Hypothesis

Plastoquinone as a mobile redox carrier in the photosynthetic membrane

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The role of plastoquinone as a mobile redox carrier linking photosystem II and the cytochrome b_6–f complex is considered. It is proposed that plastoquinone is located primarily within the fluid bilayer-midplane region of the thylakoid membrane and thus can move laterally at very fast rates corresponding to a microscopic diffusion coefficient of 10^{-6} cm^2·s^{-1}. Because of the presence of integral proteins the diffusion path will be tortuous and extended, giving rise to a sub-macroscopic diffusion coefficient which is lower than the above value. Even so it is concluded that within the half-time for electron donation to the cytochrome b_6–f complex there is adequate time for plastoquinone to diffuse over a distance equivalent to the radius of a granum membrane.

Plastoquinone *Fluidity* *Lateral diffusion* *Thylakoid membrane* *Photosynthesis*

1. INTRODUCTION

The thylakoid membrane of chloroplasts is the site for the light-driven electron transport processes of photosynthesis. The pigments which absorb photons and the majority of the redox components involved in subsequent electron transfer are incorporated into 4 macromolecular protein complexes; the light-harvesting chlorophyll *a*/chlorophyll *b* protein (LHCP), Photosystem II (PS II), Photosystem I (PS I) and the cytochrome b_6–f complex [1,2]. These complexes, together with the ATP synthase complex (CF_0–CF_1) are embedded in the lipids of the thylakoid membrane and their co-operative interactions are dependent on several diffusional processes. These processes probably involve rotational and lateral movements within the membrane and are therefore dependent on the fluidity of the lipid matrix [3]. In particular, a picture is emerging that electron transfer from PS II to the cytochrome b_6–f complex is brought about by long-range diffusion of reduced plastoquinone (PQ) [4]. In this article we examine the suitability of PQ for this diffusional role taking into account the composition and physical nature of the thylakoid membrane matrix. From this we propose probable distances traversed and suggest a likely value for the PQ diffusion coefficient.

2. LIPID COMPOSITION OF THE THYLAKOID MEMBRANE

The thylakoid lipid matrix is dominated by the uncharged lipids; monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) [5]. The majority of the remaining lipids, sulphoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG), carry net negative charge although there is a small amount of the zwitterionic phospholipid, phosphatidylcholine (PC). A striking feature of these lipids is the high degree of unsaturation of their acyl chains with α-linolenic acid ($\Delta_{9,12,15}$ C18:3) representing the major fatty acid. For example, in the thylakoid membrane of *Pisum sativum* approx. 80% of the acyl chains are linolenic acid giving more than 5 double bonds per

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lipid moleule [6,7]. Recent analyses have indicated that the distribution of various lipid species in the thylakoid is not homogeneous either across the bilayer or in different regions along the plane of the membrane [8–10]. Such an asymmetry is not surprising if, for example, the non-bilayer forming lipid MGDG is required for packaging specific proteins into the membrane as well as being necessary to satisfy the restraints imposed by the high radius of curvature at the grana ends [11,12]. In addition to this, specific protein interactions have been suggested [13,14] which will also result in heterogeneity in the lipid class distribution.

3. PHYSICAL PROPERTIES OF THE LIPID MATRIX

Work on pure phospholipid systems, using ^{2}H-NMR [15] or fluorescence anisotropy of hydrophobic membrane probe molecules such as diphenylhexatriene (DPH) [16], has shown that the effect of fatty acid unsaturation is to produce a disordered environment where the acyl chains possess substantial dynamic motion. It is therefore not surprising that in model systems employing isolated thylakoid lipids without [17] and with integral thylakoid proteins reconstituted [18], or with natural chloroplast membranes [19], the thylakoid lipids reveal themselves to be exceptionally fluid at physiological temperatures, at least when analysed using the fluorescence anisotropy properties of DPH. Indeed, in comparison with other biological membranes [20] the thylakoid is one of the most fluid, being comparable with the inner mitochondrial membrane. Apparently, even though the presence of integral proteins causes a substantial ordering of the thylakoid lipid acyl chains their dynamic motion seems to be only slightly restricted [17–19].

