Biochimica et Biophysica Acta, 680 (1982) 127–133
Elsevier Biomedical Press

BBA 41076

THE EFFECT OF REDOX POTENTIAL ON THE KINETICS OF FLUORESCENCE INDUCTION IN PHOTOSYSTEM II PARTICLES FROM *PHORMIDIUM LAMINOSUM*

SIGMOIDICITY, ENERGY TRANSFER AND THE SLOW PHASE

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(Received November 26th, 1981)

Key words: Photosystem II; Fluorescence induction kinetics; Redox Potential; (Blue-green alga)

Fluorescence induction curves in 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-inhibited Photosystem (PS) II particles isolated from the blue-green alga *Phormidium laminosum* have been analysed as a function of redox potential. Redox titration of the initial fluorescence indicated a single component with $E_{m,7.5} = +30$ mV ($n = 1$) (Bowes, J., Horton, P. and Bendall, D.S. (1981) FEBS Lett. 135, 261–264). Despite this simplified electron acceptor system and the small number of chlorophylls per reaction centre, a sigmoidal induction curve was nevertheless seen. Sigmoidicity decreased as Q was reduced potentiometrically prior to induction such that the induction was exponential when the ratio $F_i/F_m = 0.64$. These particles also showed a slow (β) phase of induction which titrated with an E_m value slightly more positive than that of the major quencher. It is concluded that the sigmoidal shape of the fluorescence induction curve observed in *Phormidium* PS II particles is not a consequence of a requirement for two photons to close the PS II reaction centre, but is generated as a result of energy transfer between photosynthetic units comprising one reaction centre per approx. 50 chlorophylls. Also, the existence of PS II heterogeneity (PS II_α, PS II_β centres) does not require a structurally differentiated chloroplast, but may only indicate the extent of aggregation of PS II centres.

Introduction

Illumination of chloroplasts or algae inhibited by DCMU produces a characteristic rise in the yield of fluorescence from Chl *a* which reflects the photoreduction of Q, the primary electron acceptor of PS II [1]. The kinetics of this induction are complex, an initial fast sigmoidal phase of the rise being followed by a slower exponential one. These phases have been attributed to the coexistence of two types of PS II centres, PS II_α and PS II_β [2,3]. In higher plants, these centres differ in their antenna size [4] and composition [5,6] as well as in the redox mid-point potential of Q [7,8]. It has been suggested that PS II_β centres are located in the non-appressed regions of the chloroplast membrane and PS II_α centres in the partitions [2,4]. However, in chloroplasts developed under intermittent light regimes [9] or in the absence of divalent cations [4], both types of which are deficient in thylakoid stacks, centres of both types α and β are still present, and this would suggest that the heterogeneity reflects an intrinsic property.

The sigmoidal part of the fluorescence rise curve

Abbreviations: PS II, Photosystem II; Chl, chlorophyll; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_o, minimum fluorescence when Q is oxidised; F_m, maximum fluorescence when Q is reduced; F_i, initial fluorescence; $F_i = F_o$ when Q is oxidised.

is the distinguishing feature of the PS II_α centres [2–5]. Sigmoidicity could be generated either by energy transfer between the antenna pigments of a 'domain' of reaction centres [10–13] or by a requirement for two photo-reactions to close the PS II trap [14–16]. The former is widely accepted, but some recent data showing a correlation between the ratio Q_L/Q_H seen in redox titration of Q and the extent of sigmoidicity, are perhaps more satisfactorily explained by the two-photon closure model [17].

Since the fluorescence induction curve is potentially a valuable diagnostic tool for probing in vivo photosynthesis, it would seem additionally important to establish firmly a criterion for explain the induction kinetics.

In this paper, we have examined fluorescence induction curves as a function of redox potential in a PS II particle preparation from a blue-green alga that contains, of course, no granal stacks and no Chl *b*. This soluble preparation is highly active in oxygen evolution and is enriched in PS II components although it is not chemically pure and retains a considerable portion of the protein and lipid components of the original membranes [18]. It has been shown to be deficient in Q_L, the primary acceptor of PS II in chloroplasts which titrates at −250 mV [19]. This preparation exhibits a high F_v/F_m ratio and a sigmoidal induction curve with a slow phase reminiscent of α- and β-centres of higher plants.

