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Plastoquinones in photosynthesis

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Several plastoquinones with different or modified side chains have been characterized in plant material: they are localized in the inner thylakoid membrane of the chloroplast. So far only plastoquinone-45 (PQ-45) has been identified as an obligatory functional component of the photosynthetic electron transport chain in chloroplasts between photosystem II and photosystem I. A special form (semiquinone) of PQ-45 acts as primary acceptor Q of photosystem II, a large pool of PQ-45 as electron buffer, interconnecting several electron transport chains. The rôle of PQ in energy conservation (ATP formation) is of particular current interest. Owing to vectorial electron flow across the thylakoid membrane, plastoquinone is thought to be reduced on the outside and plastohydroquinone to be oxidized on the inside of the membrane. This results in a proton translocation across the membrane and a build-up of a proton motive force which drives ATP formation. Old and new plastoquinone antagonists are described and the relevance of inhibitor studies on the rôle of plastoquinone in electron flow and photophosphorylation is discussed. Open questions and current problems of the mechanism of plastoquinone/plastoquinol transport across the membrane – and of proton translocation connected to it – relevant for the mechanism of energy conservation in photosynthesis, are pointed out.

Several prenyl substituted dimethyl-*p*-benzoquinones (the plastoquinone series) of different chain length have been isolated from plants and algae. The major compound, plastoquinone A_{45} or PQ-45, has nine isoprenyl units with 45 C atoms in the side chain. Plastoquinone-15 and -20 have also been found as well as the phytyl derivative of dimethylbenzoquinone. In the plastoquinone B and C series a hydroxy group in the side chain (figure 1) may or may not be esterified with various fatty acids (Redfearn 1965; Barr & Crane 1971). The biosynthesis of plastoquinone from shikimic acid, CO_2, mevalonic acid and the methyl group of methionine via homogentisic acid and polyprenylphosphate has been worked out by Threlfall & Whistance (1971). Plastoquinone(s) are localized in the chloroplast of the leaf, and there in the inner thylakoid membrane as well as in osmiophilic plastoglobuli (Lichtenthaler 1969).

Only for plastoquinone A with 45 C atoms in the side chain has a functional rôle in plant metabolism been established. It is an obligatory electron carrier in the photosynthetic electron transport system, which in the light oxidizes water to oxygen and reduces $NADP^+$ via two photosystems. In bacterial photosynthesis, plastoquinone is replaced by ubiquinone or in some bacteria by a naphthoquinone.

First results on the function of plastoquinone

The function of plastoquinone, first isolated and identified by Kofler (1946), became clear when ubiquinone and its rôle in respiratory electron flow was discovered in mitochondria and a search for it in various organisms led to the rediscovery of plastoquinone by Lester & Crane (1959). At this time the principal activities of isolated chloroplasts had been established by Arnon, Whatley and Allen (Hill reaction with $NADP^+$ as acceptor and photophosphorylation).

Early petrol ether extraction of the chloroplasts had been shown to inactivate the photosynthetic activity and a reactivation by components of the extract had been ascribed to carotene or to vitamin K (phylloquinone) (Bishop 1958). With the characterization of plastoquinone it became immediately clear that plastoquinone is the active component removed by petrol ether and required for reactivation (Bishop 1959). At a meeting of the Royal Society in 1962 the results of the rôle of plastoquinone in photosynthesis as obtained by petrol ether extraction of