Information about the fluidity of biological and artificial membranes at different depths in the bilayer can be gained by ESR spectroscopy using spin-labelled fatty acid probes where the label is positioned at different places along the hydrophobic tail [21,22]. From this approach it has become clear that in the thylakoid membrane [23,24] as with other membranes, the fluidity increases to a maximum value at the membrane bilayer midplane. Whilst it is difficult to give a precise value for the viscosity at the bilayer midplane the DPH technique gives an average value for the whole hydrophobic interior of about 0.34 P at 25°C [19]. However, it is not unreasonable to assume a midplane viscosity approaching 0.01 P which is a value proposed by Vaz et al. [25]. Such a low value is comparable with the viscosity of water at 20°C and emphasises just how fluid the environment is within the centre of a membrane, especially one which is composed of unsaturated lipids.

4. LOCATION OF PLASTOQUINONE WITHIN THE MEMBRANE

The main species of long-chain prenylquinone in the thylakoid membrane, PQ_A [26], possesses a relatively non-polar head group compared with the membrane lipids and its tail is both substantially longer (C_{36} *vs* C_{18} for the most common fatty acyl

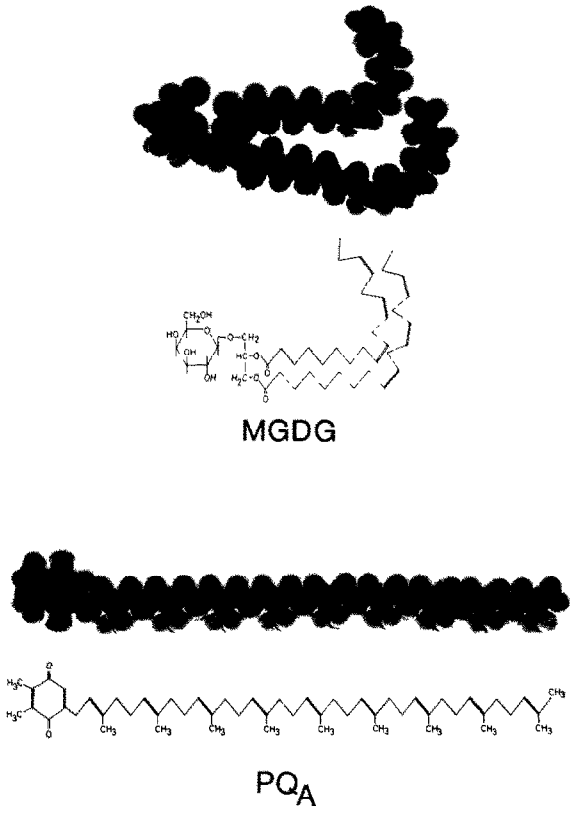

Fig.1. Corey-Pauling-Koltan (CPK) space filling models and chemical structures of monogalactosyldiacylglycerol (MGDG) and plastoquinone A_{45} (PQ_A).

chains) and bulkier due to the isoprenoid side-chain methyl groups. The chemical structure of PQ_A [27] and that of MGDG, the predominant thylakoid polar lipid, are compared in fig.1. Thus, intuitively based on structural grounds, the PQ_A molecule would not be expected to pack effectively between the acyl chains of the thylakoid lipid matrix. Indeed, this is borne out from studies where long-chain prenylquinones have been artificially introduced into a variety of membrane systems. For example, differential scanning calorimetry experiments using phospholipid bilayer membranes containing differing proportions of the prenylquinone, ubiquinone-10 (Q_{10}), showed that its presence did not cause an appreciable broadening of the lipid phase transition [28,29]. This was taken to indicate that the quinone molecules were not interposed between the lipid acyl chains which is a general conclusion also drawn from monolayer studies with similar lipid systems [30,31], although a monolayer study employing unsaturated galactolipids and PQ_A did indicate a small degree of quinone/lipid miscibility [32]. Overall these studies suggest that long-chain prenylquinones, such as PQ_A, are likely to be located relatively deep within the hydrophobic region of the lipid bilayer close to the midplane. Such a conclusion has also been reached from fluorescence quenching studies [33] as well as from ESR [34] and NMR [35] measurements.