From our results we conclude firstly that energy transfer between units with an antenna size of only approx. 50 chlorophylls is a sufficient criterion for sigmoidicity,and secondly that PS II_α and PS II_β may only differ in their aggregation state or connectivity.

Materials and Methods

Preparation of particles from *Phormidium laminosum* and redox titration of the fluorescence induction curve were performed as described previously [19].

Particles were suspended in the following medium at pH 7.5, 25% glycerol, 10 mM $MgCl_2$, 10 mM Hepes-NaOH plus 5 mM phosphate buffer, at a chlorophyll concentration of 5.3 μg/ml. DCMU and mediators were present as before.

Results

Fig. 1 shows fluorescence induction curves of *Phormidium* PS II particles recorded in the presence of 10 μM DCMU poised at different redox potentials. As Q was progressively reduced, F_i increased. Titration of F_i/F_m showed the presence of a single species with $E_{m,7.5} = +30$ mV [19]. At higher potentials when Q was fully oxidised, the induction was clearly sigmoidal.

The variable fluorescence F_v has been related to the proportional concentration of oxidised acceptor Q by the following equation:

$$F_v = (1-p)(1-[Q])/1-p(1-[Q]) \qquad (1)$$

[Q] is understood to vary between 1 and 0 as F_v increases from 0 to 1 during an induction curve. In the original formulation [10], p equalled the probability of energy transfer from the antenna of a closed PS II trap to another unit. The meaning of p may be altered in modifications of the simple model [20–22], but the same form of the equation applies. We have found it convenient when describing the induction curves for samples that have been pre-equilibrated at a series of different redox potentials, to use Eqn. 2 which has a form identical to that of Eqn. 1:

$$f_v = (1-r)(1-q)/1-r(1-q) \qquad (2)$$

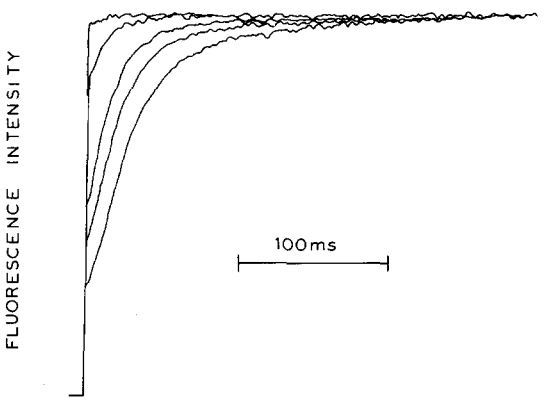

Fig. 1. Fluorescence induction curves in *Phormidium* PS II particles recorded in the presence of 10 μM DCMU and at different oxidation-reduction potentials (E_h). Conditions as described in the text. Samples were allowed to stabilize for 10 min at each potential before an induction was measured. Data are shown at +155 (bottom curve), +99, +60, −2 and −48 mV (top curve).

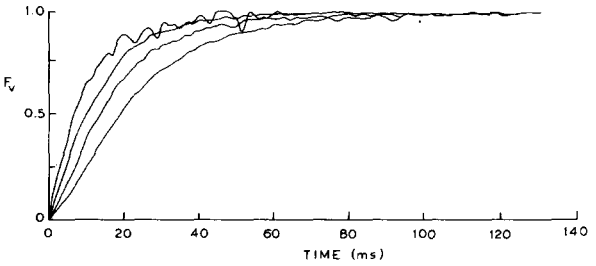

Fig. 2. Plots of normalized f_v of the α-phase of fluorescence induction at different values of E_h. Conditions as in Fig. 1 with curves at +155 (bottom), +99, +60 and −2 mV (top) shown. The slow (β) phase was subtracted and the normalized f_v presented on an expanded scale as described previously [17,35].

