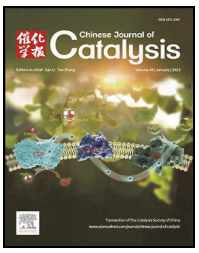
Chinese Journal of Catalysis 44 (2023) 117–126

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.sciencedirect.com/journal/chinese-journal-of-catalysis



Article

Light-driven CO_2 assimilation by photosystem II and its relation to photosynthesis

Yuehui Li [a,1], Duanhui Si [a,1], Wangyin Wang [b,*], Song Xue [c], Wenzhe Shang [a], Zhanyou Chi [c], Can Li [b], Ce Hao [a,*], Govindjee Govindjee [d], Yantao Shi [a,*]

[a] *State Key laboratory of Fine Chemicals, Frontier Science Center for Smart Materials, Department of Chemistry, School of Chemical Engineering, Dalian University of Technology, Dalian 116024, Liaoning, China*

[b] *State Key Laboratory of Catalysis, Dalian National Laboratory for Clean Energy, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, Liaoning, China*

[c] *School of Bioengineering, Dalian University of Technology, Dalian 116024, Liaoning, China*

[d] *Department of Biochemistry, Department of Plant Biology and Center of Biophysics& Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana 61801, USA*

ARTICLE INFO

Article history:
Received 10 July 2022
Accepted 18 September 2022
Available online 10 December 2022

Keywords:
Photosynthesis
Photosystem II core complex
Photoreaction
CO_2 assimilation
Methanol

ABSTRACT

For natural oxygenic photosynthesis, there is a consensus that H_2O is oxidized to molecular oxygen by photosystem II (PSII) in the grana, while CO_2 assimilation takes place, long after oxygen evolution, in light-independent reactions, for example, through the Calvin-Benson Cycle in the stroma. Here, we report, for the first time, light-driven CO_2 assimilation by the PSII core complex, where the formation of methanol, along with the oxygen evolution, is validated by *in-situ* mass spectrometry, gas chromatography and isotopic labeling experiments. Such an unusual CO_2 assimilation is likely to be a simultaneous event along with the usual electron transfer occurring in normal light–independent assimilation. This discovery is extraordinary and is of great significance as it may substantially modify our understanding of the mechanism of photosynthesis.

1. Introduction

Photosynthesis is the greatest natural process converting sunlight into chemical energy on a massive scale and maintaining the life on Earth [1,2]. There are basically two successive stages of oxygenic photosynthesis, of which the light-dependent reactions in photosystem II (PSII), and in photosystem I (PSI), enable the oxidation of H_2O into molecular oxygen, and production of reducing power (NADPH and ATP), while CO_2 assimilation is generally known to take place long after oxygen evolution, and NADP reduction, *via* light-independent reactions in the stroma [3]. Meanwhile, there is wide consensus that during light dependent reactions CO_2 (or bicarbonate) is not reduced as a substrate, but plays a unique stimulatory role in O_2 evolution, namely the so-called "bicarbonate effect" [4].

The role of bicarbonate effect as well as the bicarbonate binding sites in PSII have been intensively studied since the

* Corresponding author. E-mail: shiyantao@dlut.edu.cn (Y. Shi), haoce@dlut.edu.cn (C. Hao), wywang@dicp.ac.cn (W. Wang).
[1] Contributed equally to this work.
This work was supported by the National Natural Science Foundation of China (51773025, 21677029, 22088102), LiaoNing Revitalization Talents Program (XLYC2007038, XLYC2008032), the Fundamental Research Funds for the Central Universities (DUT22LAB602).
https://doi.org/10.1016/S1872-2067(22)64170-6

1970s, with some important conclusions reached thus far [4]. On the (electron) acceptor side of PSII, bicarbonate is known to serve as a bidentate ligand to the NHI (non-heme iron) bridging Q_A and Q_B, which can facilitate proton transfer and thereby accelerate electron flow and the plastoquinone reduction of Q_B [5–8]. Whereas the role of bicarbonate on the (electron) donor side of PSII, either functioning as a binding site, or as a structural cofactor of oxygen evolution center (OEC), or as a substrate, or as a chemical intermediate [9], is not as clear as it is on the (electron) acceptor side. Currently, the way bicarbonate is possibly involved in the events on the donor side of PSII has had many speculations. The two viewpoints considering bicarbonate as an intermediate substrate for photosynthetic water oxidation or as a direct ligand of Mn_4CaO_5 cluster are thought to be unlikely since no strong experimental evidence exists or supports it [10,11]. There are suggestions that bicarbonate may play a role in stabilizing the OEC indirectly by binding to extrinsic proteins or some other nearby protein components [12,13], which, however, needs to be investigated. Further, the universality of the above suggestions needs to be examined in higher plants. In addition, there are still other suggestions that bicarbonate may be a transient ligand to Mn ions and act as a native cofactor in the photo-assembly process of the Mn_4CaO_5 cluster [14,15]; additionally, it may even serve as a mobile exchangeable agent for proton removal during photosynthetic water oxidation [16,17]. All in all, from the current point of view, CO_2 (or bicarbonate) in PSII is thought to only play supporting roles during water oxidation, rather than being '*assimilated*' by PSII.

Herein, through a series of well-designed experiments, we, for the first time, report an extraordinary finding regarding the natural photosynthesis that CO_2 can be assimilated into methanol (CH_3OH) by the PSII core complex under illumination, in direct contradiction to the widely accepted point of view that CO_2 assimilation occurs only via light-independent reactions.

2. Experimental

2.1. Materials

All chemicals were used as received without further purification. Isotopic $C^{18}O_2$ (^{18}O content ≥ 97%) and $^{13}CO_2$ (^{13}C content ≥ 99%) were obtained from Sigma Aldrich (USA). 2-(N-Morpholino) ethanesulfonic acid (MES)E, (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (DCMU, 99%), 2,6-dichloro-1,4-benzoquinone (DCBQ), Sodium hydroxide (NaOH, 99.9%) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (China). Ethylene glycol tetraacetic acid (EGTA, BC grade), bovine serum albumin (BSA frac. V, biotech grade), 2-(2-[4-(1,1,3,3-tetramethylbutyl) phenoxy] ethoxy) ethanol (Triton X-100, 98%), n-octyl-β-D-thioglucopyranoside (OTG, 99%), PEG 6000 (molecular weight: 5000–7000, 99%), sucrose (AR) were purchased from Sangon Biotech Co., Ltd (China). Sodium chloride (NaCl, 99.5%), Potassium ferricyanide ($K_3[Fe(CN)_6]$, 99.5%), $MgCl_2{\cdot}6H_2O$ (99.9%), methanol (HPLC grade, >99.9%) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (China). The *chlorella* was provided by Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences (China). Fresh Spinach was purchased from a local vegetable market in Dalian (China).

2.2. Preparation of the chlorella cells

The *chlorella* was cultivated in BG-11 buffer solution, a standard method used frequently in this field [18]. The pH of the BG-11 suspension was maintained at 7.5 under LED light irradiation about 2000 lux at temperature of 298 K. *Chlorella* was cultivated under LED light irradiation 12 h and dark for 12 h, light and dark were cultured alternately. Before the start of photosynthesis, the chlorella in suspension was cultivated for 72 h.

