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The electron transfer pathway upon H_2 oxidation by the NiFe bidirectional hydrogenase of *Synechocystis* sp. PCC 6803 in the light shares components with the photosynthetic electron transfer chain in thylakoid membranes

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ABSTRACT

In anaerobic conditions the NiFe hydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803 catalyzes transient H_2 production upon a darkness-to-light transition, followed by a rapid H_2 uptake. We measured H_2 uptake in *Synechocystis* mutants lacking photosystem I, photosystem II or terminal oxidases and in the wild-type strain with and without active cytochrome b_6f. Rapid light-induced H_2 uptake was dependent on cytochrome b_6f and the presence of photosystem I. We propose light-dependent electron transport from H_2 to plastoquinone, probably via NAD(P)H dehydrogenase, and on to cytochrome b_6f and photosystem I. In darkness H_2 uptake is ~10-fold slower than in the light and is independent of thylakoid redox components. The plastoquinone redox state may be key in determining the ultimate H_2 redox partner. H_2 uptake and production in darkness likely use the same redox partners. NADH and NADPH, but not reduced ferredoxin, were confirmed as hydrogenase redox donors *in vitro*.

Introduction

The bidirectional hydrogenase in *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) catalyzes both H_2 oxidation and H^+ reduction [1]. It is the sole enzyme in the cell that can catalyze such reactions as a nitrogenase and an uptake hydrogenase are absent in this organism [2]. Hydrogenase in *Synechocystis* is an O_2-sensitive pentameric enzyme consisting of HoxH, HoxY, HoxU, HoxE and HoxF [1,3]. HoxH and HoxY form the hydrogenase module of the enzyme that participates in H_2 oxidation/H^+ reduction, and HoxE, HoxF and HoxU form the diaphorase module that participates in NAD(P)H/NAD(P)$^+$ oxidation/reduction [1]. While HoxH contains the NiFe-containing active site of the enzyme, all five subunits contain [Fe-S]-cluster binding motifs [4]. During H_2 production protons are delivered directly from the cytoplasm of the cell to the active site of the enzyme [5]. The delivery of electrons

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occurs from one or more redox partner(s), NADH, NADPH (collectively referred as NAD(P)H) and reduced ferredoxin/flavodoxin, to the active site likely via the [Fe-S] clusters present in various subunits [4,6–8].

H_2 production in *Synechocystis* can occur under anaerobic conditions in darkness (fermentative H_2 production) and during the transition from darkness to the light (photohydrogen production or H_2 photoevolution) [1,6,9,10]. The two modes differ in terms of the source of NAD(P)H and/or reduced ferredoxin/flavodoxin. In darkness NAD(P)H and/or reduced ferredoxin/flavodoxin are generated by fermentation of organic compounds such as glycogen [11], while in the light photosynthesis is the main source of reduced ferredoxin/flavodoxin and NADPH [12].

H_2 production in the fermentative mode is stable and can continue for several minutes or even hours [13]. However, H_2 photoevolution, which is observed during the transition from darkness to the light, lasts only for 20–30 s before net H_2 production stops. After that the enzyme catalyzes H_2 uptake that continues until the O_2-sensitive hydrogenase is inactivated by photosynthetically produced O_2 or until H_2 in the medium has been used up [6,7,10].

H_2 photoevolution in *Synechocystis* involves electrons that originated from the splitting of water in photosystem II (PS II) producing O_2, electrons and protons. These electrons are transferred via plastoquinone (PQ) to the cytochrome b_6f complex and via a lumenal electron carrier, plastocyanin (PC) or cytochrome c_{553}, to photosystem I (PS I). PS I uses these electrons to reduce ferredoxin and, via ferredoxin:NADP reductase (FNR), to reduce $NADP^+$ to form NADPH, which is the primary electron donor for photosynthetic CO_2 fixation [14–17]. However, CO_2 fixation is not immediately active upon light-induced production of NADPH as ribulose-1,5-bisphosphate carboxylase/oxygenase needs to be activated for CO_2 fixation [18]; therefore, there is a short period of time after the start of illumination when NADPH is produced but not oxidized. Hydrogenase in *Synechocystis* has been suggested to act as a valve to get rid of excess reducing equivalents (NADPH and/or reduced ferredoxin/flavodoxin) generated by photosynthesis upon illumination of a dark-adapted culture [1,9], which indeed is important under anaerobic conditions where hydrogenase is active. Under aerobic conditions the flavoproteins Flv1/Flv3 serve as powerful acceptors of reducing equivalents from PS I [19–21].