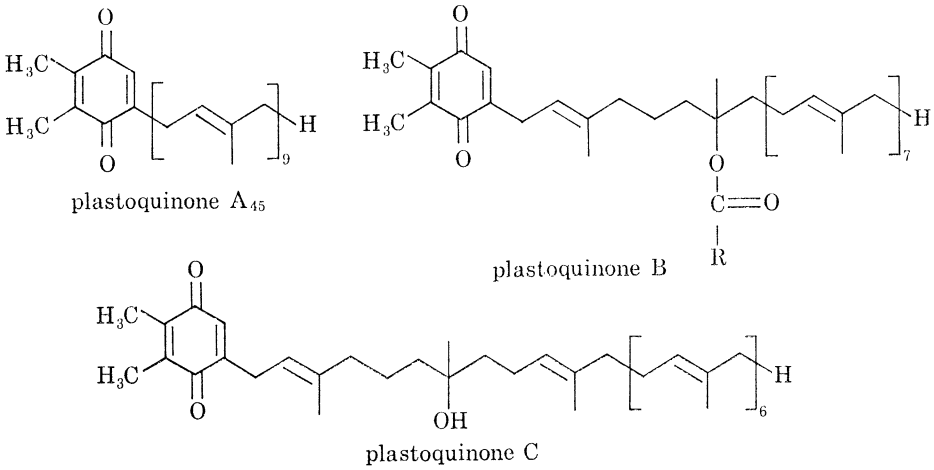

FIGURE 1. Chemical structure of plastoquinones.

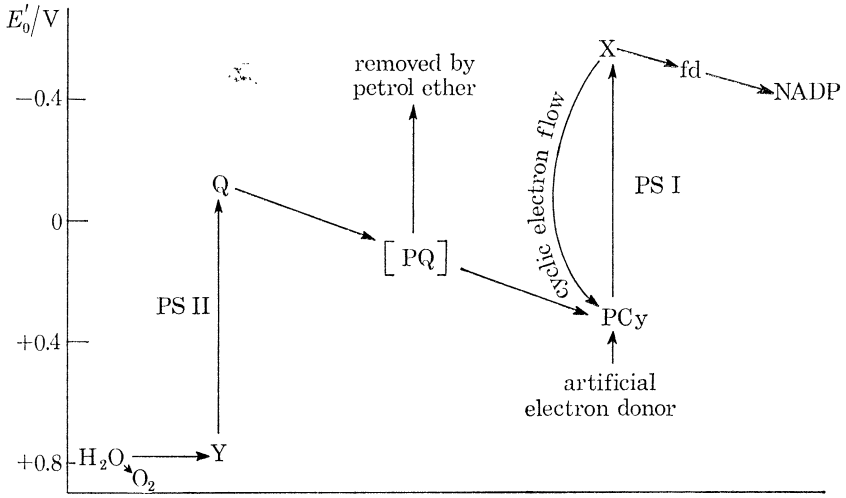

FIGURE 2. Function of plastoquinone in the photosynthetic electron transport chain. Removal of plastoquinone still permits NADP photoreduction at the expense of artificial electron donors as well as cyclic electron flow. Y, Primary donor of photosystem II; Q, quencher and primary acceptor of photosystem II; PQ, plastoquinone; PCy, plastocyanin; X, primary acceptor of photosystem I; fd, ferredoxin; PS, photosystem.

chloroplasts were summarized (Trebst 1963). The functional rôle of plastoquinone was shown to be close to photosystem II, photoreductions at the expense of an artificial donor system (like dichlorophenolindophenol–ascorbate) for photosystem I were not affected by petrol ether extraction (figure 2). This was in agreement with biophysical studies by Klingenberg, Müller, Schmidt-Mende & Witt (1962). Cyclic photophosphorylation catalysed by PMS (phenazine-methosulphate) seemed to be dependent on plastoquinone (Krogmann & Olivero 1962), but

very possibly, as we realize now, an additional effect of petrol ether on the structure of the thylakoid membrane is the cause of the inactivation of ATP formation *per se* by petrol ether. The reactivation of petrol ether-extracted chloroplasts showed a specificity for dimethyl benzoquinones with a prenyl side chain of at least 5 C atoms (Trebst 1963).