2.3. Preparation of the chloroplasts samples

For preparation of the isolated chloroplasts, first, 250.0 g fresh spinach leaves were added into 500.0 mL SMN buffer solution (400 mmol L^{-1} sucrose, 50 mmol L^{-1} MES-NaOH, 15 mmol L^{-1} NaCl, pH = 6.0), after which the mixture was ground and filtrated by blender, the isolated chloroplasts were separated out by centrifugation of the filtrate at 3500 r·min^{-1} for 10 min. The chlorophyll (Chl) concentration of chloroplasts sample is 1.47 mg chlorophyll mL^{-1} based on the optical absorption coefficients of chlorophyll a and b in 80% acetone aqueous solution [19]. The prepared chloroplasts samples were finally suspended in a SMN buffer solution, and then frozen in liquid nitrogen and stored in low-temperature refrigerator at –72 °C.

2.4. Preparation of PSII core complex samples

The PSII core complex sample was prepared by two-step detergent treatment. First, the PSII membrane fragments was isolated from fresh spinach leaves according to a reported procedure [20]. Second, the PSII core complex was collected by treating PSII membrane fragments with 0.4% (w/v) n-octyl-β-D-thioglucoside (OTG) solution [21]. The prepared PSII samples were finally suspended in a SMN buffer solution (400 mmol L^{-1} sucrose, 50 mmol L^{-1} MES-NaOH, 15 mmol L^{-1} NaCl, pH=6.0), and then frozen in liquid nitrogen and stored at –72°C. The chlorophyll concentration was determined based on the optical absorption coefficients of chlorophyll a and b in a 80% acetone aqueous solution by UV-Vis absorption spectroscopy [19]. The chlorophyll concentration of PSII core sample is 0.26 mg chlorophyll mL^{-1}. The O_2 evolution activities were measured by a Clark-type oxygen electrode in the SMN buffer with 0.53 mmol L^{-1} 2,6-dichlorobenzoqinone as electron acceptors under saturated red light irradiation [22]. The oxygen evolution activity of PSII sample is over 900 μmol O_2 (mg of chlorophyll)$^{-1}$ h^{-1} (see Fig. S1).

2.5. Experimental setup

Our setup is composed of three main parts (see Fig. S2): carrier gas system, quartz reactor equipped with a light source,

and real-time mass spectrometry (GSD301O3, pfeiffer vacuum, germany) with an with an electron ionization (EI) ion source, and the voltage between filament and the receiver is 70 eV. He as carrier gas in our experiment has a high purity of 99.999%. Before flowing into reactor, the carrier gas is further purified by a deoxidization column so as to thoroughly remove the residual oxygen. The photosynthesis is initiated by illumination with a xenon lamp with a long-pass filter (λ > 600 nm) about 100 mW cm^{-1} (1.0 Sun, AM 1.5), the temperature of the quartz reactor is maintained at around 298 K for *chlorella* system, while that for chloroplasts and PSII systems is around 283 K. The O_2 and CH_3OH were detected by RT-MS for the three systems (*chlorella*, chloroplasts, and PSII core complex). The MS parameters were set according to that of National Institute of Standards and Technology (NIST) (see Figs. S3(a) and (b)). In our MS analysis, the model of "Multiple Concentration Detection" was used and the SEM (second electron multiplier) value was set at 1400. The mass range is 1–300, the detection limit is 10^{-14} with the SEM detector, and the unit resolution was ≤ 1 amu. The interval for detection of one MS signal was 0.1 s, the injection temperature was 413 K while the inner pressure was 1.9×10^{-6} mbar. In addition to the use of mass spectrometry (MS), the CH_3OH formed in the light-driven reactions were also detected by gas chromatography (CEL-GC-7920, China) in which the chromatographic column was HP-5 capillary column (50 m × 0.32 mm, 1 μm) with N_2 serving as carrier gas. The temperature of column was kept at 328 K while that of vaporizer and flame ionization detector (FID) was kept at 453 K.

2.6. Photosynthesis experiment based on chlorella

Prior to being transferred to the reactor of photosynthesis, the *chlorella* was cultivated in BG-11 buffer suspension 72 h. Then, the *chlorella* was separated from BG-11 cultivation suspension by centrifugation at 3000 r min^{-1} for 10 min to increase *Chlorella* concentration. Subsequently, the wet chlorella precipitate was re–dispersed into 10.0 mL BG–11 solution (~0.11 mg·mL^{-1}). The *chlorella* suspension was transferred to the quartz reactor, and the suspension was feed with a mixture of He and CO_2 (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}). The residual oxygen in *chlorella* suspension was thoroughly removed by duration of aeration for 2 h prior to photosynthesis, after which the evolution of the O_2 and CH_3OH were monitored by RT-MS under saturated red light irradiation at temperature of 298 K.

2.7. $C^{18}O_2$ and $^{13}CO_2$ labeling experiment based on chlorella

The wet *chlorella* precipitate was dispersed into 10.0 mL BG–11 suspension and then transferred to the quartz reactor. The residual oxygen in *chlorella* suspension was removed by duration of aeration with carrier gas (with a flow rate of 20.0 mL min^{-1}) until reaching a steady-state MS baseline. Then, the chlorella suspension was feed with a mixture of He and $C^{18}O_2$ or $^{13}CO_2$ (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}). After the arising of a steady-state baseline, the photosynthesis was initiated by switching on the light, with the products ($^{13}CH_3OH$ and $CH_3{}^{18}OH$) being monitored by RT-MS under saturated red light irradiation at temperature of 298 K.

2.8. Photosynthesis experiment based on isolated chloroplasts

Before transfer to the reactor of photosynthesis, the isolated chloroplasts (700 μL, 1.47 mg chlorophyll mL^{-1}) was firstly revived in an ice bath for 30 min by being added into SMN Buffer solution at pH 6.0 composed containing 0.5 mmol L^{-1} DCBQ. Then, the PSII suspension (total 10.0 mL) was transferred to quartz reactor. The residual oxygen in SMN suspension was feed with a mixture of He and CO_2 (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}) until reaching a steady-state MS baseline. After the arising of a steady-state baseline, the photosynthesis experiment was initiated by switching on the light. The products (O_2 and CH_3OH) were monitored by RT-MS under saturated red light irradiation at temperature of 283 K.

2.9. $C^{18}O_2$ and $^{13}CO_2$ labeling experiment based on isolated chloroplasts

The isolated chloroplasts were dispersed the SMN Buffer solution contained 0.5 mmol L^{-1} DCBQ, after which the chloroplasts suspension was transferred to quartz reactor. The residual oxygen in chloroplasts suspension was removed by duration of aeration with carrier gas (with a flow rate of 20.0 mL·min^{-1}) until reaching a steady-state MS baseline. Then, the chloroplasts suspension was feed with a mixture of He and $C^{18}O_2$ or $^{13}CO_2$ (*V:V* = 98.5:1.5, flow rate is 5.1 mL min^{-1}). After the arising of a steady-state baseline, the photosynthesis was initiated by switching on the light, with the products ($^{13}CH_3OH$ and $CH_3{}^{18}OH$) being monitored by RT-MS under saturated red light irradiation at temperature of 283 K.

