Photosynthesis Research **73:** 273–277, 2002.

Minireview

The present model for chlororespiration

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Received 11 September 2001; accepted in revised form 24 October 2001

Key words: Pierre Bennoun, carotenoid biosynthesis, *Chlamydomonas reinhardtii*, chlororespiration, *n*-propyl-gallate

Abstract

The present model of chlororespiration deals with the dark reduction and oxidation of plastoquinone. Both stages are reviewed here for algae and higher plants. Recent data confirm the presence of a plastoquinone:oxygen oxidoreductase with features different from those of the mitochondrial oxidases. The possible involvement of the chloroplast oxidase in the pathway of carotenoid biosynthesis is discussed in view of various experimental data and on energetics considerations.

Abbreviations: NAD(P)H – nicotinamide adenine dinucleotide (phosphate); SHAM – salicylhydroxamic acid; PG – *n*-propyl-gallate

Introduction

The model of 'chlororespiration' was proposed originally to account for two sets of observations by a single model (Bennoun 1982). The first one referred to the changes of the redox state of the plastoquinone pool that can be observed in darkness, the second one to the transmembrane potential of thylakoids detected in darkness in the absence of the chloroplast F_0F_1 ATP synthase (referred below as the permanent membrane potential). In this model, plastoquinone was supposed to be reduced by an NAD(P)H dehydrogenase and reoxidized by molecular oxygen through a chloroplast oxidase. Electron transfer through this respiratory chain from NAD(P)H to oxygen was supposed to be electrogenic and thus responsible for the observed permanent membrane potential.

A careful examination of mitochondrial–chloroplast interactions led to the conclusion that the permanent membrane potential resulted mostly from a new ATP-dependent electrogenic pump present in the thylakoid membranes rather than from chlororespiration (Bennoun 1994). This conclusion was strengthened by the persistence of a permanent membrane potential under anaerobic conditions (Joliot and Joliot 1994). Unlike the chloroplast F_0F_1 ATP synthase, this new pump was shown to be specific for ions different from protons (Rappaport et al. 1999). On this basis, the actual model of chlororespiration concerns mainly the processes of reduction and oxidation of plastoquinone in darkness (Figure 1). This model was deduced from the analysis of green alga displaying a rather high rate of plastoquinone oxidation in darkness (*Chlamydomonas reinhardtii* and *Chlorella sorokiniana* showing halftimes in the range of 1–3 s). These rates of oxygen uptake remain, however, more than one order of magnitude smaller than those related to mitochondrial respiration. Therefore, the very low rates of plastoquinone oxidation observed in various cases (halftime in the range of 10–30 s) might correspond to processes of different nature. In order to avoid confusion, it would thus be wise to refer to chlororespiration only in cases where such high rates are actually observed.

The dark reduction of plastoquinone

The reduction of plastoquinone was assigned in the model of chlororespiration to the presence of an

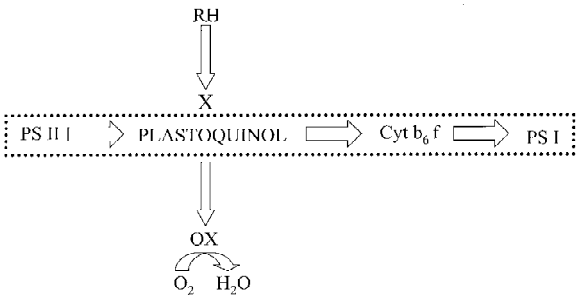

Figure 1. A scheme for chlororespiration. In light, plastoquinone is a redox carrier of the phothosynthetic electron transport chain located between Photosystem II reaction centers (PS II RCs) and cytochrome b_6f complex. In darkness, a complex X mediates the dark reduction of plastoquinone by an internal reductant RH. Plastoquinol is reoxidized at the expense of molecular oxygen via an oxidase OX sensitive to *n*-propyl-gallate. In higher plants, the complex X could possibly be the chloroplast NADH: plastoquinone oxidoreductase. In algae, the nature of this complex is not known.

