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A STUDY ON THE LATERAL DISTRIBUTION OF THE PLASTOQUINONE POOL WITH RESPECT TO PHOTOSYSTEM II IN STACKED AND UNSTACKED SPINACH CHLOROPLASTS

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The quenching of Photosystem II (PS II) chlorophyll fluorescence by oxidised plastoquinone has been used in an attempt to determine their relative distribution in the partition zone and stroma-exposed thylakoid membranes. Thus, the PS II-plastoquinone interaction was determined in stacked (2.5 mM $MgCl_2$) and largely unstacked (0.25 mM $MgCl_2$) membranes. A method to correct for spillover or other quenching changes at the different $MgCl_2$ concentrations, which would compete with the plastoquinone-induced quenching, was devised utilising the quinone dibromothymoquinone. This compound is demonstrated to behave as an ideal (theoretically) PS II quencher at both high and low $MgCl_2$ concentrations, which indicates that it distributes itself homogeneously between partition zone and stroma-exposed membrane regions. In passing from the stacked to the unstacked configuration, the PS II-plastoquinone interaction decreases less than the PS II-dibromothymoquinone interaction. This is interpreted to mean that plastoquinone is present in both the partition zone and stroma-exposed membranes, with somewhat higher concentrations in the stroma-exposed membranes. Thus, plastoquinone is well placed to transport reducing equivalents from the partition zones to the stroma-exposed membranes.

Introduction

It has been known for many years that PS I is found in high concentrations in the stromal membranes of chloroplasts and, conversely, that PS II is present in high concentrations in the granal membranes [1–3]. Upon the advent of a method which permitted the isolation of relatively clean fractions of partition zone membranes [4], Andersson and Anderson [5] were able to demonstrate that the partition zones contain the great bulk of PS II, whereas PS I is largely restricted to the stroma-exposed membranes. Such a distribution of the two photosystems would necessitate the presence of a mobile electron carrier to shuttle reducing equivalents from the partition zones to the stroma-exposed membranes according to the 'Z scheme' of photosynthetic electron transport. Plastoquinone and plastocyanin are the two main candidates for this role [6–8]. Whereas plastocyanin seems to be largely localised in the intrathylakoid space [7,9,10], which would thus permit it to exercise such a function, plastoquinone is located within the membranes themselves. Thus, it is important to know something of the lateral distribution of this compound. Past attempts [17,18] have utilised fractionation procedures which separate granal from stromal membranes. The results, however, are contradictory and suffer from

Abbreviations: PS, photosystem; DBMIB, dibromothymoquinone; PQ, plastoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine.

the defect that much plastoquinone is solubilised by the fractionation technique. In the present work we analyse plastoquinone distribution utilising an approach based on the in vivo quenching of PS II fluorescence by oxidised plastoquinone [11]. Our findings are interpreted to indicate that the plastoquinone pool is found in both partition zone and stroma-exposed membranes, with possibly somewhat higher concentrations present in the stroma-exposed membranes.

Materials and Methods

Chloroplasts were extracted from young, freshly harvested spinach leaves by blending in a solution containing Tricine (30 mM, pH 8), sucrose (0.4 M), NaCl (10 mM) and $MgCl_2$ (2.5 mM). Chloroplasts were pelleted by a brief centrifugation at $1500 \times g$ and resuspended for 2 min in the above medium minus sucrose and containing $MgCl_2$ at a concentration of either 2.5 or 0.25 mM. An equal volume of the sucrose-containing medium was then added, maintaining the $MgCl_2$ concentration unchanged, and the chloroplasts pelleted at $1500 \times g$. They were subsequently resuspended in the sucrose-containing medium ($MgCl_2$, either 2.5 or 0.25 mM) and stored in ice for 1–2 h before starting the experiment. All the above-described steps were performed at 0–4°C. The reaction medium employed consisted of the same components as were present in the storage medium minus sucrose.