2.10. Photosynthesis experiment based on PSII core complex

Prior to being transferred to the reactor of photosynthesis, the PSII core complex (1.0 mL, 0.26 mg chlorophyll·mL^{-1}) was firstly revived in an ice bath for 30 min by being added into SMN Buffer solution at pH 6.0 composed of 9.0 mL SMN containing 0.5 mmol L^{-1} DCBQ. Then, the PSII suspension (total 10.0 mL) was transferred to quartz reactor. The residual oxygen in SMN suspension was feed with a mixture of He and CO_2 (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}) until reaching a steady-state MS baseline. Then, the photosynthesis was initiated by switching on the light. The products (O_2 and CH_3OH) were monitored by RT-MS under saturated red light irradiation at temperature of 283 K.

2.11. Photosynthesis experiment based on PSII core complex containing DCMU

The PSII core complex was firstly revived in an ice bath for 30 min by being added into SMN buffer solution at pH 6.0 containing 0.5 mmol L^{-1} DCBQ and different concentration DCMU (0, 0.5, 5.0, 50, 250, 750 μmol L^{-1}). Then, the PSII suspension (total 10.0 mL) was transferred to quartz reactor with a mix-

ture of He and CO_2 (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}). After the arising of a steady-state baseline, the photosynthesis was initiated by switching on the light, with the products (O_2 and CH_3OH) being monitored by RT-MS under saturated red light irradiation at temperature of 283 K.

2.12. $C^{18}O_2$ and $^{13}CO_2$ labeling experiment based on PSII core complex

The PSII core complex was firstly revived in an ice bath for 30 min by being added into SMN Buffer solution (pH=6.0) composed of 9.0 mL SMN buffer containing 0.5 mmol L^{-1} DCBQ. Then, the PSII core complex suspension (total 10.0 mL) was transferred to quartz reactor. The residual oxygen in PSII suspension was removed by duration of aeration with carrier gas (with a flow rate of 20 mL min^{-1}) until reaching a steady-state MS baseline. Then, the suspension was feed with a mixture of He and $C^{18}O_2$ or $^{13}CO_2$ (*V:V* = 98.5:1.5, flow rate was 5.1 mL min^{-1}). After the arising of a steady-state baseline, the photosynthesis was initiated by switching on the light. The products ($^{13}CH_3OH$ and $CH_3{}^{18}OH$) were monitored by RT-MS under saturated red light irradiation at temperature of 283 K.

2.13. The detection of CH_3OH by gas chromatography (GC) for the three systems

After photosynthesis and oxygen production of the three systems (*chlorella*, chloroplasts system, and PSII core complex) upon light illumination, the atmosphere over the suspension of were collected and analyzed by GC in which the chromatographic column was TDX-01 with N_2 serving as carrier gas. The temperature of column was kept at 328 K while that of vaporizer and detector (FID) was kept at 453 K.

2.14. Purity identification of PSII core complex

The protein samples were dissolved in 6 mol L^{-1} guanidine hydrochloride /50 mmol L^{-1} HEPES solution. 5 mmol L^{-1} TCEP (Tris (2-carboxyethyl) phosphine) and 10 mmol L^{-1} IAA (Iodoacetamide) were added to open the disulfide bond. Then, the samples were digested by trypsin with a ratio of 25:1 (*W*/*W*) at 37 °C overnight. Finally, the peptides were analyzed by an Orbitrap Fusion Lumos Tribrid MS coupled with a Vanquish Flex HPLC system. All collected MS datasets files were searched by Maxquant (version 1.6.7) to match the protein sequence downloaded from Uniprot website.

3. Results and discussion

We used the green alga *Chlorella*, as well as isolated chloroplasts (from spinach leaves), for this study (Figs. 1(a) and (b)). Along with the carrier gas (He), CO_2 was passed over the photosynthesis system (see experimental set-up in Fig. S2), and the products (such as O_2 and CH_3OH) formed during light illumination were analyzed by real-time mass spectrometry (RT-MS) and gas chromatography (GC). By means of RT-MS, the generation of CH_3OH and O_2 (Figs. 1(c) and (d)) upon light illumination, was directly observed both in *Chlorella* and chloroplast suspensions, but methanol could be formed without the participation of *Chlorella* or chloroplast (Fig. S4). In addition, CH_3OH was also detected by GC in the atmosphere over the *Chlorella* cells as well as over chloroplast suspensions upon light illumination, but it was not observed when the samples were placed in dark (Figs. 1(e) and (f)). The above results clearly suggest that CH_3OH production is a light-driven event.

In fact, it had been reported previously that CH_3OH can be produced by plants and released to the atmosphere [23,24], which had been validated to be a light–dependent event while, for example, there is a daily decline in CH_3OH concentration in the late afternoon in a forest canopy [25]. The mechanism of such CH_3OH production under light illumination has remained uncertain. One popular explanation is that CH_3OH comes from the cell wall pectin, which is reported to be able to generate CH_3OH after being demethylesterified by pectin methylesterases (PMEs) [26,27]. Whereas the fact that the isolated chloroplasts, in our current work, have been shown to produce CH_3OH indicates that its source is not the cell wall pectin. Furthermore, the probability of photorespiration involved in this CH_3OH production can also be precluded since the product of photorespiration is CO_2 rather than CH_3OH. We therefore think that this CH_3OH production may be related to photosynthesis.

Subsequently, we carried out isotopic labeling experiments using $C^{18}O_2$ and $^{13}CO_2$ to further probe into this issue. For MS characterization, it is necessary to check for interference due to overlap of signals (with the same m/z value) from other molecules. In this regard, we needed to conduct MS analysis based on the exclusive m/z signal for the labeled product. For example, in $C^{18}O_2$ labeling experiment, the labeled product $CH_3{}^{18}OH$ normally has many fragment signals, such as m/z = 32, 33, 34, of which the two signals of m/z = 32, 34 overlap with that of O_2 and $^{16}O^{18}O$, respectively. Therefore, only m/z = 33 can be used exclusively to identify the formation of $CH_3{}^{18}OH$ (see detailed m/z signals assignment in Tab. S1). Similarly, in $^{13}CO_2$ labeling experiment, among the possible signals for the labeled $^{13}CH_3OH$ (such as m/z = 32 and 33), only m/z = 33 can be used to clarify whether $^{13}CH_3OH$ is produced. Upon illumination, both *Chlorella* cells and isolated chloroplasts gave the following results and conclusion: the labeled signals of m/z = 33 ($CH_3{}^{18}OH$ and $^{13}CH_3OH$) (Figs. 2(a)–(d)), as observed in $C^{18}O_2$ and $^{13}CO_2$ labeling experiments, provided important evidence that CH_3OH emission observed in our samples came from CO_2 assimilation.