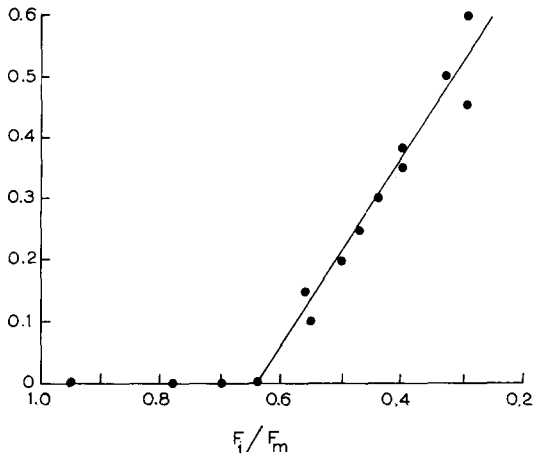

Fig. 3. Dependence of sigmoidity on the ratio F_i/F_m. Induction curves were recorded at different potentials as described in Fig. 1. Plots of the normalised f_v against area were computed for the α-phase as described in the text and Ref. 35. r was derived by fitting the relationship $f_v = (1-r(1-q)/1-r(1-q)$ (see text) to the f_v vs. area plots.

Here, f_v is the normalised variable fluorescence, after subtraction of the β-phase, which changes from 0 to 1 whatever the value of F_i. Similarly, q is the normalised concentration of acceptor, set initially at 1, whatever the starting value of [Q]. The factor r is related to the probability of energy transfer from a closed unit to another unit, but, unlike p, varies with ambient redox potential because the size of the units between which energy transfer occurs is changing during the titration (see below). At high potentials, with Q fully oxidised, $r_{max} = p$. Thus, when the potentials was +155 mV, the induction curve recorded for *Phormidium* PS II particles gave a value of $p = 0.5$. The curve recorded under similar conditions but using pea chloroplasts gave a value of $p = 0.48$ [17]. Similarly, a minimum value of F_i/F_m of approx. 0.3 was seen in *Phormidium* particles and in pea chloroplasts [17]. (As described previously, this PS II preparation is essentially devoid of accessory phycocyanin pigments which are highly fluorescent in the same region as Chl *a*.) In separate experiments we have determined the $t_{1/2}$ value for induction in pea chloroplasts and *Phormidium* membrane fragments and PS II particles at identical chlorophyll content and light intensity. Values of 16, 110 and 80 ms, respectively, were obtained for these three systems, suggesting that the PS II antenna in *Phormidium* (essentially unchanged during preparation of the particles) contains approx. one-fifth of the number of chlorophylls per PS II reaction centre as the antenna of pea chloroplasts [23].

In pea chloroplasts, the sigmoidicity disappeared as Q was titrated such that $r = 0$ when $F_i/F_m = 0.78$ [17]. This gave the impression that the presence of oxidised Q_H is necessary for sigmoidicity, since at $F_i/F_m \approx 0.75$–0.80, Q_H is reduced and Q_L oxidised. We have examined sigmoidicity in *Phormidium* PS II particles as the ratio F_i/F_m is raised by potentiometric titration. Fig. 2 shows normalised plots of f_v at different potentials, E_h. Clearly, sigmoidicity was lost ($r = 0$) and the inductions became exponential as F_i/F_m increased at lower potentials. The factor r was determined at a series of ambient redox potentials from computer-generated plots of f_v against $(1-q)$, the latter measured by the growth of area above the induction curve. A plot of r against F_i/F_m for a series of redox potentials (Fig. 3) gave a line of constant slope as F_i/F_m increased until r become zero at $F_i/F_m \geq 0.64$. Thus, the induction curves of *Phormidium* PS II particles were similar to those of chloroplasts not only in their sigmoidicity but also in that they become exponential when F_i/F_m is increased beyond a certain point by lowering of the redox potential. These similarities were found despite the differences in the composition of the acceptor complex in the two systems.