NAD(P)H-plastoquinone oxidoreductase. In line with this prediction, it was shown that most chloroplast genomes contained 11 genes (*ndhA–ndhK*) with sequence similarity to the subunits of the eubacterial and mitochondrial NADH dehydrogenase (Berger et al. 1993). These *ndh* products form a complex that can be isolated from thylakoid membranes, although the total amount of this complex in leaves is very low (Funk and Steinmüller 1995; Sazanov et al. 1995; Quiles et al. 1996). Mutagenesis of the *ndh* genes in tobacco was shown to affect both photosynthetic electron flow and carbon metabolism (Burrows et al. 1998; Koffer et al. 1998). In algae, however, there is very little molecular evidence for the existence of such a protein, although physiological evidence shows that plastoquinone reduction in darkness is catalyzed in some way. For instance, in *C. reinhardtii*, an input of electrons in the plastoquinone pool was detected in darkness in response to an inhibition of the mitochondrial electron transport (Bennoun 1994). The identification of the algal complex responsible for the dark reduction of plastoquinone remains to be achieved.

Insofar as chlororespiration reflects carotenoid biosynthesis (see below), this complex might identify with the desaturases implied in the pathway of carotene biosynthesis. These desaturases, which transfer electrons from carotene precursors to plastoquinone, belong to the class of the flavoproteins, and are bound to the thylakoid membranes (for a review, see Carol and Kuntz 2001). As described below, the rate of plastoquinol oxidation in darkness is one order of magnitude lower in leaves of higher plants than in algae, and such a quantitative difference might cover a qualitative difference. Both types of organisms might have distinct reduction pathways of the plastoquinone pool, and possibly distinct pathways of plastoquinol oxidation. This would explain why a chloroplast NADH dehydrogenase was identified in leaves of higher plants and not in algae. Furthermore, the pathways for reduction and oxidation of plastoquinone in plants might differ according to the developmental stage and to the tissues considered. The comparison of higher plants and algae with regard to the processes of reduction and oxidation of plastoquinone in darkness should thus be done with care.

The dark oxidation of plastoquinol

The plastoquinol pool is submitted to a dark oxidation at the expense of molecular oxygen (Diner and Mauzerall 1973). In the model of chlororespiration, this oxidation was assigned to the presence of a plastoquinol:oxygen oxidoreductase (Bennoun 1982). However, because of the occurrence of mitochondrial–chloroplast interactions (Bennoun 1994, 1998), it has been difficult to prove that the oxygen consumption related to plastoquinol oxidation takes place in the chloroplast compartment rather than in the mitochondrial one. Nevertheless, several lines of evidence have recently been obtained that are in favor of the existence of the chloroplast oxidase. It was proposed that the plastoquinol:oxygen oxidoreductase predicted from chlororespiration was responsible for a light-induced Photosystem II dependent oxygen uptake sensitive to *n*-propyl-gallate (PG), an inhibitor of the plant mitochondrial alternative oxidases (Cournac et al. 2000; Peltier and Cournac 2002).

As discussed in the following section, a plastid oxidase sensitive to PG might also be involved in the pathway of carotenoid biosynthesis (Josse et al. 2000). The process of plastoquinol oxidation in darkness was investigated further in whole cells of *C. reinhardtii* (Bennoun 2001). The size of the plastoquinone pool was deduced from the *in vivo* measurement of chlorophyll *a* fluorescence kinetics. As plastoquinol is reoxidized in darkness at the expense of oxygen, the rate of reoxidation of plastoquinol following light-reduction provides a specific measurement of the oxygen uptake related to plastoquinol oxidation, and thus of chlororespiration. Such a method is invaluable since direct measurements of oxygen concentration fail to distinguish the relative uptake due to mitochondrial

respiration from that due to chlororespiration. Using this approach, the sensitivity to PG and to salicylhydroxamic acid (SHAM) of plastoquinol oxidation was compared in darkness. It turned out that, whereas both PG and SHAM inhibited the mitochondrial alternative oxidase, PG but not SHAM inhibited also the output of electrons from the plastoquinol pool. It was thus concluded that the oxygen consumption linked to plastoquinol oxidation could not be ascribed to the mitochondrial oxidases, but rather to the presence of a plastoquinol:oxygen oxidoreductase sensitive to PG and insensitive to SHAM. Chlorophyll fluorescence kinetic measurements of whole cells of *C. reinhardtii* equilibrated with various mixtures of argon and air were used to evaluate the relative affinity for oxygen of the chloroplast and mitochondrial oxidases (Bennoun 2001). It turned out that plastoquinone remained largely oxidized in darkness down to 1.45% air, indicating that mitochondrial respiration was operating properly at this relative oxygen concentration. (As mentioned in the previous section, an inhibition of mitochondrial respiration induces a reduction of the plastoquinone pool.) Following light reduction, however, the dark oxidation of plastoquinol was inhibited below a level of 10% air. Thus, the oxygen concentration in a 1.45% air:argon mixture was limiting the plastoquinol oxidation but not the mitochondrial respiration. These experiments led to the conclusion that the oxygen consumption linked to plastoquinol oxidation could not be ascribed to the mitochondrial oxidases, but rather to the presence of a plastoquinol:oxygen oxidoreductase. In agreement with a previous report (Bennoun 1994), this chloroplast oxidase displayed a lower affinity for oxygen than that of mitochondrial oxidases.