Such light-driven CO_2 assimilation, shown in this study, takes place in chloroplasts, but seems incomprehensible based on the mechanisms known thus far. Here we suggest that this unusual event may be related to the photosystems in chloroplasts. As we know, chloroplast within plants and algae is the organelle carrying out photosynthesis *via* the two photosystems, PSI and PSII, which consist of structural and functional units of pigment-protein complexes involved in photosynthesis. Usually, during photosynthesis, PSI and PSII work together to synergistically carry out light absorption, and energy and electron transfer (from water to $NADP^+$ through a Z-scheme pathway) [28]. To further clarify the possible location of such unusual CO_2 assimilation, we conducted experiments using PSII

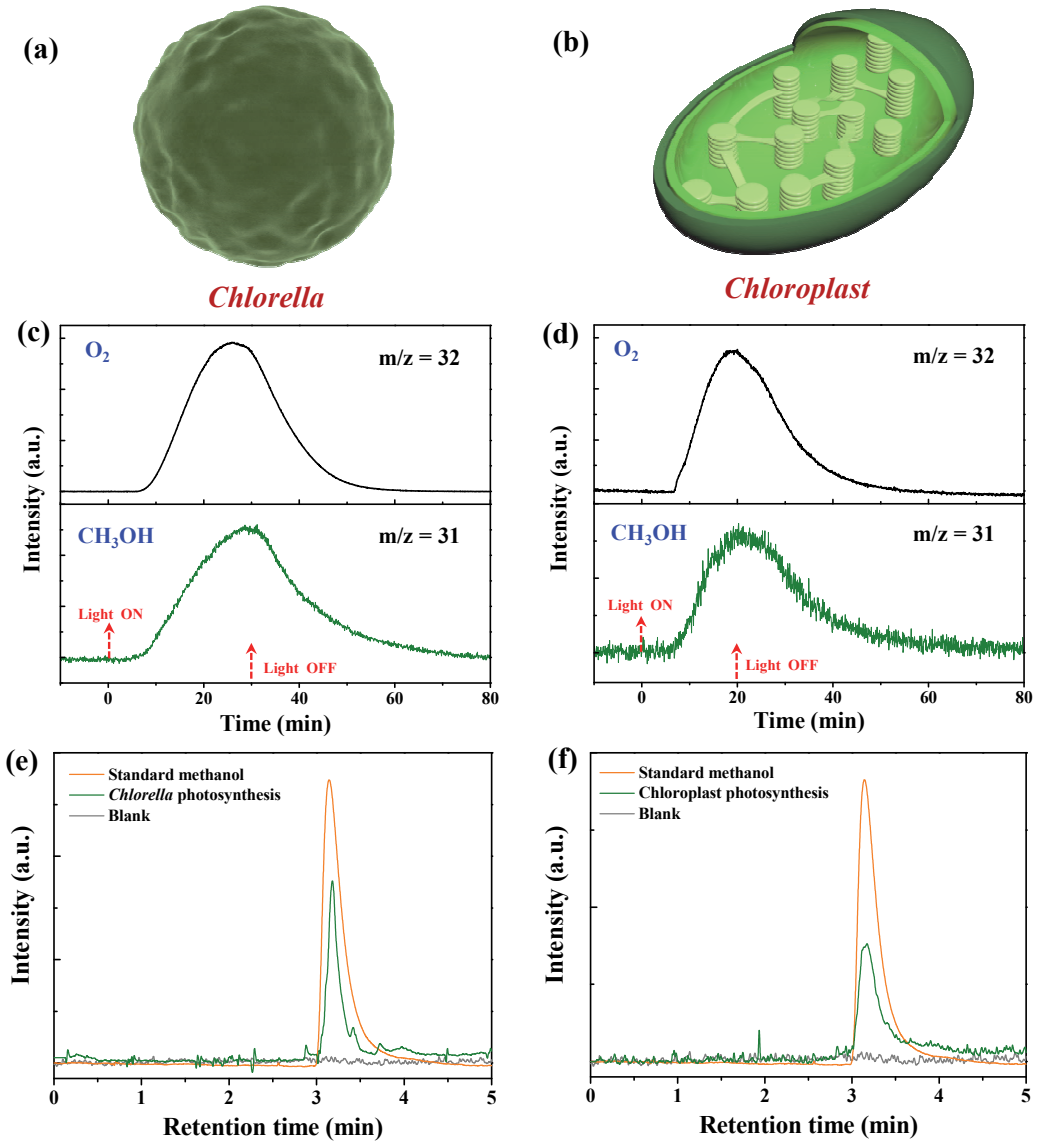


Fig. 1. The measurements of the production of O_2 and CH_3OH by the green alga *Chlorella* and chloroplasts, isolated from spinach leaves, upon light illumination. (a,b) A diagram of *Chlorella* cells and spinach chloroplasts. (c,d) The O_2 and CH_3OH detected by *in-situ* real-time MS in *Chlorella* and spinach suspensions. (e,f) The CH_3OH detected by GC in *Chlorella* cells and in spinach chloroplasts. The blank experiments E and F refer to those carried out in the dark.

prepared from spinach leaves, according to routine methods (see details in the experimental section), which was then examined by MS for purity. We showed that our PSII core complex samples had a high purity while there was only an extremely small amount (less than one percent) of residual PSI (Fig. S5).

Subsequent experiments based on PSII core complex were carried out in the same way as that in *Chlorella* and chloroplast suspensions. In PSII samples, oxygen evolution, upon light illumination, was clearly observed, while the oxygen yield was significantly reduced in the case when there was no CO_2 in the carrier gas (Fig. S6). To be noted, the important photosynthetic products (CH_3OH and O_2) targeted did not changed significantly under pure He and dark conditions (Fig. S7), indicating that the catalyst has better stability in this work. As mentioned above, the roles that CO_2 (or HCO_3^-) play in PSII are e.g., as a ligand, or as a structural cofactor. Thus far, CO_2 has never been reported to be assimilated (or fixed) by PSII in a light dependent reaction. But surprisingly, in the present study, we for the first time have detected the formation of CH_3OH during the PSII reaction, a light–driven event as verified by both *in-situ* MS (Fig. 3(a)) and GC (Fig. 3(b)) methods. The GC data showed that the total amount of CH_3OH produced by the PSII core suspensions after illumination for 15 min is 9.4 mmol·g^{-1} in this light-driven CO_2 assimilation. To be noted, in addition to CH_3OH, some other compounds were also detected by MS with relatively weak signals, possibly from CO_2 assimilation but hard to be identified at present stage. Meanwhile, we also found that the relative amounts of both O_2 and CH_3OH showed an increasing trend as we enhanced the concentration of CO_2 fed to the system (Figs. 3(c) and (d)). Such a CO_2 concentration-dependent production of CH_3OH may imply that CO_2 is consumed as substrate in the

