Artificial Energy Conservation in Bacterial Photosynthetic Electron Transport

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In photosynthesis of chloroplasts and bacterial chromatophores an induced artificial electron flow bypass may restore the inhibition of electron flow and of coupled ATP formation by two possible mechanisms. An artificial *transmembrane electron flow bypass* will lead to artificial energy conservation, when the redox reaction cycle of the added mediator across the membrane acts as proton pump. In an artificial *internal electron flow bypass* an inhibited native energy conservation may be reactivated; here an electron flow bypass induced by the mediator in the inside space restores the native proton translocation. The inhibition and the restoration of electron flow by antimycin, dibromothymoquinone and valinomycin is compared.

Introduction

Light driven electron transport in isolated thylakoid preparations from chloroplasts and in chromatophores from photosynthetic bacteria is coupled to ATP formation. The general properties of the two systems show great similarities. The present evidence supports for both systems the notion of vectorial electron flow and of a proton motive force generated in the light to form ATP, as reviewed recently [1–4]. The photosystems and electron carriers are asymmetrically oriented in the membrane. In chloroplast thylakoids as well as isolated vesicular chromatophores the primary acceptor of the photosystem is located towards the outside, the electron donor (plastocyanin for photosystem I and water for photosystem II in chloroplasts and cytochrome c_2 for the photosystem in the bacterial chromatophores) towards the inside. The two ATP forming sites in chloroplasts are connected with plastohydroquinone- and with water-oxidation on the inside of the membrane (see 1). The coupling site in cyclic photophosphorylation by chromatophores is the oxidation inside of cytochrome b and/or ubihydroquinone by cytochrome c_2 [4]. Accordingly a crossover point has been localized between cytochromes b and c_2 in chromatophores of *Rhodospirillum rubrum* [5]. Two coupling sites are proposed for *Rhodopseudomonas capsulata* (see 4). Bacterial photosynthetic electron flow is sensitive to antimycin and HOQNO. In *Rhodospirillum rubrum* the inhibition is overcome by artificial cofactors of cyclic photophosphorylation, like PMS [6] (for review see 7, 8). The coupling site(s) responsible for ATP formation in such artificial cyclic systems are a matter of debate and need new assignment in view of the recent developments, as discussed by Crofts *et al.* [4]. This review is to emphasize the analogy to photophosphorylation systems with artificial cofactors in chloroplasts and to extend concepts in chloroplast photosynthesis to bacterial electron flow.

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Artificial *vs* Native Energy Conservation in Chloroplast Electron Flow

Induced cyclic photophosphorylation, driven by just photosystem I, has been observed also in chloroplasts – it actually led to the discovery of photophosphorylation. PMS, but also many other compounds including ferredoxin, may catalyse cyclic electron flow coupled to ATP formation (for review see 1). The coupling site in such systems remained unsettled, but it was assumed that also in artificial cyclic electron flow a native coupling site of the non-cyclic electron flow was included and responsible for ATP formation. The coupling site in non-cyclic electron flow connected with photosystem I was early recognized between plastoquinone and cytochrome f [9–11]. The artificial cyclic electron flow schemes either included this site or proposed another coupling site. But all the various schemes offered, remained rather unlikely and the