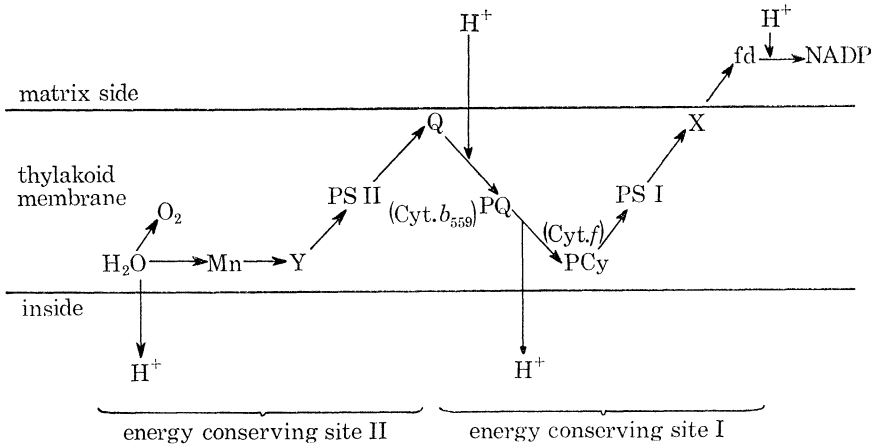

FIGURE 3. Vectorial electron flow across the thylakoid membrane of chloroplasts, indicating the two areas of energy conservation (coupling sites or proton releasing sites). According to Trebst (1974). Abbreviations as in figure 2. Inhibitors: CCP, carbonylcyanid-chlorophenylhydrazone; DCMU, dichlorophenyldimethylurea; DBMIB, dibromothymoquinone.

In the 15 years since then the central importance of plastoquinone in photosynthetic electron flow has been strengthened. As reviewed recently in the *Encyclopedia of plant physiology*, mainly by Amesz (1977), there is now detailed information on the mechanism of its reduction by photosystem II and of its function as primary acceptor and quencher of this photosystem. Furthermore, its rôle in energy conservation, i.e. in coupled ATP formation, became clear owing to the capacity of the hydroquinone form to carry hydrogens across the membrane. The mechanism of this proton translocation and build-up of a pH gradient across the membrane, via vectorial electron flow (figure 3) and the mechanism of the reoxidation of plastohydroquinone by photosystem I on the inner site of the thylakoid are now crucial points for progress in photosynthesis research. This report will summarize the main results on plastoquinone function of the past years and then touch on some current thinking on unresolved problems. It will become obvious that all this knowledge about plastoquinone function concerns the quinone part of the molecule only but perhaps we shall get closer also to an understanding why Nature chose nine prenyl units in the side chain when the binding in and translocation of plastoquinone through the membrane can be investigated in detail.

When Duysens and his colleagues studied the action of the herbicide and photosynthesis inhibitor DCMU (dichlorophenyldimethylurea), they postulated a primary acceptor of photosystem II called Q (= quencher of chlorophyll fluorescence). The identity of Q with a special form of plastoquinone, spatially and functionally separated from the main pool of plastoquinone, being reduced in the primary photoaction to the semiquinone radical only, became gradually accepted. An absorption change at 320 nm (compound X_{320}) observed by Stiehl & Witt (1969) was correlated with the primary acceptor of photosystem II on one hand and with plastosemiquinone on the other. Convincing evidence for Q's being the semiquinone was finally provided by van Gorkkom (1974) and Knaff, Malkin, Myron & Stoller (1977).

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In the sequence of electron carriers in the photosynthetic electron flow chain the small pool of the primary acceptor of photosystem II is followed by a large secondary electron acceptor pool A. No doubt remains that this pool A is plastoquinone. It is important to note that there are about seven to eight times as many plastoquinone molecules as photosystems and (most) other carriers in the chain (Stiehl & Witt 1969): plastoquinone acts as redox or electron buffer between the two photosystems. Indeed, several photosystems II are connected by this large plastoquinone pool to several photosystems I (Siggel, Renger, Stiehl & Rumberg 1972). This buffering and interconnecting property of the plastoquinone pool (figure 4) assures high efficiency of electron flow when light hits the photosystems in sequence and in different electron transport chains. Recently evidence for still another plastoquinone species has been found.