The distribution of PS I-oxidisable and PS II-reducible plastoquinone with respect to PS II was ascertained by means of the PS II fluorescence quenching associated with DCMU addition under conditions whereby the primary electron acceptor to PS II, Q, does not change its oxidation state, remaining reduced throughout the measurement. This quenching has been shown to be due to oxidised plastoquinone [11]. In order to ensure complete reduction of the plastoquinone pool before DCMU addition, the chloroplasts were bubbled with nitrogen for about 30 s to lower the oxygen concentration in the reaction medium to about 45 μM. This concentration was maintained approximately constant for the duration of the experiment by blowing nitrogen gently onto the surface of the reaction medium. Under these conditions, DCMU (25 μM) addition led to a slow fluorescence decline (Fig. 1), which if performed in the presence of normal levels of oxygen was preceded by a fluorescence rise, indicating the incomplete reduction of the system of primary and secondary electron acceptors of PS II. This slow fluorescence decline, which is accelerated by the addition of methyl viologen without changing the total extent of the quenching, was calculated as indicated in Fig. 1, where a frequently encountered, approximately linear, small downward drift of the fluorescence emission is corrected for.

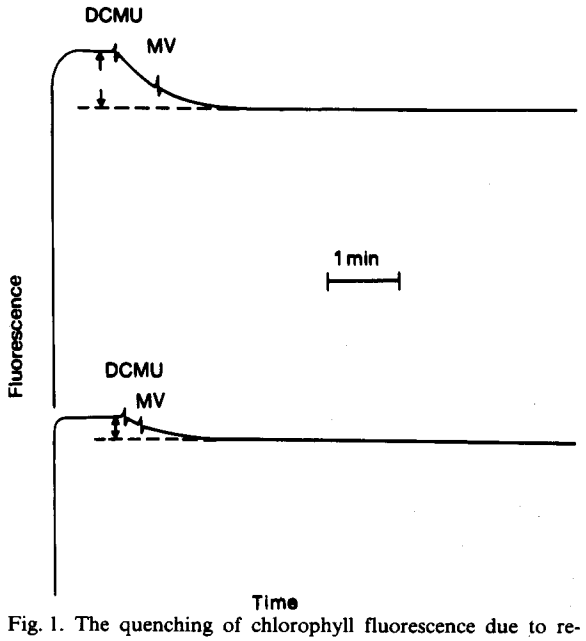

Fig. 1. The quenching of chlorophyll fluorescence due to re-oxidation of the plastoquinone pool following the addition of DCMU (25 μM) and methyl viologen (MV) (10 μM). The slow, apparently linear, downward fluorescence drift is corrected for as indicated and the total extent of quenching is represented by the double arrows. The upper curve is for chloroplasts suspended in the presence of 2.5 mM $MgCl_2$, and the lower curve is for chloroplasts suspended in 0.25 mM $MgCl_2$.

Titration of the fluorescence emission with the quinone DBMIB was also performed. This was achieved by the consecutive addition of 0.7 μM lots of DBMIB, waiting about 1 min between additions to enable complete equilibration to occur. The initial addition also contained DCMU (25 μM). The symbols used are: F_n, the fluorescence after n additions of 0.7 μM DBMIB (n = 1–5); F_0, the normalised initial fluorescence emission.

All fluorescence measurements were performed in a Perkin-Elmer MPF-3 spectrofluorimeter, with the cell compartment thermostatically maintained at 18°C. The excitation light was filtered through a Corning 4-96 filter (18000 $erg \cdot cm^{-2} \cdot s^{-1}$) and the fluorescence was measured at 681 nm using a Baltzers interference filter in addition to the instrument monochromator. The chlorophyll concentration was 4 $\mu g/ml$.

Results and Discussion

This work is based on the assumption that in stacked membranes the bulk of PS II is concentrated in the partition zones [5,7,8,12,13] and that upon unstacking it becomes approximately randomised throughout all the membranes [12,13]. Thus, in stacked membranes one can envisage a number of possible situations when plastoquinone is taken into account.

(1) Like the PS II complex, it is also largely restricted to the partition zones in stacked membranes. In this case, unstacking is expected to lead to a dilution of the concentration of plastoquinone and hence a decreased interaction.