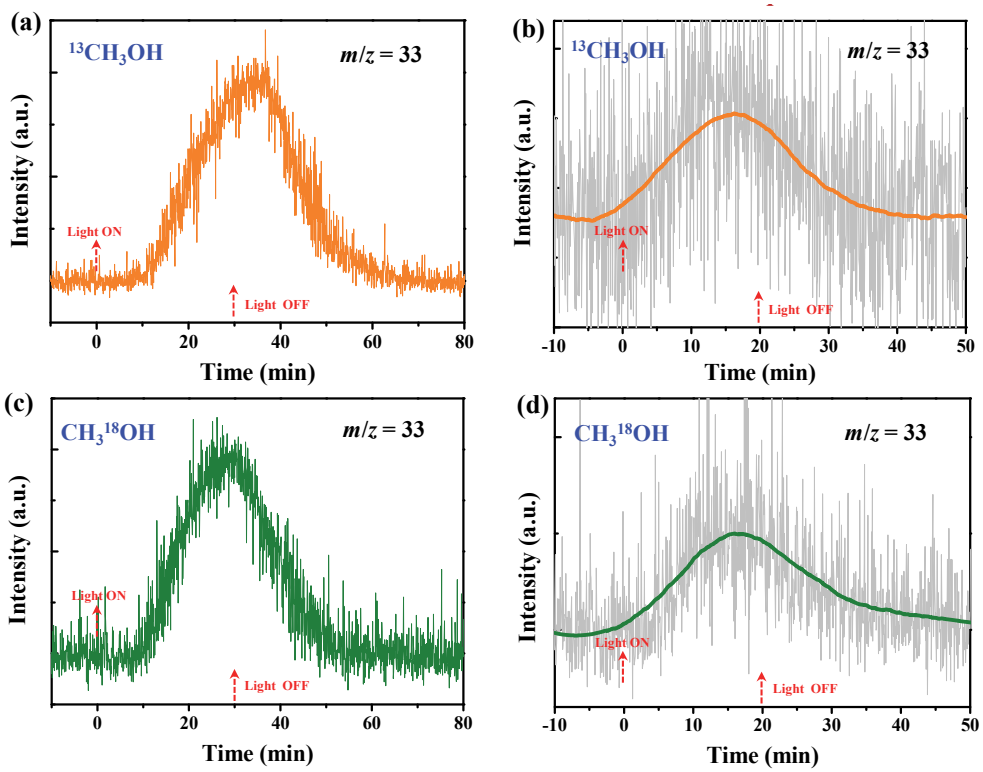


Fig. 2. The labeled CH_3OH detected by *in-situ* RT-MS in *Chlorella* cells and spinach chloroplasts upon light illumination. (a,b) The $^{13}CH_3OH$ (m/z = 33) detected by RT-MS for *Chlorella* and spinach chloroplasts labeled with $^{13}CO_2$. (c,d) The $CH_3{}^{18}OH$ (m/z = 33) detected by RT-MS for *Chlorella* and spinach chloroplasts, labeled with $C^{18}O_2$.

light reactions of PSII. We then carried out labeling experiments to further understand the source of CH_3OH detected in PSII reactions. In the experiments using $^{13}CO_2$ or $C^{18}O_2$, we clearly observed the exclusive signal of m/z = 33 for $^{13}CH_3OH$ and $CH_3{}^{18}OH$ (Figs. 3(e) and (f)), suggesting that the CH_3OH detected in PSII came from CO_2.

The above finding was unexpected, but it is a novel discovery and is very important since it provides evidence to conclude that CO_2 can be assimilated by PSII through light-dependent reactions. The traditional mechanism for CO_2 assimilation by plants and other photosynthetic organisms through light-independent reactions has been well established, for example, the C3 pathway (Calvin-Benson cycle), C4 pathway (Hatch-Slack cycle), or Crassulacean Acid Metabolism pathway (CAM cycle) [29–32], which occur in the stroma of chloroplasts rather than in PSII. But at this stage, the pathway for this extraordinary light-driven CO_2 assimilation is unknown, and we do not yet know where precisely it takes place, i.e., what is the exact location where CO_2 accepts electrons from PSII. Considering that our PSII core complex is highly purified, besides non-heme iron (in PSII), no site is available to bind CO_2, let alone do assimilation. However, when we added 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a well-known inhibitor of the electron acceptor site of PSII, which can occupy the Q_B site to block the electron transfer from Q_A to plastoquinone and quinone-quinol exchange [33], we observed that both O_2 and CH_3OH were still detectable. The amounts of O_2 and CH_3OH decreased by almost 50%, but the rates remained steady with continuously increasing DCMU concentration (Fig. S8). These results indicate that CO_2 reduction to methanol is dependent on the electron transfer rate of the acceptor side, and an alternative (and parallel) pathway occurs specially for CO_2 or bicarbonate interaction with the electron donor side of PSII. Due to the thermodynamic requirement of CO_2 reduction and the redox potential of the electron mediator, we infer that the site for the light-powered CO_2 reduction, in our case, is likely to be before the Q_A site. We all know that the more negative the redox potential is, the easier the reduction reaction proceeds. Considering that the reduction of CO_2 into CH_3OH requires sufficient energy, we suggest that the location is likely to be near the excited P680*, or even at reduced pheophytin, which can offer the most powerful reducing force [34,35].

Here, an important issue arises as to the relation of this light-driven CO_2 assimilation to the known reactions and the electron transfer pathway occurring in PSII. For this, we note the following: artificial electron acceptor such as $K_3[Fe(CN)_6]$ or 2,6-dichloro-1,4-benzoquinone (DCBQ) may be used to guarantee the occurrence of O_2 evolution by PSII core complex. We have found that CH_3OH or O_2 could not be generated without using the artificial electron acceptor (Figs. 4(a) and (b)). Second, we found that the relative amount of CH_3OH in MS was consistent with that of photosynthetic O_2. The rate of light-driven CO_2 assimilation (reflected by the relative amount of CH_3OH in MS) can be modulated by using different acceptors. When $K_3[Fe(CN)_6]$ was used, the relative amount of O_2 or