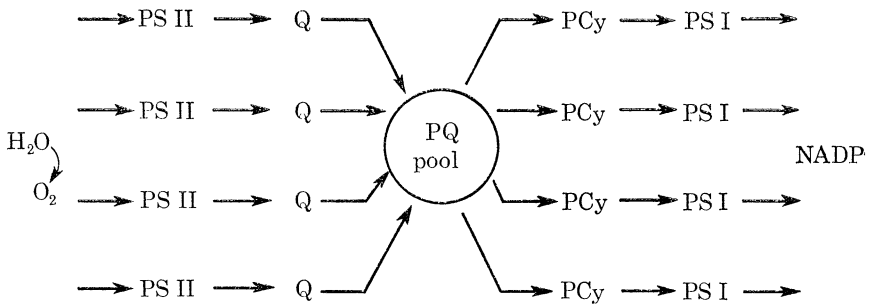

FIGURE 4. The large plastoquinone pool interconnects several electron transport chains and acts as electron buffer between the photosystems. According to Stiehl & Witt (1969) and Siggel *et al.* (1972). Mn, Managnese in the water-splitting system; Cyt, cytochrome.

A compound B (Bouges-Bocquet 1973) or R (Velthuys & Amesz 1974), accumulating two electrons from photosystem II, is located between the primary quencher Q and the main plastoquinone pool A and is possibly a plastoquinol anion. These events in the reduction of plastoquinone via the semiquinone radical and the dianion followed by proton uptake to form the hydroquinone is thought to occur on the matrix side of the thylakoid membrane (figure 3) (for reviews see Trebst 1974; Witt 1975). Plastoquinone is not exposed to the hydrophilic environment, because a protein shield covers this functional area of the membrane (Renger 1976); the shield also slows down the proton uptake in plastoquinone reduction to 60 ms (Ausländer & Junge 1974). The oxidation of plastohydroquinone by photosystem I on the other hand is assumed to occur on the inside of the thylakoid membrane (figure 3).

THE RÔLE OF PLASTOQUINONE IN ATP FORMATION AND DBMIB AS A PLASTOQUINONE ANTAGONIST

In photophosphorylation a crossover point in the electron transport system under coupling conditions, i.e. a coupling site, has been identified between plastoquinone and cytochrome *f* plastocyanin (Avron & Chance 1966; Reinwald, Stiehl & Rumberg 1968; Böhme & Cramer 1972). In the theory of vectorial electron flow according to a chemiosmotic mechanism the limiting step in coupling electron flow is either the crossing of plastohydroquinone/plastoquinone across the membrane or the reoxidation of plastohydroquinone on the inside of the membrane accompanied by proton liberation into the inside thylakoid space. The coupling site envisaged between plastoquinone and cytochrome *f*/plastocyanin would consist, according

to a chemiosmotic view, of the electrogenic photosystem I and proton translocation via plastohydroquinone. The segment between Q and photosystem I constitutes a loop in the electron flow system and one of the 'sites' for ATP formation in photosynthesis (figure 3).

In the identification of the other second energy conserving site plastoquinone was also involved. This was because a valuable plastoquinone antagonist had been found in dibromothymoquinone (DBMIB) (Trebst, Harth & Draber 1970). DBMIB at 1 μM inhibits all photosystem I-dependent Hill reactions. The inhibition is specifically reversed by addition of exogenous plastoquinone. DBMIB inhibits the reduction of cytochrome *f* and P_{700} by photosystem II but not their oxidation by photosystem I (figure 5). Neither reductions by

