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A semi-artificial model of photosynthetic oxygen evolution and $NADP^+$ reduction using thylakoid membrane proteins and a semiconductor

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Introduction

In photosynthesis solar energy is converted into chemical free energy. The process produces oxygen and organic compounds indispensable for maintenance of all life on earth. The most attractive feature of this process from the point of view of human exploitation is its raw material: photosynthesis utilizes the cheapest and most easily available starting materials, i.e., water and air. Oxidation of water results in the evolution of molecular oxygen and a release of electrons and protons from water as expressed in following formula:

$$2H_2O \xrightarrow[\text{Chloroplast}]{h\nu} O_2 + 4H^+ + 4e^-$$

This is a basis for creating a hydrogen resource.

Because of the need for environmental-friendly energy, much research on photosynthesis has been conducted during recent decades. The mechanism of photosynthesis is becoming understood [1]. Also the construction of artificial photosynthesis systems has become feasable [2]. The water oxidation step is among the least understood, although many models of the partial reactions related to it have been proposed. The main reason for the lack of success in mimicking water oxidation is that the reaction is catalyzed by a complicated molecular assembly and the reaction involves multiple events of photons, electrons and holes. The molecular machinery consists of, among other units, a Mn-protein complex which collects and stores four oxidising equivalents (i.e., holes) formed by the photochemical events in the photosynthetic reaction centre. This four-electron chemistry needed for water splitting would not be possible without the Mn-protein complex.

The idea in the present work is to disassemble the thylakoid protein complexes into small parts and to find the smallest assembly of thylakoid proteins which will play the same role as the Mn-protein complex, in order to mimic photosynthetic electron transfer, aiming at the ultimate achievement of artificial oxygen evolution reaction by using a minimum macinery and an artificial semiconductor as photosensitizer. We started this work with $NADP^+$ photoreduction and preliminary results are presented.

Design of the present model

Water oxidation in photosynthesis involves two necessary steps: the charge separation and the charge accumulation. The former is fulfilled by (1) a light-harvesting pigment-protein antenna which absorbs photons and converts light energy into excitation energy (molecular excitons), and (2) transfer of the excitation energy to chlorophyll-a (designated as P680) in the reaction centre of photosystem II of chloroplasts. An electron is lost from an occupied orbital of the donor molecule (P680) and transferred to an unoccupied orbital of a primary acceptor molecule, pheophytin (Pheo) in the reaction centre. Thereby the excitation energy is converted into electrochemical free energy. This is charge separation step (see Scheme 1 in Fig 1).

Pheophytin, the primary electron acceptor in the reaction centre, then hands over its electron to later electron acceptors, such as, QA, QB and a series of electron carriers, and finally to $NADP^+$, forming NADPH, while $P680^+$ receives one electron per absorbed photon from the Mn-protein complex through Tyrosine-z of polypeptide D1. This electron donation to $P680^+$ must occur four times and four holes are thus accumulated in the complex, enabling the oxidation of two molecules of water with the release of one oxygen molecule as shown in the scheme. Thus, the charge accumulation step is finished [1] (see Scheme 1 in Fig 1).

The attempt will be made in such a manner that the charge separation step will mainly be mimicked through the photocatalytic properties of semiconductors while the charge accumulation step will be reconstructed by thylakoid membrane proteins disassembled from Photosystem II complex. The general idea is illustrated by Scheme 2 in Fig 1.