specific coupling site of cyclic electron flow could not be identified. Studies with the plastoquinone antagonist dibromothymoquinone (DBMIB) finally led to a resolution of the discrepancies. It became apparent, that DBMIB blocks plastohydroquinone oxidation and therefore very likely also blocks the coupling site coupled to it. Still a number of cyclic photophosphorylation systems are DBMIB insensitive [12, 13]. Therefore a concept of artificial versus native energy conservation in chloroplast photophosphorylation was proposed [13–15]. In native energy conservation, according to a chemiosmotic mechanism, both electrogenic photosystems are connected with a proton translocating step to constitute a Mitchellian loop (Fig. 1). In photosystem I phosphorylation the latter is the plastoquinone-plastohydroquinone reaction, in photosystem II phosphorylation it is the water splitting reaction. The "site" connected with plastoquinone is blocked by DBMIB. In artificial energy conservation the cofactor in cyclic electron flow itself carries protons across the thylakoid membrane during an oxidation-reduction cycle, thus replacing the native proton translocation blocked by DBMIB. In induced cyclic electron flow then, not only artificial electron flow but also artificial energy conservation occurs [13, 15]. Also non-cyclic electron flow, insensitive to DBMIB or DCMU in donor systems for photosystem I (like DAD/ascorbate or DCPIP/ascorbate as donors for $NADP^+$-reduction) may be coupled to ATP formation via an artificial energy conservation [14]. The comparison of the chemistry of the donor of uncoupled to coupled electron flow makes the point clear, that artificial proton translocation by lipophilic quinoid hydrogen carriers provides energy for ATP synthesis [16]. N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) restores electron flow to photosystem I, but not ATP formation, when electron flow from photosystem II is blocked by DCMU, because TMPD is not a proton carrying redox ompound, *i. e.* it is not able to sustain artificial energy conservation, as against DAD, which supports coupled electron flow and which liberates protons and electrons upon oxidation [14]. The same situation is possible for photosystem II donor systems. If in photosystem II phosphorylation the water splitting reaction is blocked, artificial energy conservation may be restored also by a pro-

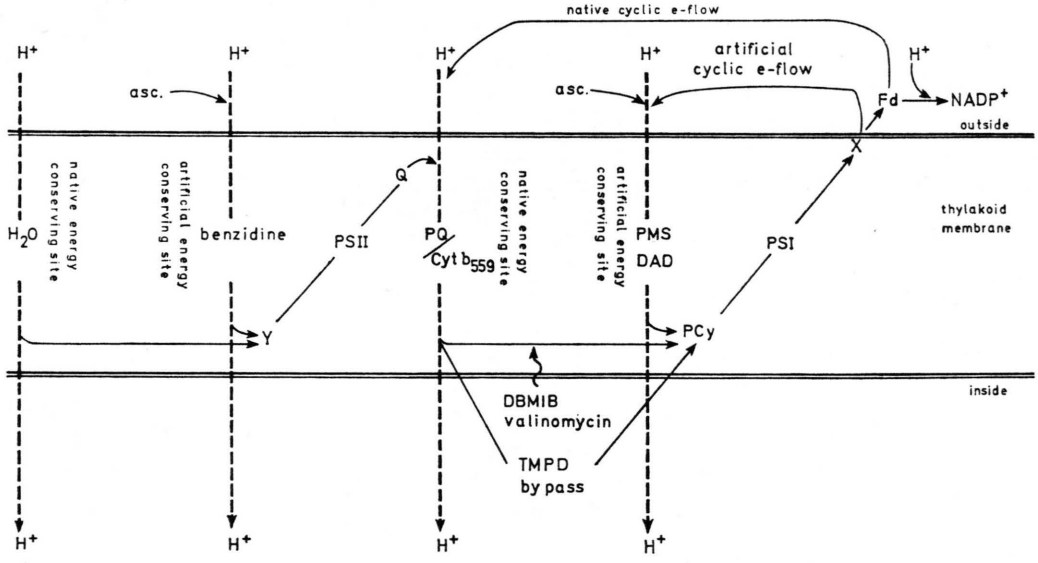

Fig. 1. Native and artificial energy conservation in photosynthetic electron transport across the thylakoid membrane of chloroplasts. A *transmembrane electron flow bypass* leads to artificial energy conservation in a. artificial cyclic e-flow systems (PMS, DAD), b. donor systems for photosystem I (DAD) and c. donor systems for photosystem II (benzidine). An *internal electron flow bypass* (TMPD) restores native energy conservation in the presence of electron flow inhibitors (DBMIB, valinomycin). Abbreviations used are: PS II for photosystem II, PS I for photosystem I, Y for the water splitting complex Q for the primary electron acceptor in photosystem II, PQ for plastoquinone, Cyt b_{559} for cytochrome b_{559} PCy for plastocyanin, X for the primary reductant in photosystem I, Fd for ferredoxin, asc. for ascorbate, PMS for N-methylphenazonium methosulfate, DAD for diaminodorene (2,3,5,6-tetramethyl-*p*-phenylenediamine), TMPD for N,N,N′,N′-tetramethyl-*p*-phenylenediamine and DBMIB for dibromo-thymoquinone.

ton carrying electron donor like benzidine [17]. Tetramethylbenzidine, an electron donor for photosystem II not carrying hydrogens, is unable to induce artificial energy conservation [17]. In all these cases artificial donors or cyclic cofactors induce a *transmembrane electron flow bypass*, connected (or not) with an artificial proton pump across the membrane.