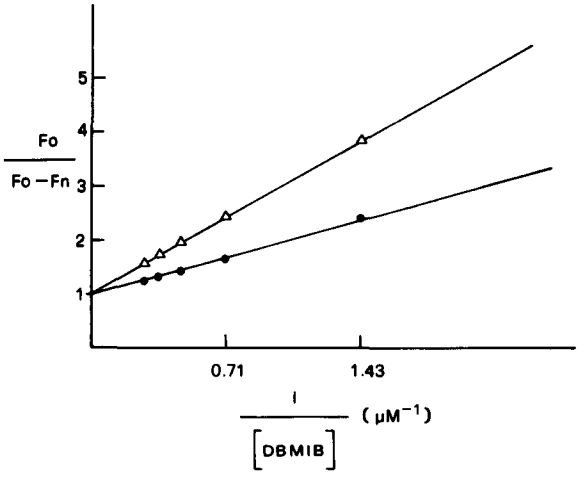

Fig. 2. The reciprocal of the fluorescence quenching ($F_0/[F_0 - F_n]$) vs. the reciprocal of the DBMIB concentration for chloroplasts suspended in 0.25 or 2.5 mM $MgCl_2$. DBMIB was added at concentrations between 0.7 and 3.5 μM. (●) 2.5 mM $MgCl_2$, (△) 0.25 mM $MgCl_2$. Data have been averaged from nine separate determinations using five different chloroplast preparations. The chlorophyll fluorescence was 56 units in the presence of 2.5 mM $MgCl_2$ and 26.9 units in the presence of 0.25 mM $MgCl_2$.

(2) It is evenly distributed throughout the membranes when they are in both the stacked and unstacked configuration. In this case, the interaction between PS II and plastoquinone is not expected to change upon membrane unstacking.

(3) The concentration of plastoquinone is higher in the unstacked membrane regions, in which case unstacking would lead to an increased PS II-plastoquinone interaction.

In order to estimate the extent of the PS II-plastoquinone interaction in the presence of different concentrations of Mg^{2+}, it is necessary to correct for the effect of spillover and other possible quenching changes associated with different levels of Mg^{2+}. Increased spillover, in the presence of low concentrations of Mg^{2+}, is expected to lead to decreased quenching by plastoquinone (or any other quencher which competes with the spillover for energy within PS II). This problem has been overcome by titration of the fluorescence with the quinone DBMIB. The results (Fig. 2) confirm the observation of Kitajima and Butler [14] that the plot of $F_0/(F_0 - F_n)$ vs. 1/[DBMIB] yields a straight line, which extrapolates towards $F_0/(F_0 - F_n) = 1$ as 1/[DBMIB] approaches zero. This is expected from a theoretical point of view, as Kitajima and Butler [14] have pointed out using a simple fluorescence theory in which the fluorescence yield is the simple ratio of the rate constant of fluorescence to the rate constants for all deactivating processes at the level of the PS II antenna chlorophyll. It is easily demonstrated that the subsequent refinement and elaboration of the fluorescence theory by Butler and his colleagues [15,16] leads to the same conclusion.

Fig. 2 illustrates two important points. (a) In the presence of low spillover (2.5 mM $MgCl_2$), quenching by the same concentration of DBMIB is greater than in the presence of high spillover (0.25 mM $MgCl_2$) due to competition effects. (b) Quenching by addition of the same concentration of quencher decreases as the background quenching increases.

The latter point is made more clearly in Fig. 3, in which data from the same experiment as Fig. 2 are plotted as $(F_{n-1} - F_n)/(F_{n-1})$ vs. $(F_0 - F_n)/F_0$. The value of $(F_{n-1} - F_n)/(F_{n-1})$ approaches zero as $(F_0 - F_n)/F_0$ approaches 1. The intercept on the y-axis, at $(F_0 - F_n)/F_0$ equal to zero, gives a value

