

Supporting Information

Bioluminescence as Light Source for Photosynthesis

Huanxiang Yuan, Libing Liu*, Fengting Lv and Shu Wang*

Experimental Section

Materials and Measurements: All chemicals were purchased from Acros, Aldrich Chemical Company or Alfa-Aesar and used as received. Geranium was used for the photosynthesis experiments. Spinach was purchased from market. UV-Vis absorption spectra were taken on a JASCO V-550 spectrophotometer. Fluorescence spectra were measured on a Hitachi F-4500 fluorometer equipped with a xenon lamp excitation source.

Spectral measurements for luminol bioluminescence: 1 μL of 1mg/mL HRP, 20 μL of 1 mg/mL enhancer, and 100 μL of 20 mM luminol were added into 679 μL of carbonate buffer (pH 9.0). Vortex the mixture for 5 s, and 200 μL of 50 mM hydrogenperoxide was added. Luminescence intensity changes at 425 nm were recorded. The luminescence could last more than one hour.

Photosynthesis of *Geranium* under luminol bioluminescence: Put a pot of *Geranium* into a dark room overnight to consume the starch in the leaves before the photosynthesis experiments. Choose four pieces of leaves with the same area. Take

one leaf as the control group and other three as experimental groups. The three experimental leaves were soaked in the Petri dishes filled with 20 mL of carbonate buffer (pH 9.0), 600 μ L of 1 mg/mL enhancer, 3 mL of 20 mM luminol, 6 mL of 50 mM hydrogenperoxode, and 30 μ L of 1mg/mL HRP; and 600 μ L of 1 mg/mL enhancer, 3 mL of 20 mM luminol, and 6 mL of 50 mM hydrogen peroxide were added into the Petri dishes every hour to make the luminescence last six hours. The diameter of the Petri dishes was 90 mm. As for the case of control group, the leaf was soaked in the Petri dish filled with only 20 mL of carbonate buffer (pH 9.0). The photosynthesis experiments of the *Geranium* leaves soaked in higher substrates concentrations solutions which provided brighter light were performed, where 5 mg/mL enhancer, 100 mM luminol, 250 mM hydrogen peroxide and 10 mg/mL enhancer, 200 mM luminol, 500 mM hydrogen peroxide were used respectively. All the operations were performed in the dark.

Starch measurement after the photosynthesis of *Geranium* under luminol bioluminescence: The experimental leaves were picked after irradiation of the luminol bioluminescence system. Washed leaves were cut into pieces and then were ground with a mortar and pestle in 80% ethanol. The suspension was centrifuged for 2 min at 4000 rpm to discard the supernatant. The sediment was washed twice by water. 80% $\text{Ca}(\text{NO}_3)_2$ was added to resuspend the sediment and then put the specimens into boiling water for ten minutes. The specimens were centrifuged for 4 min at 6000 rpm to collect the supernatant. Repeat the boiling-step twice and collect the supernatant.

To 2 mL of the extracted starch solution in a spectrophotometer cuvette was added 100 μ L of I₂-KI solution (2 g of KI and 0.2 g of I₂ dissolved in 100 mL of distilled water). The absorbance recorded at 620 nm after 20 min. The starch content ratio was calculated according to the following equation:

$$R = \frac{A}{A_0}$$

Where A is the absorbance of the experimental groups with irradiation of luminol bioluminescence system and A₀ is the absorbance of the control group treated in dark.

Chloroplasts isolation and chlorophyll determination: Intact chloroplasts were prepared from market spinach leaves (*Sponacea oleracea* L). Fresh leaves were cleaned and cut to pieces about 1 cm². These were then ground with a mortar and pestle in buffer containing 0.01 M tris-HCl at pH 8.0, 0.04 M sucrose, and 0.01 M NaCl. The suspension was filtered through four layers of gauze, and centrifuged for 1 minute at 1000 rpm to remove the cell debris. The supernatant was centrifuged at 3000 rpm for 3 minutes, and the precipitate was collected and resuspended with the above buffer. All chloroplast preparations were carried out at temperatures between 0 and 4 °C. To 4.9 mL of acetone was added 0.1 mL of chloroplast suspension and intensively mixed. The suspension was centrifuged for 2 minutes at 3000 rpm. Collect the supernatant to measure its absorbance at 652 nm. The chlorophyll concentration (mg chlorophyll/mL chloroplast suspension) was calculated according to the following equation:¹

$$C = \frac{OD_{652} \times 50}{34.5}$$

Where C is the chlorophyll concentration and OD₆₅₂ is the absorbance of supernatant at 652 nm.

Effect of the chloroplasts irradiated by luminol bioluminescence on Hill reaction

measurement: The photochemical activity of the chloroplasts was determined by measuring the rate of reduction of potassium ferricyanide. The ferricyanide was prepared immediately before use in distilled water. 10 mL of Hill reaction solution contained 0.01 M tris-HCl at pH 8.0, 0.02 M KFe(CN)₆, 0.01 M NaCl, 0.01 M MgCl₂, and 20 µg/mL chlorophyll. To two spectrophotometer cuvettes were both added 2 mL of Hill reaction solution, one as the control in the dark without illumination and the other as the illuminated group with luminol bioluminescence. The absorbance at 420 nm was recorded before illumination. The luminol bioluminescence solution contained 0.03 mg/mL enhancer, 3 mM luminol, 15 mM hydrogen peroxide, and 0.0015 mg/mL HRP. Put the illuminated cuvette in the luminol bioluminescence solution in the dark room and the absorbance changes at 420 nm were recorded every 30 minutes.

References

1. S. Saha, R. Ouitaku, S. Izawa, N. E. Good, *J. Biol. Chem.* **1971**, 246, 3204.