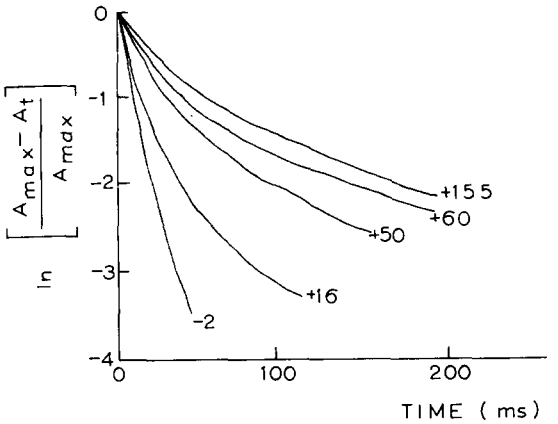

Fig. 4. Semilogarithmic plots of the area growth above the fluorescence induction curve. Analysis was performed by the method of Melis and Homann [2,3] as described in Refs. 7, 17 and 35. Curves at five different E_h values are shown.

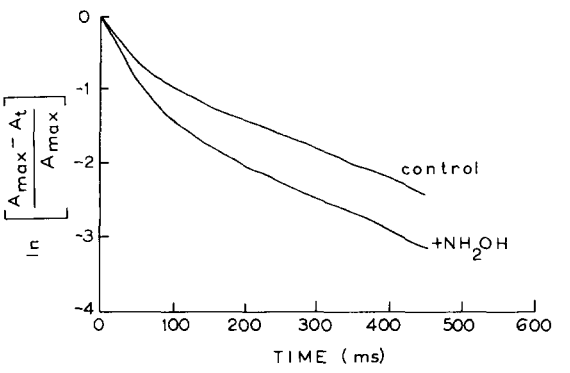

Fig. 6. Effect of NH_2OH on the slow (β) phase of fluorescence induction. Data were obtained as in Fig. 1 except that no redox mediators were present. After 10 min dark adaptation, NH_2OH (10 mM) and DCMU (10 μM) were added. Analysis as in Fig. 4.

The fluorescence induction curves shown in Fig. 1 were analysed by the method of Melis and Homann [2,3] to look for any biphasicity. Semilogarithmic plots of area growth above the curves against time were remarkably similar to those in pea chloroplasts (Fig. 4 and Ref. 7). Clearly, the fast sigmoidal (α) phase was followed by a slow exponential (β) phase. The proportions of Q_α and Q_β, determined from the y-axis intercept of these plots, changed as Q was reduced. This indicates that Q_α and Q_β have different E_m values. In Fig. 5, values for β_{max}, the proportion of Q_β, are plotted against redox potential. Assignment of an accurate E_m value is difficult due to the spread of the data points, but we estimate a value of approx. +45 mV, about 15 mV more positive than Q_α [19]. The spread of data is due to the closeness of the two E_m values which means analysis of small F_v amplitudes became necessary. In contrast, in pea chloroplasts Q_α and Q_β are separated in potential by over 100 mV [7,8].

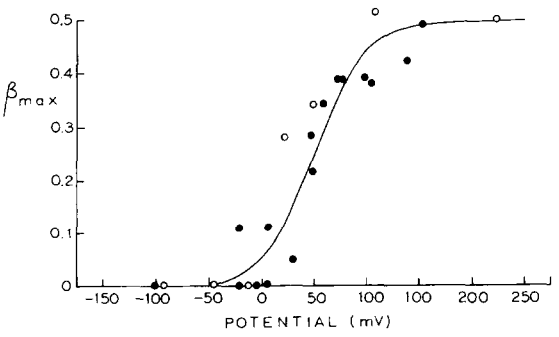

Fig. 5. Redox titration of the proportion of PS II_β, β_{max}, the proportion of PS II due to PS II_β, was derived from the y-axis intercept of the extrapolated linear phase of the curves of the band shown in Fig. 4. The dotted line is an $n=1$ Nernst plot with $E_m = +45$ mV. (●) Reductive titration, (○) oxidative titration.

