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Short communication

Linking chlorophyll biosynthesis to a dynamic plastoquinone pool

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ABSTRACT

Chlorophylls are essential cofactors in photosynthesis. All steps in the chlorophyll pathway are well characterized except for the cyclase reaction in which the fifth ring of the chlorophyll molecule is formed during conversion of Mg-protoporphyrin IX monomethyl ester into Protochlorophyllide. The only subunit of the cyclase identified so far, is AcsF (Xantha-l in barley and Chl27 in Arabidopsis). This subunit contains a typical consensus di-iron-binding sequence and belongs to a subgroup of di-iron proteins, such as the plastid terminal oxidase (PTOX) in the chloroplast and the alternative oxidase (AOX) found in mitochondria. In order to complete the catalytic cycle, the irons of these proteins need to be reduced from Fe^{3+} to Fe^{2+} and either a reductase or another form of reductant is required. It has been reported that the alternative oxidase (AOX) and the plastid terminal oxidase (PTOX) utilize the di-iron center to oxidise ubiquinol and plastoquinol, respectively. In this paper, we have used a specific inhibitor of di-iron proteins as well as Arabidopsis and barley mutants affected in regulation of photosynthetic electron flow, to show that the cyclase step indeed is directly coupled to the plastoquinone pool. Thus, plastoquinol might act as an electron donor for the cyclase reaction and thereby fulfil the role of a cyclase reductase. That would provide a functional connection between the redox status of the thylakoids and the biosynthesis of chlorophyll.

1. Introduction

Chlorophylls are key cofactors of the photosynthetic apparatus, involved in capturing light and harnessing the energy harvested for splitting water, driving electron transport and generation of chemical energy and reducing power in the form of ATP and NADPH, respectively. Chlorophylls are therefore highly abundant in all photosynthetic organisms and their synthesis is a major anabolic pathway.

Most steps in the chlorophyll biosynthetic pathway are rather well studied through genetic and biochemical analysis of mutants and by in vitro reconstitution of individual reactions (von Wettstein et al., 1995; Stenbaek and Jensen, 2010; Chen, 2014). Nevertheless biosynthesis of chlorophyll still comprises a number of challenging and unsolved topics.

Among all the reactions in the chlorophyll biosynthetic pathway, the formation of the fifth ring of the chlorophyll molecule yielding Protochlorophyllide (Pchlide) from Mg-protoporphyrin IX monomethyl ester (MgPMME) remains the least understood reaction at the molecular level (Fig. 1). The overall reaction, catalysed by a cyclase system, is a six-electron oxidation proposed to occur in three sequential steps involving (i) hydroxylation of the methyl-esterified ring-C propionate by incorporation of atmospheric oxygen, (ii) oxidation of the resulting alcohol to the corresponding ketone, and (iii) reaction of the activated methylene group with the γ-meso carbon of the porphyrin nucleus in an oxidative reaction involving removal of two protons to yield the "fifth" ring (Walker et al., 1988; Porra et al., 1996) (Fig. 2). However, the enzymatic mechanism as well as the involved enzymes remains to be elucidated.

In purple bacteria, such as *Rhodovulum sulfidophilum* and *Rubrivivax gelatinosus*, distinct cyclase systems have been shown to coexist – an anaerobic enzymatic pathway and an aerobic pathway. The anaerobic cyclase is encoded by the *bchE* gene, whereas the aerobic cyclase is encoded by *acsF*, which has homologous only in oxygenic photosynthetic species (Ouchane et al., 2004).

In aerobic photosynthetic species, the isocyclic ring formation is catalysed by an aerobic oxidative cyclase in a reaction requiring iron, O_2 and reducing power (Tottey et al., 2003). The cyclase

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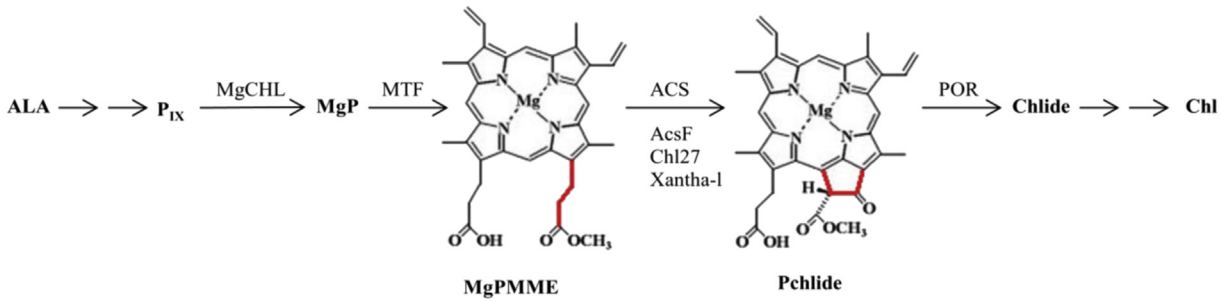

Fig. 1. Schematic representation of the chlorophyll biosynthetic pathway in plants. Aminolevulinic acid (ALA) is the universal precursor in the tetrapyrrole biosynthetic pathway. The chlorophyll branch starts with the insertion of Mg^{2+} into protophorphyrin IX (P_{IX}) by Mg chelatase (MgCHL). Methylation of Mg-protoporphyrin IX (MgP) into Mg-protoporphyrin IX monomethyl ester (MgPMME) by a methyl transferase (MTF) follows and subsequently the aerobic cyclase (ACS) introduces the fifth ring characteristic of the chlorophyll molecule yielding protochlorophyllide (Pchlide). Then Pchlide is converted in chlorophyllide (Chlide) by the light dependent enzyme protochlorophyllide oxidoreductase (POR). After this step Chlide undergoes other reactions to finally be transformed into chlorophyll *a* and *b*. Different species-specific names of the catalytic subunit of the ACS are shown: AcsF in purple bacteria, Chl27 in Arabidopsis and Xantha-l in barley.