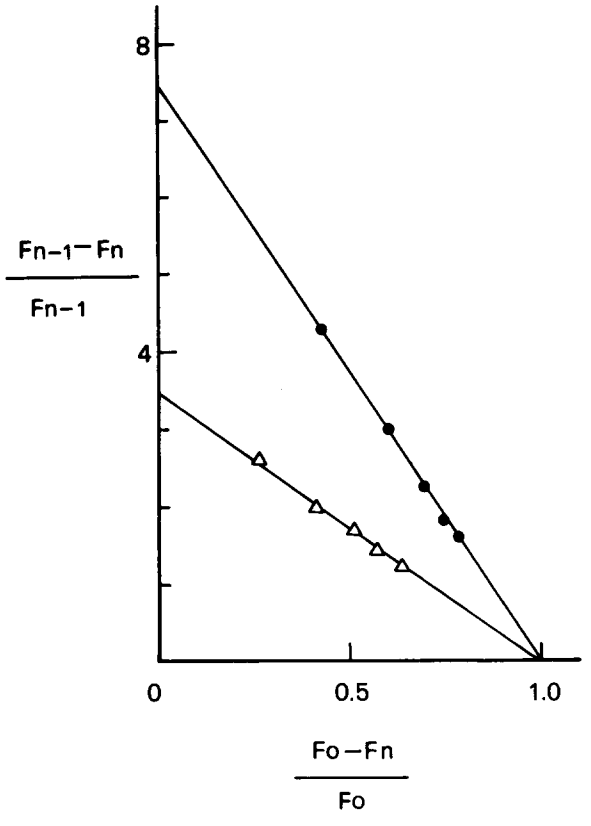

Fig. 3. Plot of the fluorescence quenching due to each addition of 0.7 μM DBMIB ($[F_{n-1} - F_n]/F_{n-1}$) vs. the total fluorescence quenching accumulated due to all additions of DBMIB made to the point ($[F_0 - F_n]/F_0$). The data are from the same series of experiments as those of Fig. 2.

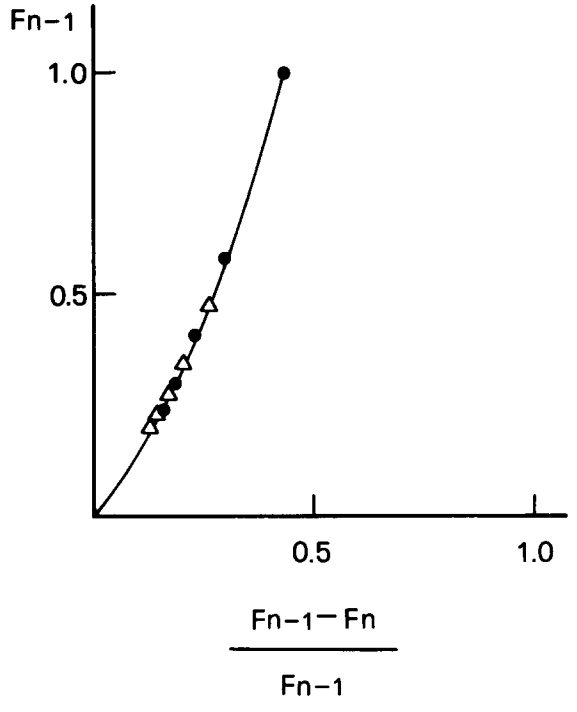

Fig. 4. Plot of the fluorescence yield prior to each 0.7 μM addition of DBMIB (F_{n-1}) vs. the quenching brought about by each single addition of DBMIB ($[F_{n-1} - F_n]/F_{n-1}$). The data are from the same series of experiments as those of Fig. 2. (●) 2.5 mM $MgCl_2$, (△) 0.25 mM $MgCl_2$.

which is directly proportional to the extent of the PS II-quinone interaction (Appendix). This value will be referred to here as the PS II-quinone relative interaction index. Thus, it is seen that the quenching produced by a finite concentration of quencher is always an underestimate of the interaction of that particular concentration of quencher with PS II. This can be obtained by means of the $(F_{n-1} - F_n)/(F_{n-1})$ vs. $(F_0 - F_n)/F_0$ plot.

In Fig. 4 are presented data from the DBMIB experiment in the form of an F_{n-1} vs. $(F_{n-1} - F_n)/F_{n-1}$ plot in the presence of 2.5 and 0.25 mM $MgCl_2$. As discussed before, with increased quenching (lower F_{n-1}), the subsequent effect of the same addition of quinone decreased in both cases. Interestingly, data obtained in the presence of high and low concentrations of $MgCl_2$ fall on the one curve, which is expected from the theoretical consideration that from the point of view of PS II an increase in the rate constant for radiationless decay due to an added quencher or an increase in the rate constant for spillover is the same thing. The fact that these data obey the theoretical predictions means that DBMIB partitions itself homogeneously between the appressed and nonappressed membranes. Thus, its use here to correct the plastoquinone data for interactions with spillover is entirely justified.