The mechanism of light-induced H_2 uptake catalyzed by hydrogenase after the initial burst of H_2 photoevolution is unclear. Obviously the enzymatic reaction reverses its direction, but the identity of the electron sink during this light-induced H_2 oxidation is as yet unknown. CO_2 assimilation, which is inactive during periods of darkness until shortly after the start of illumination, was suggested to use H_2 as an initial source of electrons after turning on the light [6,22]. However, the light-induced H_2 uptake profile was unchanged upon flushing cyanobacterial cells with pure N_2 (i.e., no CO_2 present, and therefore no CO_2 assimilation possible) [23], indicating that H_2 uptake in the light occurs even in the absence of CO_2 assimilation. Therefore, CO_2 assimilation cannot be the only sink of electrons during H_2 uptake in the presence of light.

The goal of this study was to gain a better understanding of the electron transfer pathways involved in H_2 metabolism, particularly in H_2 uptake in *Synechocystis*. To this end, we examined the effects of various members of the photosynthetic and respiratory electron transport chains in thylakoid membranes on H_2 uptake and production in darkness and in the light. In this paper we show that electrons released upon H_2 oxidation in the light reach the cytochrome b_6f complex, and that H_2 oxidation in the light depends on having an oxidized PQ pool and/or cytochrome b_6f.

Materials and methods

Construction of the hox*-deletion mutant*

In order to generate a strain lacking hydrogenase, the *hox* operon containing all five *hox* genes was deleted from the *Synechocystis* genome. Primers used for the construction of this Δ*hox* strain are listed in Table 1. PCR was used to amplify portions of the *Synechocystis* genome containing the two flanking regions of the *hox* operon on both ends (1679265-1678582 and 1672239-1671416; nucleotide numbering according to CyanoBase; Fig. 1). A 1024-bp DNA fragment containing a pACYC184-derived chloramphenicol-resistance cassette was amplified as well.

A pUC19-based plasmid (pΔ*hox*) was constructed with the PCR-amplified 684-bp upstream (FR-1) and 824-bp downstream (FR-2) flanking regions of the *Synechocystis hox* operon, with the chloramphenicol-resistance cassette in between (Fig. 1). The PCR fragments were fused by the extension PCR method [24] and the fused PCR product was cloned into pUC19 using restriction enzymes SacI and SphI creating the plasmid pΔ*hox*. A wild-type *Synechocystis* culture was then transformed with pΔ*hox* following the procedure described by Vermaas et al. [25]. Transformants were grown on BG-11 plates with increasing chloramphenicol concentrations starting from 5 to 100 μg ml^{-1}. Fully segregated transformants were obtained from BG-11 plates with 100 μg ml^{-1} chloramphenicol and their complete segregation was confirmed by PCR.

Cyanobacterial growth conditions

Cultures of the *Synechocystis* wild-type and hydrogenase deletion (Δ*hox*) strains were grown at 30 °C, in 200-ml culture flasks in standard BG-11 medium [26]. A *psaAB*-deletion strain (Δ*psaAB*) [27], a *psbB*-deletion strain (Δ*psbB*) [28] and a strain lacking the terminal oxidases (*ctaDIEI*$^-$/*ctaDIIEII*$^-$/*cydAB*$^-$, or Δ*ox*) [29] were available in our laboratory and were grown in BG-11 medium supplemented with 5 mM glucose. For experiments that compared H_2 uptake rates in the wild-type and the obligate photoheterotrophic mutant strains, the wild-type strain was also grown in the presence of 5 mM glucose. The light-sensitive Δ*psaAB* strain was grown at a light intensity of 3–5 μmol photons m^{-2} s^{-1}. All other cultures were grown at a light intensity between 40 and 45 μmol photons m^{-2} s^{-1}. All light intensities in this study are reported as photosynthetically active radiation (PAR).

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