{m(7)}$ of +120 mV (though not the $n = 1$ character) suggests plastoquinone could be involved or low potential cytochrome *b*-559 [17]. The slow phase, in this scheme, would be merely indicative of the existence leak or side pathway through the DCMU block that keeps Q partially oxidized and need not necessitate the postulation of an additional Q. A similar suggestion was made recently concerning the +400 mV 'quencher' first described by Ikegami and Katoh [26] as an explanation for the lower F_{max} and exagerated slow phase seen after incubation with ferricyanide [27]. That the +120 mV component is only an apparent E_m for Q_β could also arise if there was no direct redox equilibration of Q, but only through B; in this case a proportion of Q would be reduced upon addition of DCMU when the redox potential was sufficiently negative to reduce B [28]. Presumably Q_H would be preferentially affected and thus would titrate with the potential of B (i.e. +120 mV?). This is unlikely to be a major factor however because, firstly, titration of F_i is the same with and without DCMU and secondly, it seems that β centres do not show the presence of B [29].

It is concluded that more experimentation is required before connections are made between the PS II heterogeneity observed by Joliot and Joliot [14], that described by Melis and co-workers [9—13] and that observed during redox titration of Q [15—19].

Acknowledgement

I wish to thank Barrie Naylor for technical assistance and the Science Research Council (U.K.) for support (Grant No. Gr/A/79956).

References

1 Duysens, L.N.M. and Swears, H.E. (1963) in Studies on Microalgae and Photosynthetic Bacteria (Japanese Society of Plant Physiologists, ed.), pp. 353—372, University of Tokyo Press, Tokyo
2 Morin, P. (1964) J. Chim. Phys. Phys. Chim. Biol. 61, 624—680
3 Joliot, A. and Joliot, P. (1964) C.R. Acad. Sci. Paris Ser. D 278, 4622—4625
4 Doschek, W.W. and Kok, B. (1972) Biophys. J. 12, 832—838
5 Lavorel, J. (1972) C.R. Acad. Sci. Paris Ser. D 274, 2909—2912
6 Lavoral, J. and Joliot, P. (1972) Biophys. J. 12, 815—831
7 Paillotin, G. (1976) J. Theor. Biol. 58, 237—252
8 Hipkins, M.F. (1978) Biochim. Biophys. Acta 502, 514—523
9 Melis, A. and Homann, P.H. (1975) Photochem. Photobiol. 21, 431—437
10 Melis, A. and Homann, P.H. (1976) Photochem. Photobiol. 23, 343—350
11 Melis, A. and Homann, P.H. (1978) Arch. Biochem. Biophys. 190, 523—530
12 Melis, A. and Duysens, L.N.M. (1979) Photochem. Photobiol. 29, 373—392
13 Melis, A. and Schreiber, U. (1979) Biochim. Biophys. Acta 547, 47—57
14 Joliot, P. and Joliot, A. (1979) Biochim. Biophys. Acta 546, 93—105
15 Cramer, W.A. and Butler, W.L. (1969) Biochim. Biophys. Acta 172, 503—510
16 Ke, B., Hawkridge, R.M. and Sahu, S. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2211—2215
17 Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188—201
18 Malkin, R. and Barber, J. (1979) Arch. Biochem. Biophys. 193, 169—178
19 Golbeck, J.H. and Kok, B. (1979) Biochim. Biophys. Acta 547, 347—360
20 Melis, A. (1978) FEBS Lett. 95, 202—206
21 Homann, P.H. (1969) Plant Physiol. 44, 932—936
22 Malkin, R. (1978) FEBS Lett. 87, 329—333
23 Horton, P. and Croze, E. (1977) Biochim. Biophys. Acta 462, 86—101
24 Etienne, A.L. (1974) Biochim. Biophys. Acta 333, 497—508
25 Horton, P. and Naylor, R.B. (1979) Photobiochem. Photobiophys. 1, 17—23
26 Ikegami, I. and Katoh, S. (1973) Plant Cell Physiol. 14, 829—836
27 Bowes, J.M., Crofts, A.R. and Itoh, S. (1979) Biochim. Biophys. Acta 547, 320—335
28 Velthuys, R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85
29 Thielen, A.P.G.M. and van Gorkom, H.J. (1981) Proc. 5th Int. Congr. Photosynthesis (Akoyunoglou, G., ed.), International Science Services, Jerusalem, in the press