In Table Ia are presented data from a series of experiments in which the quenching due to the oxidised plastoquinone pool was measured at high and low concentrations of $MgCl_2$. These data are compared with the quenching induced by DBMIB, equalising the two values obtained in the presence of 2.5 mM $MgCl_2$ and from Fig. 2 calculating the quenching expected at that particular concentration of DBMIB in the presence of 0.25 mM $MgCl_2$ (Table Ib). Clearly, the plastoquinone-induced

TABLE I

FLUORESCENCE QUENCHING DUE TO THE PLASTOQUINONE POOL COMPARED WITH THAT BROUGHT ABOUT BY DBMIB IN CHLOROPLASTS SUSPENDED IN 2.5 OR 0.25 mM $MgCl_2$

Quenching is expressed as the fluorescence decrease divided by the initial fluorescence. (a) Quenching due to plastoquinone (PQ) (average of 21 measurements made with five different chloroplast preparations). (b) Quenching due to DBMIB and calculated from Fig. 2 (average of nine measurements made with five different chloroplast preparations). (c) The PS II-plastoquinone relative interaction index. (d) The PS II-DBMIB (0.7 μM) relative interaction index. (e) The normalised chlorophyll fluorescence emissions prior to quinone-induced quenching.

	Values obtained with		B/A ratio
	2.5 mM $MgCl_2$ (A)	0.25 mM $MgCl_2$ (B)	
(a) Quenching (PQ)	0.157	0.112	0.71
(b) Quenching (DBMIB)	0.157	0.083	0.53
(c) PS II-PQ relative interaction index	0.185	0.126	0.68
(d) PS II-DBMIB relative interaction index	0.745	0.345	0.46
(e) Chlorophyll fluorescence	1.0	0.48	0.48

quenching decreased less than the DBMIB-induced quenching in passing from the stacked configuration (2.5 mM $MgCl_2$) to the largely unstacked one (0.25 mM $MgCl_2$). However, as discussed above, these values will be underestimates of the PS II-plastoquinone interaction, so we have also used the graphical method represented in Fig. 3 (Table Ic and d). Here, also, we see that the decrease in the PS II-quinone relative interaction index on passing from stacked to unstacked membranes is greater for DBMIB than for PQ. The ratio of the DBMIB-induced quenching is practically the same as that of the decrease in chlorophyll fluorescence yield due to Mg^{2+} removal (Table Id and e), which is expected from theoretical considerations.

We conclude that plastoquinone is located throughout the membrane with a somewhat higher concentration in the parts of the membranes where PS II is not located in the fully stacked configuration, i.e., in the stroma-exposed membranes. Thus, this substance is suitably distributed to function as a mobile electron carrier between the partition zone and the stroma-exposed membranes. It should be mentioned, however, that evidence has been presented [7,8] which suggests that the cytochrome *b-f* complex is distributed evenly between partition zone and stroma-exposed membranes and that this may support the idea that plastocyanin carries electrons between the two membrane compartments. Cox and Andersson [7] furthermore suggest that the diffusion rate of a protein with the dimensions of plastocyanin in a medium with the same viscosity as water is compatible with the turnover time of P-700. Nesbitt and Berg [19], however, indicate that in the light the internal thylakoid space may have a viscosity which is 20–30-times greater than that of water. Thus, to date, it is not possible to definitively reach a conclusion regarding the nature of the mobile electron carrier and it may be that both plastocyanin and plastoquinone can potentially transport reducing equivalents from the partition zones to the stroma-exposed membranes.

Appendix

With all PS II traps closed one can write:

$$F_0 = \frac{k_f}{k_f + k_x}$$

$$F_n = \frac{k_f}{k_f + k_x + nDk_q}$$

$$F_{n-1} = \frac{k_f}{k_f + k_x + (n-1)Dk_q}$$

where k_f is the rate constant for fluorescence emission, k_q the rate constant for quinone-induced quenching, k_x the rate constant for all other processes which compete for the PS II excitons, D the number of molecules of quencher/PS II unit per addition of quencher and n the number of such additions. Thus:

$$\frac{F_0 - F_n}{F_0} = \frac{nDk_q}{k_f + k_x + nDk_q}$$

and

$$\frac{F_{n-1} - F_n}{F_{n-1}} = \frac{Dk_q}{k_f + k_x + nDk_q}$$

When

$$\frac{F_0 - F_n}{F_0} = 0$$

$$\frac{F_{n-1} - F_n}{F_{n-1}} = \frac{Dk_q}{k_f + k_x}$$

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