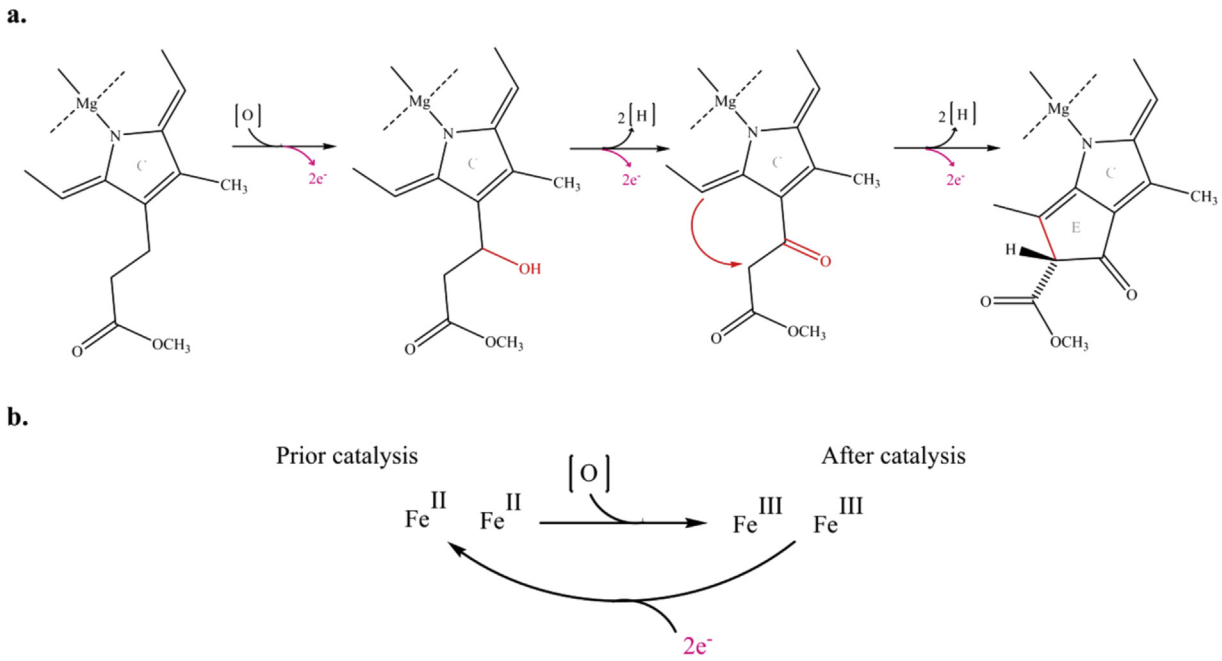

Fig. 2. Conversion of MgPMME substrate into Pchlide and aerobic cyclase catalytic cycle. (a). Conversion of MgPMME to Pchlide requires three sequential two-electrons oxidation steps: (i) hydroxylation of the methyl-esterified ring-C propionate by incorporation of atmospheric oxygen; (ii) oxidation of the resulting alcohol to the corresponding ketone; and (iii) the activated methylene group reacts with the γ-meso carbon of the porphyrin nucleus yielding ring-E (Walker et al., 1988; Porra et al., 1996). (b). Proposed aerobic cyclase catalytic cycle. During catalysis the irons get oxidised and, in order to complete the enzymatic cycle, they need to be reduced from Fe^{3+} to Fe^{2+}. For this step two electrons are required.

enzyme is indeed shown to be iron dependent as iron chelators inhibits its activity (Nasrulhaq-Boyce et al., 1987) and MgPMME, the substrate of the reaction, accumulates in iron deficient plants (Spiller et al., 1982). In vitro, NADPH stimulates the enzymatic activity and O_2 is directly involved in the hydroxylation step (Wong et al., 1985). The aerobic oxidative cyclase is expected to be active as a multisubunit complex, and was shown to require both soluble and membrane-bound plastid fractions (Wong et al., 1985; Bollivar and Beale, 1996; Rzeznicka et al., 2005). In barley, two mutants which are defective in the membrane components have been identified: *xantha-l* and *viridis-k* (Rzeznicka et al., 2005). Therefore it is speculated that the cyclase may be a complex of three gene products: a soluble protein and two membrane bound components – one encoded by the *Xantha-l* locus and the other by the *Viridis-k* locus. Recently, a new component of the aerobic oxidative cyclase machinery was independently discovered in cyanobacteria and tobacco by two different research groups. Ycf54 was identified in *Synechocystis* by Hollingshead et al. (2012) and the homologous in tobacco was named LCAA by Albus et al. (2012). Subsequently, Bollivar et al. (2014) univocally pinpointed Ycf54/LCAA as a membrane localized component of the cyclase system. This new component, most likely, plays a critical role in the synthesis/maturation of the cyclase catalytic subunit or in the process of forming a catalytic complex between the cyclase and preceding or following enzymes, rather than being a true catalytic subunit of the cyclase enzyme complex (Hollingshead et al., 2012; Albus et al., 2012). Furthermore, Ycf54 exhibits a structure similar to Psb28, a well-known photosystem II assembly factor (Hollingshead et al., 2012) and it has been shown to connect the cyclase activity with ALA synthesis (Albus et al., 2012). Therefore Ycf54 might be involved in the coordination of photosystem biogenesis and chlorophyll biosynthesis.