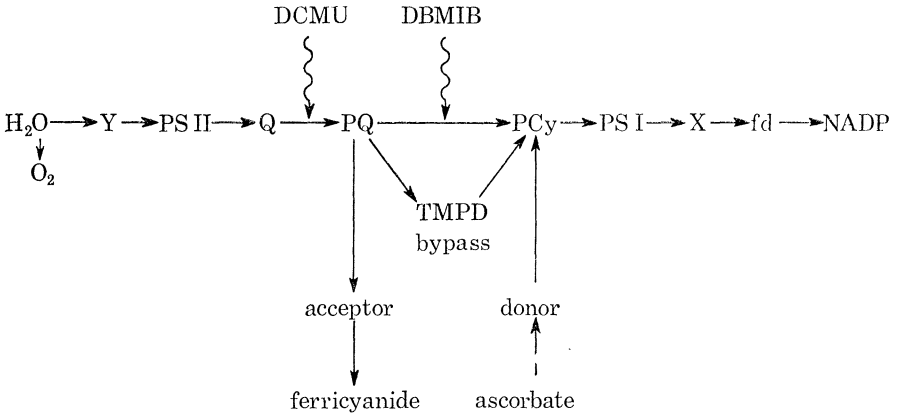

FIGURE 5. Inhibition sites of DCMU (dichlorophenyldimethylurea) and DBMIB (dibromothymoquinone) before or after plastoquinone function respectively. In the presence of DBMIB, an artificial acceptor system driven by photosystem II (and sensitive to DCMU) as well as an artificial donor system driven by photosystem I are still operative. Catalytic amounts of TMPD (*N*-tetramethyl-*p*-phenylene-diamine) will bypass the inhibition site of DBMIB and restore electron flow from water to NADP.

photosystem I at the expense of artificial electron donors nor cyclic photophosphorylation systems are sensitive to DBMIB, except for the ferredoxin and menadione catalysed systems (for reviews see Amesz 1977; Trebst 1974). From the area above the induction curve of variable fluorescence of photosystem II (Bauer & Wijnands 1974) and the amount of oxygen burst (de Kouchkovsky 1975) in the presence of DBMIB it is possible to see that 1 μM DBMIB does not disconnect the large secondary acceptor pool from photosystem II (as compared with DCMU action). Direct measurements of plastoquinone absorption at 265 nm finally proved that DBMIB inhibits the reoxidation of plastohydroquinone by photosystem I (Haehnel 1977). The recent finding of Wood & Bendall (1976), that a protein fraction from the thylakoid membrane catalysing a plastocyanin reduction by reduced plastoquinone-5, is affected by DBMIB, substantiates this further.

The advantage of DBMIB was that it was possible to identify and then characterize photoreductions driven only by photosystem II (figure 5). As it turned out, DBMIB does not inhibit the photoreduction (accompanied by oxygen evolution) of certain lipophilic electron acceptors of appropriate electropositive potential (class three acceptors) (for review see Trebst 1974). Even more important, it was shown that such photosystem II driven Hill reactions (usually with *p*-phenyelenediimine as primary and ferricyanide as terminal electron acceptors) are coupled to ATP formation (Trebst & Reimer 1973*a*, *b*; Izawa *et al.* 1973). The stoichiometry of ATP formation to electron flow, the $P:e_2$ ratio, of such systems in the presence of DBMIB to prevent

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photosystem I participation, is in the order of 0.6 (in washed thylakoid preparation) (Trebst & Reimer 1973*a*; Izawa *et al.* 1973) and 0.9 in class I chloroplasts (Heathcote & Hall 1975); that is, the P:e_2 ratio is exactly half the total stoichiometry of non cyclic electron flow to a photosystem I acceptor in the respective chloroplast preparations. Because the known coupling site (I) located between plastoquinone and photosystem I, discussed above, is inoperative in the presence of DBMIB, a new and long sought-after coupling site (II) had been identified and located between oxygen evolution and the endogenous electron donor for the photosystem II acceptors (figures 3 and 5). In chemiosmotic terms this coupling site II consists of the electron flow loop of the electrogenic photosystem II and the protons liberated in the water splitting reaction on the inside of the thylakoid membrane (figure 3).