In addition to artificial energy conservation connected with artificial transmembrane electron flow a restoration of an inhibited native energy conservation is possible [17] (Fig. 1). This is the case, when a TMPD oxidation-reduction cycle substitutes for the DBMIB inhibited redox reaction and acts as an electron flow short circuit on the inside of the membrane restoring electron flow as well as ATP formation [18]. This will be called an *internal electron flow bypass*. In this restoration, the original native proton translocating reaction is again operative because TMPD accepts electrons from plastohydroquinone on the inside of the membrane before the DBMIB block and donates the electron back to plastocyanin also on the inside of the membrane (Fig. 1). Such internal electron flow bypass is possible in noncyclic as well as in cyclic electron flow [13, 18].

Artificial Energy Conservation in Bacterial Electron Flow

The same concept can be applied to bacterial photosynthesis when artificial electron carriers are employed. The concept is able to rationalize results which are in the literature already for some time, but not yet satisfactorily explained. The role of DBMIB – a plastoquinone antagonist – in chloroplast systems may be compared with the role of antimycin – a cytochrome b inhibitor – and of HOQNO in bacterial photosynthesis. In chromatophores of *Rhodospirillum rubrum* cyclic photophosphorylation is inhibited by antimycin and HOQNO [7, 8]; electron flow and ATP formation is restored by the addition of compounds like PMS, DAD or TMPD [6–8, 19–22]. With these compounds either an artificial energy conservation in a transmembrane electron flow bypass or an internal bypass of the inhibition site might be responsible for restoration. It is proposed that in antimycin (or HOQNO) resistant cyclic photophosphorylation with PMS or DAD artificial energy conservation occurs *i.e.* these two cofactors catalyse transmembrane proton translocation when protons are liberated upon oxidation of the reduced cofactor by cytochrome c_2 on the inside of the bacterial membrane vesicles similar to the chloroplast system (Fig. 2). This way the redox reaction cycle of the cofactor shuttle completes a Mitchellian loop by replacing the inhibited native proton translocating step at cytochrome b/ubiquinone by an artificial proton translocation.

The restoration of cyclic phosphorylation in chromatophores of *Rh. rubrum* in the presence of antimycin by TMPD [20] cannot be explained in the same way, because TMPD, being oxidized to a cation-radical, does not release protons upon oxidation inside as discussed above. Therefore TMPD cannot sustain artificial energy conservation neither in chloroplasts nor in the bacterial chromatophores in