5. RATE OF LATERAL MOTION OF PLASTOQUINONE

Most discussions of the lateral diffusion coefficient (D_L) of plastoquinone in the plane of the thylakoid membrane [4] and of ubiquinone in the mitochondrial membrane [36] have been based on values of D_L found for fluorescent analogues of polar lipid molecules. These are of the order of 10^{-8} $cm^2 \cdot s^{-1}$ for lipids in biological membranes (table 1). However, such comparisons have presupposed that PQ_A molecules move laterally in an analogous fashion to a polar lipid. Accepting the argument that PQ_A is likely to be located in the midplane region of the membrane and not packed vertically across the bilayer between the lipid acyl chains, it is almost certainly incorrect to adopt D_L values as low as 10^{-8} $cm^2 \cdot s^{-1}$. In fact we suggest that PQ_A and related molecules like ubiquinone

Table 1

Lateral diffusion coefficients of fluorescent lipid analogues measured by fluorescence recovery after photobleaching

Lipid analogue	Lipid bilayer	T (°C)	D_L ($cm^2 \cdot s^{-1}$) ($\times 10^8$)	Ref.
NBD-PE	Soybean lecithin	36	11.0	25
NBD-PE	DMPC	36	8.8	
NBD-PE	DMPC multibilayer	30	5.5	47
NBD-PE	Egg PC	25	4.0	48
DiI-C_{16}	Fibroblast plasma membrane lipids	37	>6.0	49
DiI-C_{16}	Fibroblast plasma membrane	37	2.0	
DiI-C_{18}	Rat myotube	30	0.6	50
DiI-C_{18}	Hamster V79 cell line	22	0.6	51

NBD-PE, (4-nitrobenz-2-oxa-1,3-diazole)phosphatidylethanolamine; DiI-C_{16} and DiI-C_{18}, 3,3′-dihexadecyl- and 3,3′-dioctadecylindocarbocyanine iodide, respectively

diffuse laterally in the centre of the bilayer where the local viscosity is likely to be very low. Thus it is not unreasonable, given no direct measurement, to assume that D_L for PQ_A could be as high as 10^{-6} $cm^2 \cdot s^{-1}$. A value of this order of magnitude has been quoted previously for quinone diffusion [37] based on the work of Marcus and Hawley [38]. Using the Einstein two-dimensional diffusion equation $\bar{r}^2 = 4D_L t$, where t is time and $\bar{r}$ is the mean diffusion path length and taking $D_L = 10^{-6}$ $cm^2 \cdot s^{-1}$, then a PQ_A molecule may diffuse about 2800 nm in 20 ms, which is the half-time for cytochrome f reduction at room temperature after a series of saturating flashes [39].

6. ACTUAL DISTANCE TRAVERSED BY PQ_A

For normal functioning of linear electron flow in photosynthesis, PQ_A must traverse the distance between its site of reduction at the PS II complex to its site of oxidation at the cytochrome b_6–f complex. Whilst the exact position of the latter is uncertain [40,41], if we accept its location in the thylakoid granal margins [2,42] and a typical radius of 250 nm for a granum then the 'average'

PS II will be about 73 nm from the nearest cytochrome b_6–f complex. The value of 73 nm represents the difference between the radius of the granum (250 nm) and an inner circle which gives 50% of the total area and thus 50% of PS II complexes (fig.2).