The DBMIB inhibition site may be bypassed by a catalytic amount of tetramethyl-*p*-phenylenediamine (TMPD), i.e. oxygen evolution as well as the reduction of a photosystem I acceptor is restored, but the redox reaction between plastohydroquinone and plastocyanin remains inoperative (figure 5). From the coupling of the TMPD bypass to ATP formation it was concluded that an internal rather than a transmembrane TMPD bypass occurs (Trebst & Reimer 1973*b*). Furthermore, the properties of electron transport chain segments including photosystem I in the presence of DBMIB led to the concept of artificial energy conservation via artificial proton translocation by means of electron donors and cofactors of cyclic photophosphorylation (see Hauska & Trebst 1977). It follows then that plastoquinone itself plays a central rôle in coupled ATP formation in photosynthetic electron flow and that studies on its function led to supporting evidence for vectorial electron flow.

Mechanism of proton translocation via plastoquinone and the Q cycle

Many important questions on the rôle of plastoquinone in photosystems remain to be solved. An important problem currently under investigation is the nature of the mechanism of plastoquinone/plastoquinol movement in the membrane. A number of possibilities, like diffusion, catalysis by a proton pumping carrier, cooperation of two carriers for the two electron steps in reduction or oxidation, or the movement of the semiquinone instead, have been suggested, as reviewed by Hauska & Trebst (1977). Studies with model liposomes suggest that a benzoquinone easily moves across a lipid barrier and that a prenyl side chain may greatly facilitate this (Hauska 1977). Other problems currently under investigation include (i) the mode of the participation of plastoquinone in the photoreductions by photosystem II in the presence of DBMIB, (ii) whether the artificial acceptor is reduced by the new compound B or by plastoquinol on the outside or on the inside of the membrane (the TMPD bypass argues for the latter), (iii) whether the protons liberated in plastoquinol oxidation are released right into the inside thylakoid space or (first) into a space within the membrane, (iv) whether the pH gradient is distributed evenly and the membrane potential delocalized over the inside thylakoid space, (v) whether there is a heterogeneity of the pH space and of coupling factors for the two coupling sites, (vi) whether such a special domain of protons liberated in plastoquinol oxidation is identical with the domain in which the protons of water are liberated, (vii) whether certain conditions, i.e. a conformational change of the membrane are required to liberate proton release into specific domains, and (viii) whether the stoichiometry of ATP formation in photosystem II Hill reactions and of coupling site II is lower than measured because coupling site I

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has to contribute to the efficiency of coupling site II. The problems also touch on the mechanism of coupling, whether chemiosmotic or microchemiosmotic or via a conformational change. These questions have been raised and considered in reviews by Hauska & Trebst (1977), Dilley & Giaquinta (1975) and Dilley, Giaquinta, Prochaska & Ort (1977).

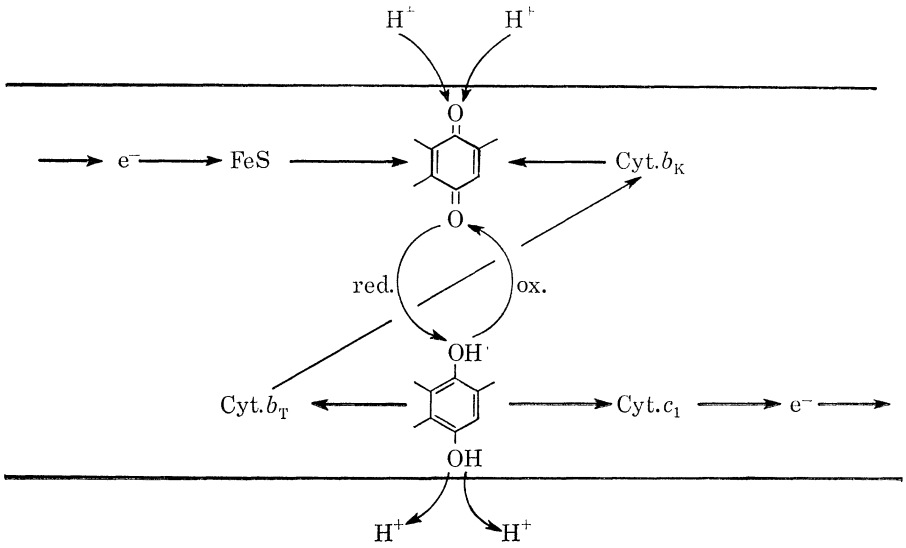

FIGURE 6. The Q cycle for respiratory electron flow according to Mitchell (1975). The benzoquinone is reduced by the cooperation of two carriers on one side of the membrane as is the oxidation of its hydroquinone form on the other side. Cytochrome *b* transports one electron back across the membrane, yielding a stoichiometry of 2 H^+ translocated per one electron moved from FeS (non-haem iron) to cytochrome c_1.