Chlororespiration and carotenoid biosynthesis

A gene product (labeled as IM) was recently reported in *Arabidopsis thaliana*, showing amino acid similarity to a quinol:oxygen oxidoreductase (Carol et al. 1999; Wu et al. 1999). The IM protein was shown to be located in the thylakoid membranes and to display an iron-binding motif similar to that of the plant mitochondrial alternative oxidase. The IM protein is thought to be essential for carotenoid biosynthesis at certain stages of plant development. It was proposed that phytoene desaturation would be coupled with the reduction of plastoquinone, and plastoquinol oxidation carried out by the IM protein acting as a plastoquinol:oxygen oxidoreductase (Figure 2). In addition to its role during early chloroplast differentiation, the IM protein was shown to be essential for carotenoid accumulation in petal and fruit chromoplasts, a process linked to a respiratory redox pathway as well (for a review, see Carol and Kuntz 2001). Upon expression in *E. coli*, the IM protein was shown to confer a cyanide-resistant electron transport to isolated membranes, thus acting as an alternative terminal oxidase. Interestingly enough, this activity appeared to be sensitive to PG, a feature that is shared by the plastoquinol:oxygen oxidoreductase of chlororespiration. Therefore, chlororespiration and carotenoid biosynthesis might involve the same oxidase, and plastoquinone oxidation in darkness might reveal the process of carotenoid biosynthesis.

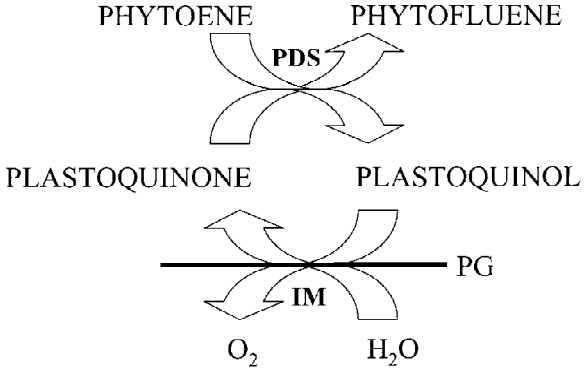

Figure 2. A scheme for carotenoid biosynthesis and respiratory redox pathway. The biosynthetic pathway of carotenoid synthesis involves several steps of desaturation requiring free energy, for instance the transition from phytoene to phytofluene achieved by phytoene desaturase (PDS). This reaction is associated with a reduction of plastoquinone. The oxidation of the plastoquinol is then achieved by a plastoquinol:oxygen oxidoreductase (IM) sensitive to PG in an exergonic reaction. From a thermodynamic point of view, the desaturation step is made possible because it is coupled with the plastoquinol oxidation. This coupling can take place because both reactions share the plastoquinone pool as a common intermediate. Both PDS and IM are bound to the thylakoid membrane, and IM would identify with the oxidase predicted from chlororespiration (labeled OX in Figure 1). The flavoprotein phytoene desaturase (PDS) might additionally mediate the reduction of plastoquinone by internal reductants.

In higher plants, the amount of IM detectable in thylakoids isolated from leaves is very low as compared to that of the photosynthetic complexes (Funk and Steinmüller 1995; Sazanov et al. 1995; Quiles et al. 1996). At the same time, plastoquinol oxidation displays a half-time of 15 s in young leaves of spinach and tobacco (Pierre Joliot, personal communication), as compared to 1.2 s in *C. sorokiniana* and 3 s in *C. reinhardtii* for the exponential stage of growth

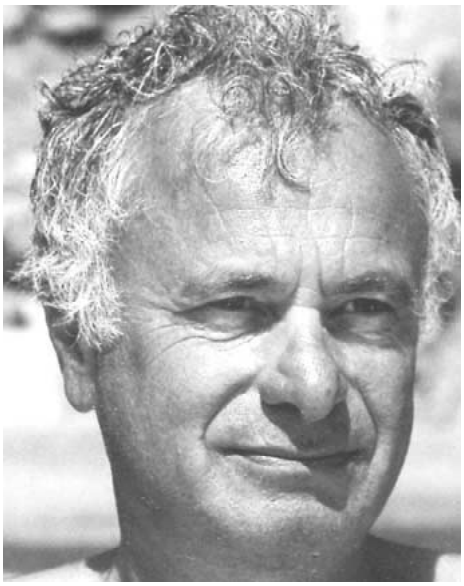

Figure 3. Pierre Bennoun (2000).