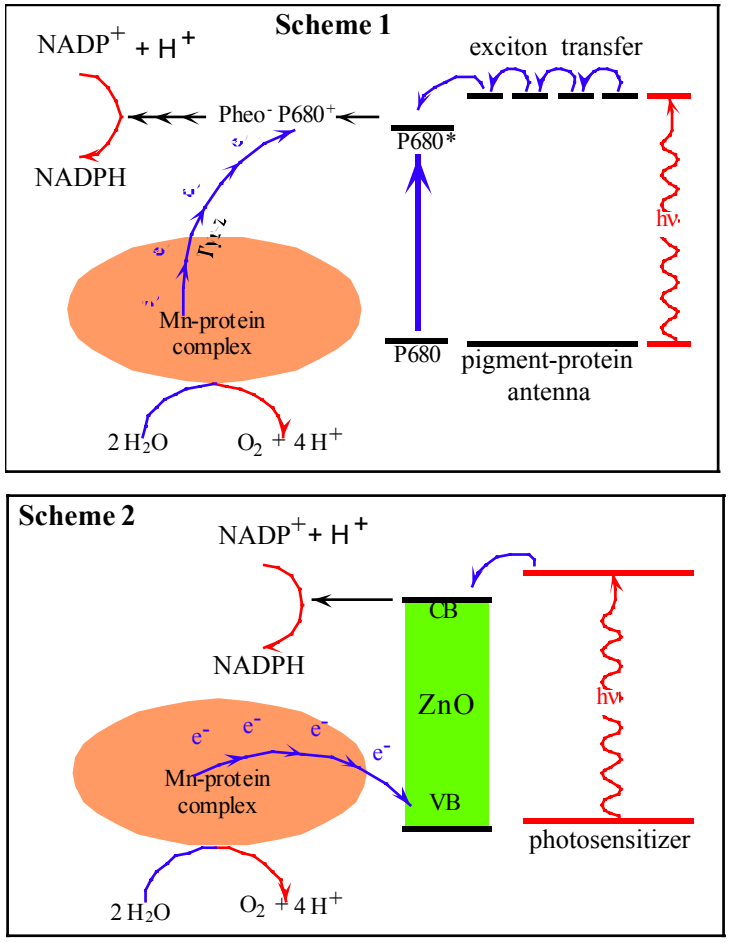


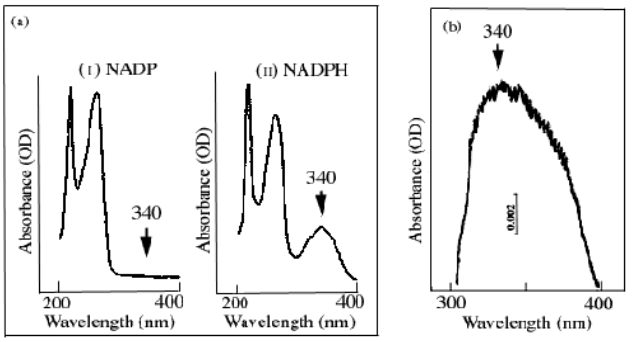
Figure 1. Scheme of the present model. For details see text.

Results and discussion

[1] Electron transfer from H_2O to $NADP^+$ with semiconductors

The essence of the light-driven reaction is the conversion of a very transient form of redox energy into energy carried by two more stable energy carriers (ATP and NADPH). NADPH is a final product of the light reaction, generated through a series of electron transfer steps.

We have mimicked this fundamental reaction of $NADP^+$ reduction by using an inorganic semiconductor, ZnO. The experimental details are shown in Figure 2.



[a] Increase of absorbance at 340 nm as a measure of NADP $^+$reduction.
[b] Absorbance increase at 340 nm for the sample which is composed of the semiconductor powder, ZnO, and $NADP^+$ in the Tris buffer, pH 8.8, after the illumination of UV-light from the deuterium lamp for 1 h.

Figure 2. Spectrophotometric determination of $NADP^+$ reduction using ZnO instead of chloroplasts. The result is shown in Figure 1b. The reaction medium is composed of 30 mM Tris pH 8.8, 1.3 mM $NADP^+$, and 30 mg ZnO in a total volume of 1 ml.

[2] Acceleration of $NADP^+$ reduction by chloroplast membrane proteins.

The above experiments were repeated using not only the semiconductor, ZnO, but also a protein mixture obtained from thylakoid membranes of spinach. It shows a remarkable effect of thylakoid proteins on the rate of $NADP^+$ reduction (Figures 3 and 4).