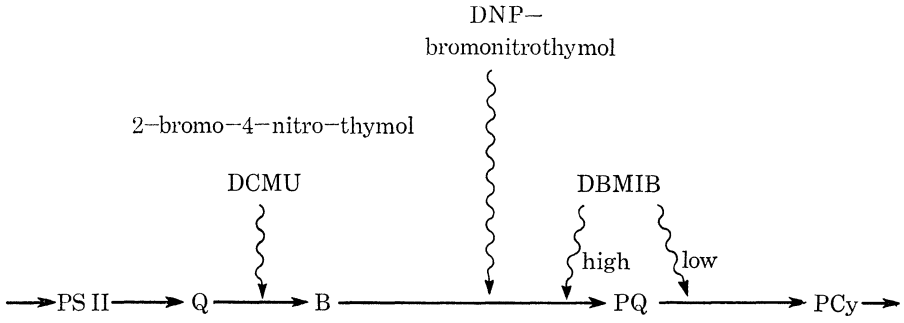

FIGURE 7. Inhibition sites in the electron flow segment of plastoquinone function. Compound B is a two-electron carrier between plastosemiquinone (Q) and plastoquinone, perhaps a plastoquinol anion; see text.

Another important open question is the existence of a Q cycle in photosynthesis. According to the formulation by Mitchell (1975) for a Q cycle in respiratory electron flow of mitochondria (figure 6), the reduction of ubiquinone as well as the oxidation of ubiquinol requires the cooperation of two one-electron transfer carriers. In addition, one of these, cytochrome *b*, could cross the membrane in an electrogenic step. In this way ubiquinone would translocate two protons per electron in a double loop in the electron flow sequence leading to a doubling of the ATP:electron ratio. Evidence for a Q cycle in plant photosynthesis involving plastoquinone has not yet been found, but the cooperative scheme is seriously considered (see Hauska & Trebst 1977). However, in bacterial photosynthesis, where ubiquinone is functioning instead of plastoquinone, evidence for a double loop and a function of ubiquinone before and after

cytochrome *b* is indeed accumulating. DBMIB again has been very useful in this problem (Baltscheffsky 1975; Crofts, Crowther & Fierney 1975; Gromet-Elhanan & Gest 1977; Baccarini-Melandri & Melandri 1977).

Possibly a new plastoquinone antagonist may help to resolve some of the questions. Recently halogenated alkyl substituted nitrophenols, represented by 2-bromo-4-nitrothymol, have been found to be effective inhibitors (not uncouplers!) of photosynthetic electron flow (Draber, Knops, Trebst & Reimer 1977). Their inhibition site is identical to that of DCMU, i.e. between Q and B (figure 7). The dinitrophenyl derivative of bromonitrothymol, however, behaves very much like DBMIB (Trebst, Reimer, Draber & Knops 1977). DNP-bromonitrothymol does not inhibit acceptor systems for photosystem II, not a TMPD bypass, but effectively inhibits electron flow towards photosystem I (figure 7). There is one important difference between this inhibitor and DBMIB: the coupling of the systems has a lower stoichiometry. From the evidence presently available it may be concluded that DNP-bromonitrothymol inhibits the reduction of plastoquinone by compound B on the outside of the membrane. With this compound, proton uptake during plastoquinone reduction in protons liberated in the water-splitting reaction may be separated from each other. This may clarify some of the questions as to the possibility of different domains for the two coupling sites as discussed above.

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