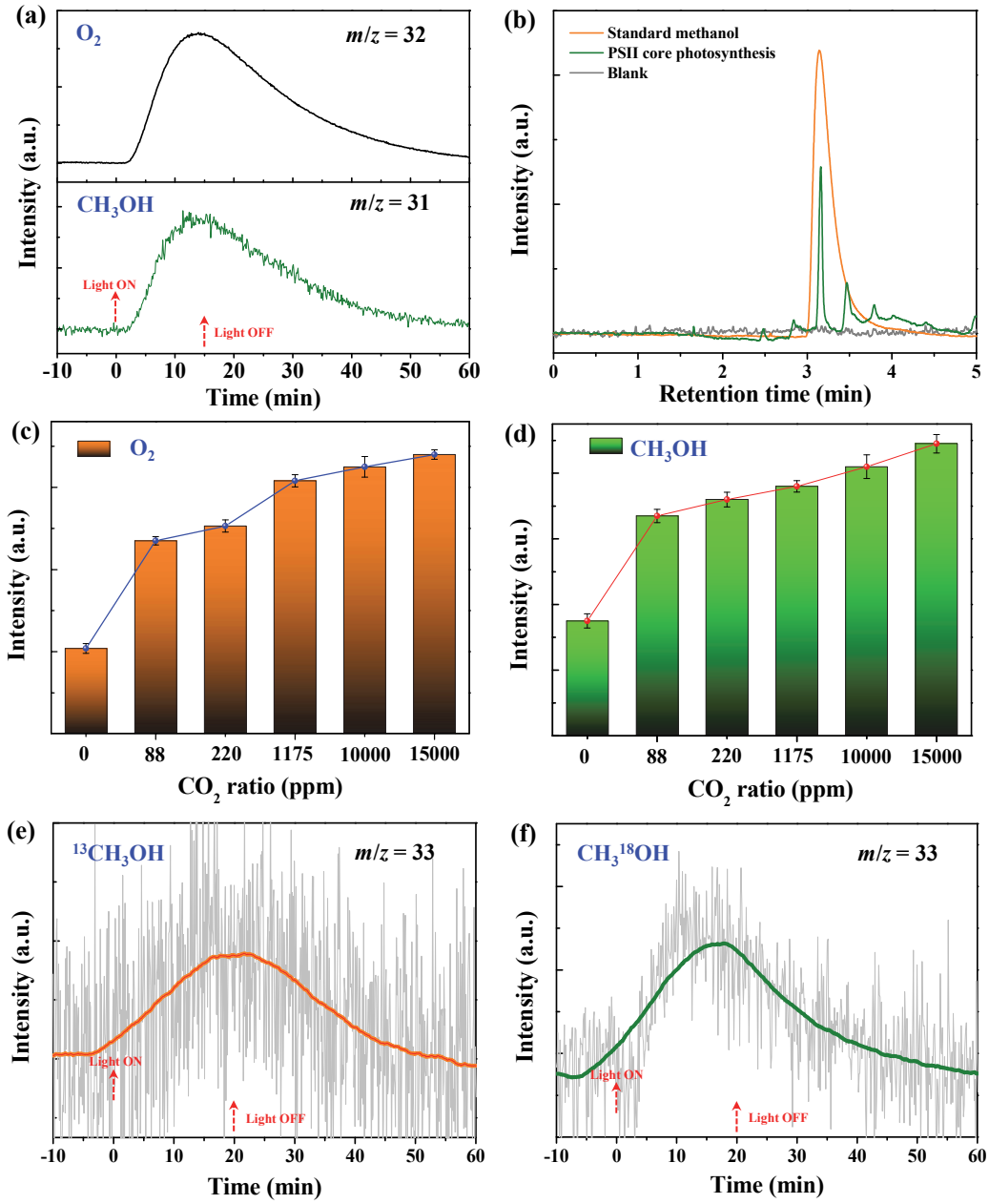


Fig. 3. The measurements of the production of O_2 and CH_3OH by the PSII core complex upon light illumination. (a) The O_2 and CH_3OH detected by *in-situ* RT-MS. (b) The CH_3OH detected by GC; The O_2 (c) and CH_3OH (d) relative amounts detected by RT-MS under different concentration of CO_2 in carrier gas (0, 88, 220, 1175, 10000, 15000 ppm). The $^{13}CH_3OH$ (e) and $CH_3{}^{18}OH$ (f) detected by *in-situ* RT-MS in $^{13}CO_2$ and $C^{18}O_2$ labeling experiments.

CH_3OH in MS was only about one fifth that observed when DCBQ was used. We suggest that the novel light-driven CO_2 assimilation (discovered in this work) likely occurs simultaneously with the normal electron transfer pathway, or in other words, it does not operate independently of the main pathway of electron flow. Further, we speculate that the electrons feeding to the light-driven CO_2 assimilation are possibly part of the total electrons obtained from water oxidation (Fig. 4(c)). We wonder whether the occurrence of this newly discovered light–driven CO_2 assimilation is due to the need of lowering of the reducing pressure along the electron transfer chain, by which plants can prevent photodamage to PSII core complex. Traditionally, CH_3OH emitted by plants has been explained to be a by product of pectin metabolism during cell wall synthesis [25]. By comparison, our observation that CH_3OH comes from light-driven CO_2 assimilation, by PSII, seems reasonable because (i) CH_3OH emission is a light-dependent event; and (ii) our isotopic labeling experiments confirm CO_2 to be the source of CH_3OH.

Despite being identified to be a product of light-driven CO_2 assimilation, we cannot be sure whether CH_3OH is the only product formed in this process. Based on the GC and MS data presented in this paper, we can hardly identify whether other C1 compounds (formaldehyde or formate) are also formed. This could be due to their very low yield or because their MS signals overlap with CH_3OH. Further, for this light driven CO_2

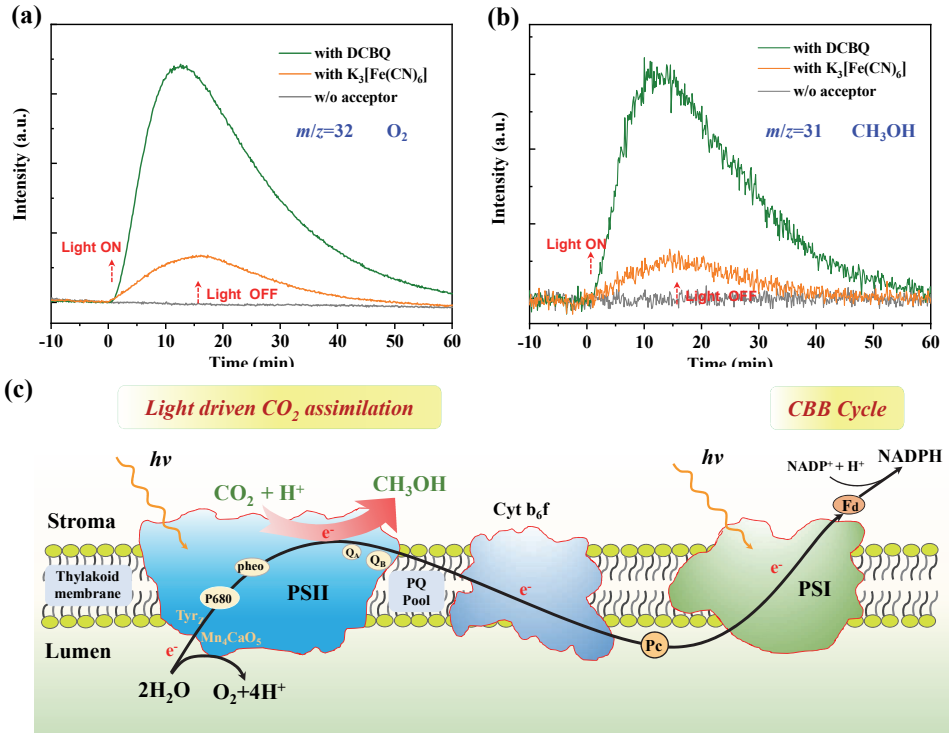


Fig. 4. The O_2 (a) and CH_3OH (b) detected by RT-MS for the PSII core complex, upon light illumination, involving different electron accepters. (c) A schematic depiction of the electron flow across the three protein complexes, as well as the light-driven CO_2 light assimilation possibly occurring prior to Q_A.

assimilation, CH_3OH may not be the end product, and other advanced assimilation products (such as C2 compounds) may also be formed. We thus tentatively conclude that CH_3OH may be an intermediate towards higher level assimilation product(s). In fact, CH_3OH has been tested to have many positive effects on the growth of plants [36–39]. For example, external application of CH_3OH to leaves can enhance the growth and yield of C3 plants. Although the mechanism for this phenomenon is still under debate, we are inclined to support the idea that CH_3OH may serve as a carbon source for plant growth.