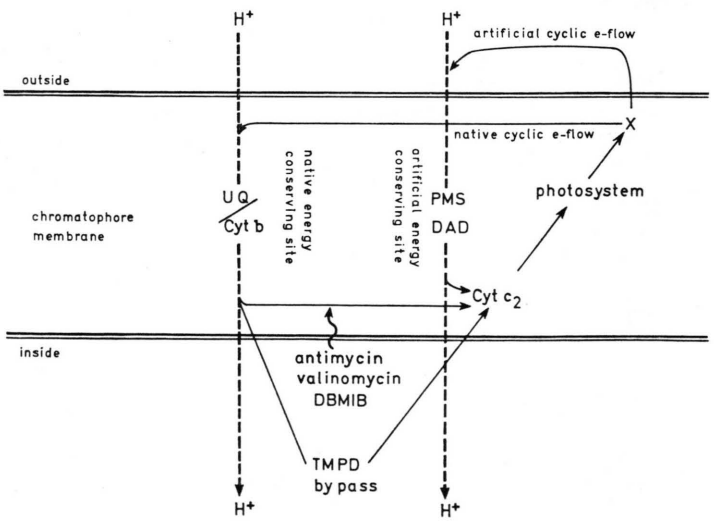

Fig. 2. Native and artificial energy conservation in photosynthetic electron transport across the membrane of chromatophores from *Rhodospirillum rubrum*. A transmembrane electron bypass (PMS or DAD) leads to artificial energy conservation. An internal electron flow bypass (TMPD) restores native energy conservation in the presence of electron flow inhibitors (antimycin, valinomycin and DBMIB). Abbreviations used are the same as in Fig. 1 plus UQ for ubiquinone, Cyt b for cytochrome b and Cyt c_2 for cytochrome c_2.

the presence of DCMU or antimycin, respectively. But as in the DBMIB-inhibited chloroplast system TMPD might be able to bypass the antimycin inhibition site by inducing an electron flow short circuit on the inside of the membrane (Fig. 2). In this internal electron flow bypass the native energy conserving site at cytochrome b/ubiquinone is restored, the proton translocation site remains the same as the one in the uninhibited system. It is possible, that also PMS and DAD act via an internal bypass, as perhaps implicit in schemes of Ramirez [22] and Prince [23]. Possibly DBMIB experiments on non-cyclic flow systems might help distinguish between the possibilities. In chloroplasts there is an easy distinction between the two possible bypasses because in the internal bypass non-cyclic flow from water to photosystem I is restored, in the transmembrane bypass only electron flow from an artificial donor.

In that context it is noteworthy that cyclic phosphorylation in chromatophores from *Rhodopseudomonas capsulata* or *spheriodes* is not restored by artificial redox compounds after inhibition by antimycin [21, 23]. This might reflect less efficient reactions of the compounds with ubiquinone or X for reduction and cytochrome c_2 for oxidation and/or less efficient proton translocation.

Valinomycin as Inhibitor of Electron Flow in Bacterial Chromatophores and Chloroplasts

The generalisation of chloroplast and bacterial photosynthesis equalises antimycin inhibition in bacterial chromatophores with DBMIB inhibition in chloroplasts. DBMIB is also an inhibitor of photophosphorylation in *Rhodospirillum* chromatophores with comparable properties as in chloroplasts, *i. e.* inhibition at the cytochrome b/ubiquinone level [24]. Another inhibitor which inhibits both systems in the same way is valinomycin. As reported by Keister [25] and Gromet-Elhanan [26] valinomycin, at high concentrations, is an electron transport inhibitor in *Rhodospirillum rubrum*. The inhibition is overcome by PMS but also by TMPD, as already observed earlier by Baltscheffsky [19]. At that time no explanation of the valinomycin experiments could be offered. Keister [25] also concluded already that valinomycin is an inhibitor of chloroplast electron flow (rather than acting as uncoupler or energy transfer inhibitor, as suggested before) and found that PMS catalysed cyclic photophosphorylation is valinomycin insensitive. Our own recent results [27], reported elsewhere, show that valinomycin inhibition of chloroplast electron flow is comparable to DBMIB inhibition because it does not inhibit photosystem II phosphorylation and electron flow nor certain photosystem I systems. Particularly suggestive, TMPD can bypass the inhibition of non-cyclic electron flow by valinomycin in chloroplasts [27] as it does the inhibition by DBMIB (Figs 1 and 2).

Conclusion

TMPD is able to overcome inhibition by antimycin and valinomycin in bacterial photophosphorylation and inhibition by DBMIB and valinomycin in chloroplast photophosphorylation by bypassing the inhibition site on the inside of the membrane and restoring native energy conservation. PMS and DAD on the other hand restore ATP formation in chloroplasts and possibly also in bacterial chromatophores by inducing artificial energy conservation.

The similarities of the inhibition of electron flow by antimycin – a cytochrome b inhibitor – and by DBMIB – a plastoquinone inhibitor – seems very indicative [24]. It might be taken as suggestive support, that, as recently proposed by Mitchell [28, 29], a cooperation of cytochrome b and a quinone is essential in order to couple electron flow to energy conservation.

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