In spinach chloroplasts, addition of hydroxylamine decreased β_{max} from 0.64 (a value seen consistently in spinach; (Horton, P., unpublished observations) with DCMU alone, to 0.31 with 10mM hydroxylamine (not shown). Similarly, a decrease was observed in *Phormidium* particles (Fig. 6). β_{max} decreased from 0.55 to 0.32 (the rate constant k_β was unchanged). Thus, the contribution of β-centres to the induction was initially greater in chloroplasts, but hydroxylamine removed the slow phase to give approximately the same final extent in both systems.

Discussion

Sigmoidicity

In this paper we have shown that approximately the same degree of sigmoidicity as defined by the value of p [10], seen in the fluorescence induction curve of higher-plant chloroplasts, can

be seen in a PS II particle prepared from a blue-green alga that contains Q_H but is deficient in Q_L. Previously, it has been proposed that the coexistence of Q_H and Q_L in reaction centres of PS II_α would require two photons to close the PS II trap [6]. The postulated equivalence of Q_H and Q_L and Q_1 and Q_2 [24] which has been strengthened by recent experiments using *Phormidium* [19] would also indicate a requirement for more than one photon to close PS II in higher plant chloroplasts, since Q_1 and Q_2 can coexist in the same centre [25]. A requirement for more than one photon could result in sigmoidal kinetics if the rate constants for $Q_HQ_L \rightarrow Q_H^-Q_L$ and $Q_H^-Q_L \rightarrow Q_H^-Q_L^-$ are appropriately related [15,17]. Recent studies have revealed situations in which a correlation was seen between the ratio Q_H/Q_L and the degree of sigmoidicity which were consistent with this proposal [17]. However, it was also suggested that because sigmoidicity is related to the ratio F_i/F_m [10], i.e., to the density of open reaction centres and, because Q_H and Q_L may have different quenching efficiencies, this correlation could be accommodated in a model in which sigmoidicity is due to energy transfer between PS II units.

In the present study, sigmoidicity was seen in the absence of Q_L or Q_2 [19], indicating that a two-photon closure is not obligatory for observing sigmoidal kinetics. Thus, in chloroplasts, energy transfer is at least a partial explanation for the shape of the induction curve. However, the similarity of the values of r_{max} (p) in the two systems requires further comment since it is probably, to some extent, fortuitous. Energy transfer from one unit is another in a pigment bed is governed by the arrangement of the photosynthetic units which in turn governs the centre-to-centre distance [13]. It may be imagined that this distance would be larger in pea chloroplasts in which each centre is associated with approx. 240 chlorophylls than in *Phormidium* (see above) or other systems (see below) with approx. 50 chlorophylls per centre; the probability of trap closure by a photon transferred from the antenna of another closed trap would therefore seem intuitively less likely in the system with the larger number of chlorophylls per centre. However, in our *Phormidium* PS II particles and in other PS II preparations [26,27], it is almost inevitable that the treatment with detergent during preparation will have disrupted the original structural configuration of the pigment bed, and the reaggregation may have produced a less ideal arrangement in terms of energy transfer. The overall efficiency of energy transfer will thus represent the combination of these factors, and as it turned out, the sigmoidicity was only slightly greater in the *Phormidium* preparation, with an antenna size of 40–50 chlorophylls (see above, and Ref. 28), than in pea chloroplasts. In the mutant III-C of *Cyanidium caldarium* which lacks phycocyanin, aggregation of the PS II particles, as observed electron microscopically, was shown to result in a density of reaction centres about twice that in the wild type [29]; together with a small antenna size (approx. 50 chlorophylls per centre) the efficiency of energy transfer is greater in this mutant than in the wild type [23].

Fig. 3 shows that r becomes zero when F_i/F_m becomes equal to or greater than 0.64 in *Phormidium*; the physical significance of this intercept is that at this point, the effective unit size has increased to such an extent that energy transfer between these units has become insignificant and the system behaves as one of separate units of fixed (maximal) optical cross-section. The growth in unit size arises because energy transfer, in effect, is limited to nearest neighbours, and this results in the formation of clusters (islets [30]) of closed centres, increasing the effective antenna size of the remaining centres up to a limiting maximum. We do not wish to attach particular significance to the actual value of the x-axis intercept, since the configuration of reaction centres in our preparation is unlikely to be the same as the arrangement in vivo (see above).