The stimulation role of bicarbonate on photosynthetic O_2 evolution has been well studied, whereas, whether CO_2 (or bicarbonate) can directly take part in PSII reactions as one substrate has long been in dispute. In earlier times, Willstätter *et al.* [40] (in 1918), Franck *et al.* [41] (in 1941), and Warburg [42] (in 1964), did speculate that CO_2 is involved in oxygen evolution as one substrate. Very recently, Wu [43] has also presented his reasons for re-considering "photolysis of bicarbonate" from the aspects of thermodynamics and Earth's environment evolution. Wu has further re-examined the results of previous isotopic labeling experiments, and, then, he has proposed that bicarbonate is a direct substrate participating in photosynthetic O_2 evolution. Although we are uncertain whether CO_2 takes part in O_2 evolution as a direct substrate, our present study does offer important evidence for CO_2 taking part as a substrate for making CH_3OH, but it is still unclear whether this light-driven CO_2 assimilation has a direct or indirect relation to O_2 evolution since we have reached a tentative conclusion that the location where CO_2 accepts electrons is unlikely to be at the donor side of PSII.

4. Conclusions

In summary, apart from the known pathway for light-independent CO_2 assimilation, we have shown, in this paper, that there is an additional unknown pathway for light-driven CO_2 assimilation. This discovery is of great significance as it may substantially modify our understanding of photosynthesis. Moreover, our observation helps us explain some phenomenon whose mechanism is still under debate, such as CH_3OH emission by plants [44]. But at the present, the mechanism for this light-driven CO_2 assimilation is still poorly understood. Thus, our discovery on CO_2 assimilation by PSII presented here calls for further studies to obtain more evidence and to probe into the precise site where methanol is made from CO_2 by PSII. Meanwhile, this discovery may also give us inspiration to develop natural-artificial combined photosynthetic systems for solar fuel production from CO_2 and H_2O.

Notes

The authors declare no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (51773025, 21677029, 22088102), Liao-Ning Revitalization Talents Program (XLYC2007038,

XLYC2008032), the Fundamental Research Funds for the Central Universities (DUT22LAB602). Govindjee thanks the staff of the Information Technology in Life Sciences at the University of Illinois at Urbana-Champaign for support.

Electronic supporting information

Supporting information is available in the online version of this article.

References

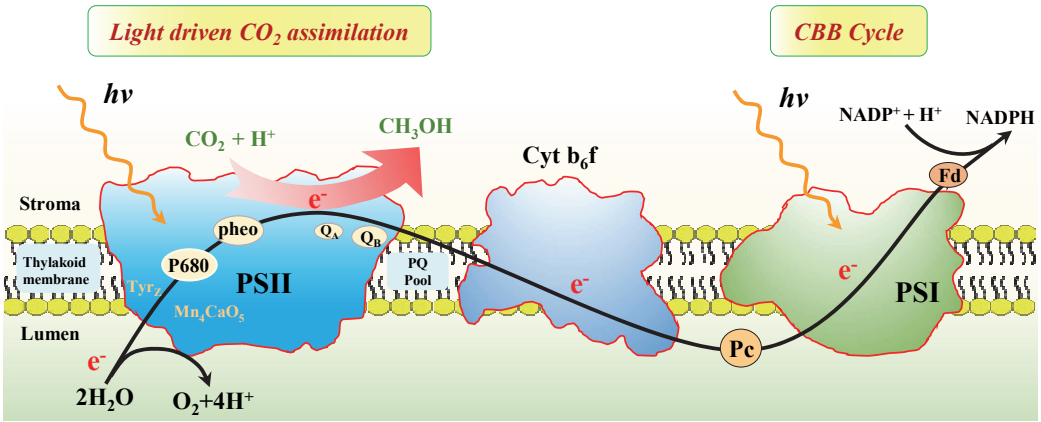
[1] R. E. Blankenship, *Molecular Mechanisms of Photosynthesis*, 3rd Edition, Wiley, **2021**, 1–9.
[2] D. Shevela, L. O. Björn, Govindjee, *Photosynthesis: Solar Energy for Life*, World Scientific, **2018**, 1.
[3] M. P. Johnson, *Essays Biochem.*, **2016**, 60, 255–273.
[4] D. Shevela, J. J. Eaton-Rye, J. R. Shen, Govindjee, *Biochim. Biophys. Acta-Bioenerg.*, **2012**, 1817, 1134–1151.
[5] D. J. Blubaugh, Govindjee. *Biochim. Biophys. Acta-Bioenerg.*, **1988**, 936, 208–214.
[6] D. J. Blubaugh, Govindjee. *Photosynth. Res.*, **1988**, 19, 85–128.
[7] R. Hienerwadel, C. Berthomieu, *Biochemistry*, **1995**, 34, 16288–16297.
[8] Y. Umena, K. Kawakami, J. R. Shen, N. Kamiya, *Nature*, **2011**, 473, 55–60.
[9] V. V. Klimov, S. V. Baranov, Bicarbonate requirement for the water-oxidizing complex of Photosystem II. *Biochim. Biophys. Acta-Bioenerg.*, **2001**, 1503, 187–196.
[10] A. Stemler, *Biochim. Biophys. Acta-Bioenerg.*, **1980**, 593, 103–112.
[11] P. A. Castelfranco, Y. K. Lu, A. J. stemler, *Photosynth. Res.*, **2007**, 94, 235–246.
[12] V. V. Klimov, S. I. Allakhverdiev, S. V. Baranov, Y. M. Feyziev, *Photosynth. Res.*, **1995**, 46, 219–225.
[13] V. V. Klimov, S. I. Allakhverdiev, Y. M. Feyziev, S. V. Baranov, *FEBS Lett.*, **1995**, 363, 251–255.
[14] S. I. Allakhverdiev, I. Yruela, R. Picorel, V. V. Klimov, *Proc. Natl. Acad. Sci. U. S. A.*, **1997**, 94, 5050–5054.
[15] R. J. Hulsebosch, S. I. Allakhverdiev, V. V. Klimov, R. Picorel, A. J. Hoff, *FEBS Lett.*, **1998**, 424, 146–148.
[16] G. Ananyev, G. C. Dismukes. *Photosynth. Res.*, **2005**, 84, 355–365.
[17] T. Shutova, H. Kenneweg, J. Buchta, J. Nikitina, V. Terentyev, S. Chernyshov, B. Andersson, S. I. Allakhverdiev, V. V. Klimov, H. Dau, W. Junge, G. Samuelsson, *EMBO J.*, **2008**, 27, 782–791.
[18] R. Y. Stanier, R. Kunisawa, M. Mandel, *Bacteriological reviews*, **1971**, 35, 171–205.
[19] H. K. Lichtenthaler, *Methods Enzymol.*, **1987**, 148, 350–382.
[20] D. A. Berthold, G. T. Babcock, C. F. Yocum, *FEBS Letters*, **1981**, 134, 231–234.
[21] K. Nakazato, C. Toyoshima, I. Enami, Y. Inoue, *J. Mol. Biol.*, **1996**, 257, 225–232.
[22] W. Wang, J. Chen, C. Li, W. Tian, *Nat. Commun.*, **2014**, 5, 4647.
[23] M. Nemecek-Marshall, R. C. MacDonald, J. J. Franzen, C. L. Wojciechowski, R. Fall, *Plant Physiol.*, **1995**, 108, 1359–1368.
[24] R. MacDonald, R. Fall, *Atmos. Environ.*, **1993**, 28, 1709–1713.
[25] R. Fall, A. A. Benson, *Trends Plant Sci.*, **1996**, 1, 296–301.
[26] Y. L. Dorokhov, E. V. Sheshukova, T. V. Komarova, *Front. Plant Sci.*, **2018**, 9, 1623.
[27] C. Frenkel, J. S. Peters, D. M. Tieman, M. E. Tiznado, A. K. Handa, *J. Biolog. Chem.*, **1998**, 273, 4293–4295.
[28] G. Govindjee, D. Shevela, L. O. Björn, *Photosynth. Res.*, **2017**, 133, 5–15.
[29] M. J. West-Eberhard, J. A. C. Smith, K. Winter, *Science*, **2011**, 332, 311–312.
[30] M. Calvin, A. A. Benson, *Science*, **1948**, 107, 476–480.