(Bennoun 2001). This might indicate the presence of large amounts of IM in thylakoid membranes of these algae. An antibody raised against the IM protein of *A. thaliana* was shown to react with a thylakoid membrane preparation of *C. reinhardtii* (Cournac et al. 2000). However, this antibody could react as well with the homologous mitochondrial alternative oxidase, and mitochondrial membranes from *C. reinhardtii* are known to co-purify with thylakoids (Atteia et al. 1992). The presence of IM in *C. reinhardtii* thus remains an open question.

The rate of plastoquinol oxidation in darkness, as revealed by chlorophyll fluorescence kinetics, was shown to decrease drastically when the stationary stage of culture was reached (Bennoun 2001). As thylakoid membrane biosynthesis follows the growth rate and depends on carotene biosynthesis, one could assume that the concentration of the proteins involved in carotenoid biosynthesis, and especially that of IM, declines when growth stops. If the oxidase involved in carotenoid biosynthesis identifies with that involved in chlororespiration, the slow rate of plastoquinol oxidation observed in the stationary stage might be the result of this decline. The rate of plastoquinone oxidation would reflect in this way the growth rate. In agreement with this assumption, a correlation between plastoquinone oxidation and growth rate could be observed. The generation time of *C. reinhardtii* is about three times that of *C. sorokiniana* (Lacambra et al. 1984). In line with this difference, the rate of plastoquinol oxidation observed during the exponential phase of growth is almost three times higher in *C. sorokiniana* than in *C. reinhardtii* (see above).

It was shown in *C. reinhardtii* that following light reduction, plastoquinol oxidation in darkness proceeded very slowly in open-cell preparations in comparison with intact cells (Bennoun 1982). This feature would be expected for an oxidase involved in a biosynthetic process, for such a process very likely requires cofactors that would be lost upon cell breakage.

On the whole, the identification of the terminal oxidase involved in carotenoid biosynthesis with that of chlororespiration remains tentative but appears as an attractive working hypothesis. This identification leads, for instance, to predict a high concentration of IM in thylakoids from *C. reinhardtii* and *C. sorokiniana* to account for their fast rate of plastoquinone oxidation in darkness. It may also help to clarify the strong differences observed between algae and higher plants regarding their patterns for plastoquinone reduction and plastoquinol oxidation in darkness.

Concluding remarks

A model is, for one thing, a way of representing available data in a consistent manner, and for another a way of suggesting new experiments. In that sense, the original model of chlororespiration did play its role, leading, for instance, to the discovery of a new ATP-dependent electrogenic pump in the thylakoid membranes. The new formulation of this model integrating the dimension of a pathway for carotenoid biosynthesis will surely allow new prospects. The present model is yet closer to the original model of chlororespiration that it may seem. The steps of desaturation of the carotene precursors are coupled to a respiratory redox pathway, namely to the reduction of plastoquinone and the reoxidation of plastoquinol via a terminal oxidase IM. Moreover, this oxidase is homologous to the alternative oxidases involved in mitochondrial respiration. Last but not the least, in nonphotosyntetic tissues of higher plants, carotenoid accumulation requires respiratory redox pathways as well. Respiratory processes are most generally devoted to the chemiosmotic synthesis of ATP, an energy source available for a wide variety of biological processes. This synthesis is a striking example of the high sophistication that biological processes can reach. However, energy can be directly recovered from respiratory redox pathways in less advanced ways.

For instance, detoxification processes that eliminate a variety of harmful organic compounds make use of the sole reducing power involved in respiratory redox pathways. In carotenoid biosynthesis, the recovery of energy from a redox pathway would not be so direct. The highly endergonic reaction of carotene desaturation would be permitted on account of its coupling to the highly exergonic reaction of plastoquinol oxidation (Figure 2). Such a coupling requires a common intermediary to both reactions – in this case the plastoquinone pool. These various ways of recovering energy from respiratory redox pathways illustrate the versatility of biological systems and refer to their gradual evolution.

At the request of the editor Govindjee, I have included here a recent photograph of myself (Figure 3).

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