PS II_β and the slow phase of fluorescence induction

A slow phase of fluorescence induction is present in *Phormidium* PS II particles. The shape of the semi-logarithmic plot in Fig. 5 is remarkably similar to that of chloroplasts, with the rate constants for the two phases, k_α and k_β, difering by about 5-fold in magnitude. This result is difficult to understand if PS II_α and PS II_β differ in antenna size, since it would suggest a population of centres with antennae with much less than 40–50 chlorophylls described above.

Thielen et al. [5] have shown that the antennae

of PS II_β centres in tobacco chloroplasts lack Chl *b*, but suggest that the two types may have a similar Chl *a*-containing core. Since the blue-green algae have no Chl *b*, a difference in antenna composition of this nature is not possible.

If a single saturating flash is given to *Phormidium* PS II particles in the presence of DCMU and hydroxylamine 10ms prior to an induction measurement, the fluorescence is found to be practically at its maximal level [19]. In chloroplasts, it has been reported that a single flash reduced the α-component of the area above the curve by approx. 98% and the β-component by approx. 70% in the presence of DCMU [31]. However, in a recent paper by Thielen and Van Gorkom [32], it is reported that the β-component is completely removed by a single flash, which left PS II_α only partially (88%) reduced. The latter experiments, performed in the presence of hydroxylamine and DCMU [8], are more consistent with our findings in *Phormidium* [19].

Some caution should perhaps be applied in equating the slow phase observed here with the slow phase in pea chloroplasts. However, the similarity extends beyond the similar kinetics relative to PS II_α, the major phase of the induction. Thus, hydroxylamine can suppress the slow phase in both systems (which would account for the differences in the flash experiments described above [31,32]). In chloroplasts, the effect is greater than in *Phormidium*. It is not quite clear what the origin of the action of hydroxylamine is; electron donation by hydroxylamine reduces the probability of back reaction between Q^- and P-680$^+$ [33] but this should not suppress Q_β preferentially, since the quantum efficiency for its reduction is equal to that of Q_α [4]. One possible explanation is that hydroxylamine chemically reduces Q_β. Examination of Fig. 6 suggests that hydroxylamine establishes a reducing potential of about 50 mV. Reduction of Q_β in chloroplasts ($E_m + 120$mV) [7] by hydroxylamine would thus be more effective than the reduction of Q_β in *Phormidium* ($E_m \approx +45$ mV); however, this cannot be the exclusive mechanism, since in both cases, a significant proportion of the β-centres remained even after treatment with hydroxylamine.

Thus, the difference between PS II_α and PS II_β cannot be due to thylakoid stacking per se. This is consistent with their presence in agranal chloroplasts, in magnesium-depleted chloroplasts [4,9] and in *Phormidium*. In *Phormidium*, it is also unlikely that the difference between PS II_α and PS II_β is due either to a large difference in antenna size or to its composition. The nature of the heterogeneity which is the basis of their existence therefore remains undefined. One possibility is that PS II_α and PS II_β differ in their aggregation state or more precisely in their connectivity [34]. This would affect the probability of energy transfer between centres, perhaps allowing interaction between PS II_α centres but not between PS II_β centres. One possible factor influencing the aggregation state, at least in green plants, is the presence of phosphoproteins; data obtained after phosphorylation of chloroplast thylakoids could be interpreted as conversion of PS II_α into PS II_β [35]. Phosphorylation could inhibit the ability of the core complexes to aggregate with each other, so giving rise to their segregation in the chloroplasts and their different antenna size and redox properties and hence fluorescence induction kinetics.

Acknowledgements

We wish to thank Dr. Derek S. Bendall for helpful discussions and critical reading of the manuscript. This research was supported by grants from the U.K. Science Research Council to P.H. and D.S.B.

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