Graphical Abstract

Chin. J. Catal., 2023, 44: 117–126 doi: 10.1016/S1872-2067(22)64170-6

Light-driven CO_2 assimilation by photosystem II and its relation to photosynthesis

Yuehui Li, Duanhui Si, Wangyin Wang *, Song Xue, Wenzhe Shang, Zhanyou Chi, Can Li, Ce Hao *, Govindjee Govindjee, Yantao Shi *
Dalian University of Technology, China; Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China; University of Illinois at Urbana-Champaign, USA



An unusual light-driven CO_2 assimilation by the PSII core complex in photosynthesis is found, which is likely to be a simultaneous event along with the normal CO_2 assimilation taking place in dark reaction.

[31] M. D. Hatch, C. R. Slack, *Biochem. J.*, **1966**, 101, 103–111.
[32] K. Winter, J. A. C. Smith, Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution, Springer-Verlag, Berlin, **1996**, 1.
[33] M. Yao, Y. Liu, L. Fei, Y. Zhou, F. Wang, J. Chen, *J. Phys. Chem. B*, **2018**, 122, 10478–10489.
[34] D. J. Vinyard, G. M. Ananyev, G. C. Dismukes, *Annu. Rev. Biochem.*, **2013**, 82, 577–606.
[35] H. Dau, I. Zaharieva, *Acc. Chem. Res.*, **2009**, 42, 1861–1870.
[36] A. M. Nonomura, A. A. Benson, *Proc. Natl. Acad. Sci. U. S. A.*, **1992**, 89, 9794–9798.
[37] R. N. Rowe, D. J. Farr, B. A. J. Richards, *New Zealand J. Crop Horticul. Sci.*, **1994**, 22, 335–337.
[38] R. M. Devlin, P. C. Bhowmik, S. J. Karczmarczyk, *PGRSA Quart.*, **1994**, 22, 102–108.
[39] L. Panella, J. N. Nishio, S. S. Martin, *J. Sugarbeet Res.*, **2000**, 37, 55–72.
[40] R. Willstätter, A. Stoll, *Untersuchungen über die Assimilation der Kohlensäure*, Springer-Verlag. Berlin, **1918**.
[41] J. Franck, K. F. Herzfeld, *J. Phys. Chem.*, **1941**, 45, 978–1025.
[42] O. Warburg, *Annu. Rev. Biochem.*, **1964**, 33, 1–14.
[43] Y. Wu, *Acta Geochim.*, **2021**, 40, 650–658.
[44] E. Gout, S. Aubert, R. Bligny, F. Rebeille, A. R. Nonomura, A. A. Benson, R. Douce, *Plant Physiology*, **2000**, 123, 287–296.

光系统II光驱动CO_2同化的光合作用

李跃辉[a,1], 司端惠[a,1], 王旺银[b,*], 薛　松[c], 商文喆[a], 迟占有[c], 李　灿[b], 郝　策[a,*], Govindjee Govindjee [d], 史彦涛[a,*]

[a]大连理工大学化工学院化学系, 智能材料化工前沿科学中心, 精细化工国家重点实验室, 辽宁大连116024, 中国
[b]中国科学院大连化学物理研究所, 催化基础国家重点实验室, 洁净能源国家实验室(筹), 辽宁大连116023, 中国
[c]大连理工大学生物工程学院, 辽宁大连116023, 中国
[d]伊利诺伊大学厄巴纳香槟分校, 生物化学系, 植物生物学系和生物物理学与定量生物学中心, 伊利诺伊州, 美国

摘要: 光合作用作为地球上最重要的化学反应, 是一切生命活动赖以生存的基础. 光合作用分为光反应和暗反应两个阶段. 通常认为, 光反应阶段产生O_2, 暗反应阶段CO_2被还原(也称"CO_2同化"). 尽管这一观点已被公众所熟知, 但也存在诸多疑点, 一些科学家(包括1931年的诺贝尔生理学或医学奖得主Otto Warburg)认为, CO_2也可能在光反应阶段作为反应底物参与了产氧并被还原. 然而, 该观点至今没有在实验上获得充足的证据支持. 那么, 在光反应阶段是否能够进行CO_2同化? 如果能够发生, 产物和机理是什么? 毫无疑问, 这些科学问题具有十分重要的研究价值, 对这些问题的探索能帮助我们更加充分认识光合作用机制. 然而, 自上世纪十年代以来, 相关研究已陷入停滞状态.

为了解开光合作用领域的这个重要科学谜团, 即在光合作用中CO_2是否能通过光反应被还原, 本文选取三类不同层次的光合作用体系(小球藻、叶绿体、PSII中心复合体)为研究对象, 结合原位质谱、气相色谱和同位素标记等手段, 设计了一系列实验, 排除了呼吸作用和其它因素干扰, 实验发现在光反应阶段PSII中心复合体不但产生O_2, 还能产生C1化合物CH_3OH. $^{13}CO_2$和$C^{18}O_2$标记实验结果表明, CH_3OH来源于CO_2光还原, 排除了CH_3OH来自于光呼吸或细胞壁果胶脱甲基分解的可能. 说明光合作用光反应阶段能够进行CO_2还原, 反应场所是PSII中心复合体, 这与CO_2的同化只能发生在暗反应阶段的传统观点相矛盾. 因此, 除了非光依赖性CO_2同化这一已知路径外, 还有一条未知的光驱动CO_2同化路径. 进一步推测, 这种CO_2光还原路径可能与暗反应下的CO_2同化同时进行. 目前, 对这种光驱动下CO_2同化机制仍需进一步深入研究. 综上, 本文丰富了人们对光合作用机理以及CO_2同化路径的认知, 并为长期以来存在争议的CH_3OH来源问题提供了新解释.

关键词: 光合作用; PSII中心复合体; 光反应; CO_2同化; 甲醇

收稿日期: 2022-07-10. 接受日期: 2022-09-18. 上网时间: 2022-12-10.

*通讯联系人. 电子信箱: shiyantao@dlut.edu.cn (史彦涛), haoce@dlut.edu.cn (郝策), wywang@dicp.ac.cn (王旺银).
[1]共同第一作者.

基金来源: 国家自然科学基金(51773025, 21677029, 22088102); 辽宁省兴辽英才青年拔尖人才项目(XLYC2007038, XLYC2008032); 中央高校基础研究经费(DUT22LAB602).