Both from a theoretical view point [43] and from direct measurements [44] the granal membranes have a high protein:lipid ratio. Thus although on average a PQ_A molecule has only to diffuse linearly about 73 nm, in fact its diffusion path may be tortuous as a result of the presence of integral proteins (see fig.2). As calculated above, a D_L value of 10^{-6} $cm^2 \cdot s^{-1}$ would allow a long diffusion path and this should compensate for the problem of a high density of PS II complexes sequestered in the appressed granal membrane [45]. The proposed extended path for PQ_A diffusion would express itself as an apparent decrease in a measured D_L value as noted when comparing the diffusion coefficients of polar lipids in artificial or natural biological membranes. However, as can be seen in table 2 this effect is not as large as might have been expected, and it seems unlikely that the apparent (submacroscopic) D_L value will drop below 10^{-7} $cm^2 \cdot s^{-1}$. Thus it seems probable that the postulated lateral separation of PS II and cytochrome b_6–f complexes can readily accommodate the concept of PQ_A acting as an obligatory mobile redox intermediate. Moreover, even a D_L = 10^{-7} $cm^2 \cdot s^{-1}$ would give an $\bar{r}$ value of ~900 nm (in 20 ms) which could mean that the plastoquinol oxidation reaction is the rate-determining step of linear electron flow and not the PQ_A diffusion process itself.

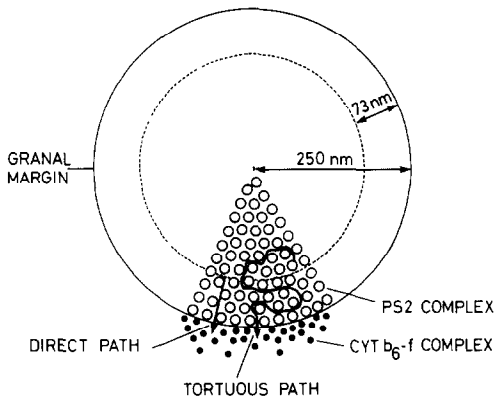

Fig.2. Diagrammatic representation of a typical granum disc of radius 250 nm showing direct and tortuous PQ_A diffusion paths from Photosystem II (PS2) complexes (○) to cytochrome b_6–f complexes (●) positioned at the granal margin. The inner circle (250–73 nm) divides the granal discs into equal areas and is taken to represent the position of an average Photosystem II complex. The position of the cytochrome complex at the margin is based on arguments previously given [2,42].

The above discussions have taken no account of the lipid class composition differences between the appressed and non-appressed membranes which may influence the rate of PQ_A diffusion. However, the two lamella types have remarkably similar fatty acid contents and levels of unsaturation [9,44] which is probably more pertinent than head group properties when considering midplane diffusion. It is also worth noting that non-appressed membranes are more fluid than appressed granal membranes [44] due to a lower protein:lipid ratio. This difference in fluidity indicates that the resistance to PQ_A motion and the lateral diffusion of other components (e.g., pigment–protein complexes [45,46]) would be less in the stromal and end-granal membranes compared with PS II-enriched appressed membranes.

7. SUMMARY

We propose the following points regarding the role of plastoquinone as a mobile redox carrier in photosynthetic intersystem electron flow.

(i) Plastoquinone is located primarily within the extremely fluid bilayer-midplane region of the thylakoid membrane.

(ii) Plastoquinone lateral diffusion occurs by 'tunnelling' along the midplane region and is likely to be extremely rapid with a real (microscopic) diffusion coefficient as high as 10^{-6} $cm^2 \cdot s^{-1}$.

(iii) The presence of integral proteins, particularly the PS II complex located in the appressed granal membranes would be expected to increase the path length for plastoquinol/plastoquinone diffusion to and from the cytochrome b_6–f complex.

(iv) Because of the tortuous diffusion path the observed radial (submacroscopic) diffusion coefficient may appear lower than

10^{-6} $cm^2 \cdot s^{-1}$ but that it is unlikely to pose a problem in satisfying the known kinetics of this step in intersystem electron transfer.

(v) The concept of an extended diffusion path length deserves a closer theoretical analysis taking into account likely protein packing and a midplane diffusion process.

(vi) Although not discussed above and bearing in mind the postulated midplane position, vectorial transport of reducing equivalents across the membrane by the PQ_A suggests that the quinone/quinol head group has some probability of moving towards the surface of the membrane or alternatively electron/proton exchanges occur at the quinone binding sites within the PS II and cytochrome b_6–f complexes positioned relatively deeply in the hydrophobic core of the lipid matrix.

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