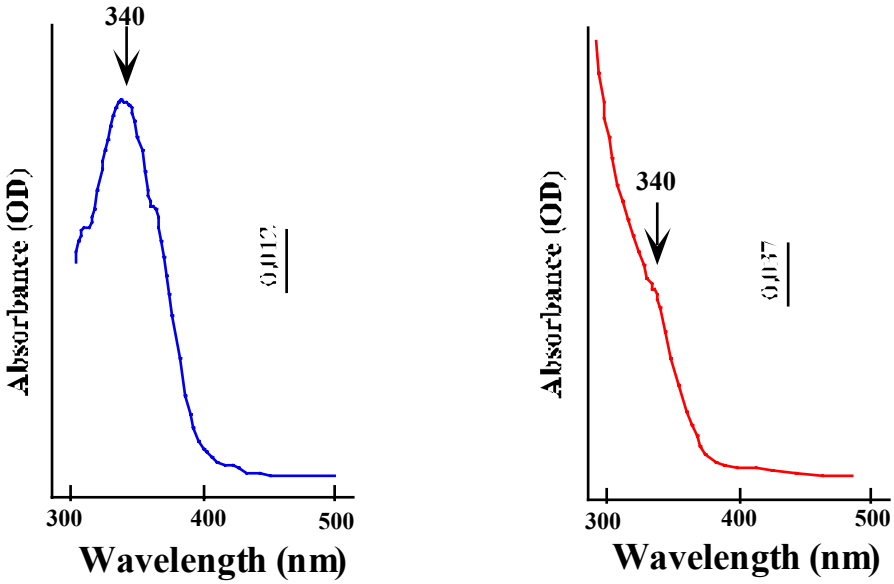


Figure 3. Photoreduction of $NADP^+$ in the presence of thylakoid proteins (blue) and in the absence of the proteins (red). The increase of absorbance at 340 nm is a measure of $NADP^+$ reduction. The irradiation was for 30 min from a deuterium lamp (Philips type 126138). The reaction medium is composed of ZnO (15 mg/ml), Tris buffer pH 8.0 (30 mM), $NADP^+$ (1.3 mM), with or without thylakoid proteins (2 mg/ml). Other conditions as in Figure 2.

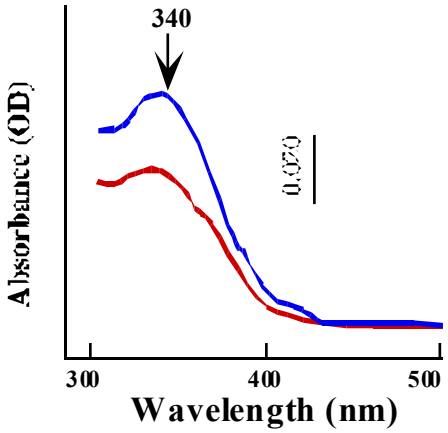


Figure 4.
Photoreduction of $NADP^+$ in the presence of thylakoid proteins (blue) and in the absence of the proteins (red). The experimental conditions were the same as those in Figure 3 except that: laser flashes of 266 nm wavelength (35 mJ/flash/cm_2. 10 flashes/s) were used for 2 min and the concentration of ZnO was 0.5 mg/ml.

We attribute the accelerating effect of the membrane proteins on the electron transfer to the interaction of proteins with the semiconductor. In general, photochemical reactions are driven by absorbed photons, and photoexcitation of an electron donor (D) or an acceptor (A) results in an increase in free energy. The interaction between proteins and semiconductors may stabilize the radical ions formed by photoexcitation and accelerate the rate of photoinduced electron transfer.

Materials and Methods

Chloroplasts were prepared from spinach (*Spinacia oleracea* L., cv. Medania) leaves as previously described (3).Pigment-free thylakoid membrane proteins were isolated from chloroplasts according to the procedure described earlier (4).

The experiment was carried out at room temperature. In order to mix the semiconductor thoroughly with the proteins, the sample was sonicated six times for 30 s (before adding $NADP^+$) using a Branson apparatus equipped with a 1/2-inch tip. The sample was divided into two parts: one subjected to radiation from a deuterium lamp (30 min, about 1 µmol m^{-2}. s^{-1}) with continuous stirring, and another one kept in the dark under the same conditions. After this incubation both sample parts were immediately centrifuged, supernatants were collected and differential spectra of the supernatants in the range 300-400 nm were measured in a Shimadzu UV-3000 spectrophotometer.

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