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Photobioelectronic Studies with Thylakoid Membranes

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ABSTRACT

The research described in this paper presents a method for chemically modifying the surface of plant photosynthetic membranes in such a way that electrical contact can be made. Colloidal platinum was prepared, precipitated directly onto thylakoid membranes from aqueous solution, and entrapped on fiberglass filter paper. This composition of matter was capable of sustained simultaneous photoevolution of hydrogen and oxygen when irradiated at any wavelength (400–700 nm) in the chlorophyll absorption spectrum. Experimental data support the interpretation that part of the platinum metal catalyst is precipitated adjacent to the photosystem-I reduction site of photosynthesis and that electron transfer occurs across the interface between photosystem I and the catalyst. When contacted with metal electrodes, the thylakoid-platinum combination was capable of generating a sustained flow of current through an external load resistor. Procedures for preparing this material and experimental data on its catalytic and electronic properties are presented. Also presented is an analysis of the flow of photocurrent in terms of the interfacial electron transfer reactions that occur at the interfaces of the components of the assembly.

Index Entries: Biomolecular electronics; photocurrents; platinum; chloroplast; electron transport; contact.

INTRODUCTION

Embedded in the thylakoid membranes of all higher plant tissue are specialized photochemical reaction centers that are the location of the primary electron transfer reactions of photosynthesis. On absorption of light, these reaction centers set in motion the movement of electrons that are derived from water and eventually enter the molecules of carbon dioxide fixation compounds.

Unlike metals and semiconductors, whose electronic properties are determined by bulk properties of matter, electron flow in the photosynthetic electron-transport chain takes place between discreet molecular entities. This novel method of electron flow and its occurrence in a thin membrane provide for a highly versatile system of charge separation and energy transduction. One example of this versatility is the differential affinity of some discreet membrane components for protons—depending on the presence or absence of an electron on the molecule. The ability to bind and release protons by plastoquinone results in the coupled translocation of protons across the photosynthesis membrane with the flow of electrons within the membrane. Under the influence of light, the flow of electric current in photosynthetic membranes occurs in two mutually orthogonal directions and with different charge carriers. The two currents are normally linked by the molecular nature of the particular electron transport chain. It is possible, however, to uncouple the electron flow from the proton translocation.

In normal photosynthesis, electrons emerge at the surface of the membrane and are used to reduce $NADP^+$ to NADPH, the electron carrier for the Calvin cycle, in which carbon dioxide is enzymatically reduced to a carbon dioxide-fixation compound. The unique feature of the research described in this paper is the direct electrical contact with emergent electrons and the utilization these electrons in two ways: for the electrosynthesis of molecular hydrogen, and for the flow of electrical current in an external circuit.

MATERIALS AND METHODS

Type C chloroplasts were prepared according to the procedure described by Reeves and Hall (*1*). In this preparation, the chloroplast envelope is osmotically ruptured, exposing the thylakoid membranes to the external aqueous medium. A solution of chloroplatinic acid (5.34 mg/mL), at pH 7 was prepared. One mL of this solution was combined with 5 mL of chloroplast suspension (containing 3 mg of chlorophyll) in Walker's assay medium (*2*). The 6-mL volume was placed in a temperature-controlled, water-jacketed chamber fitted with O-ring connectors to provide a hermetic seal and with inlet and outlet ports for hydrogen flow. The mixture was gently stirred and purged with molecular hydrogen in the headspace above the liquid. The temperature of the sample was maintained at 21°C.

In the platinum precipitation step, it was determined empirically that a hydrogen incubation time of ~30 min was needed to obtain photoactive material. Times of 60–90 min typically were used. After incubation, the

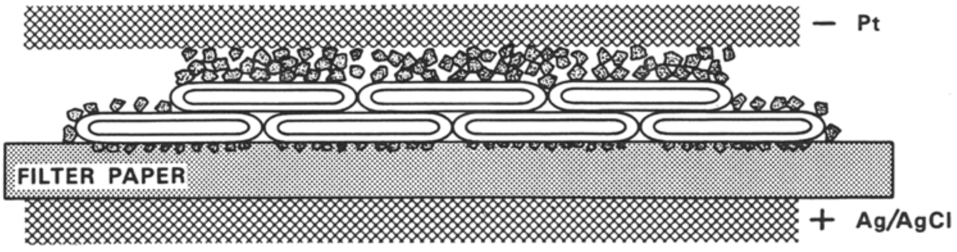

Fig. 1. Schematic illustration of the entrapped platinized chloroplast-electrode assembly. Based on the data presented in the text, it is believed that the platinum gauze electrode on the chloroplast side makes pressure contact with the reducing end of Photosystem I via the platinum colloid particles that are formed by *in situ* precipitation. The counter electrode, is in contact with the electrolyte-impregnated filter paper. Both silver and platinum gauze screens were used for these electrodes.

reactor chamber was opened to air and the contents were filtered onto fiberglass filter paper (Millipore, AP40). The platinum precipitation reaction had a marked effect on filtration properties. Control experiments without hydrogen incubation produced a chloroplast mixture that filtered immediately, whereas the platinized chloroplasts required a considerably longer time—typically, 5–30 min. Also, the platinized chloroplasts were dark green, as opposed to the normal bright green of higher plant chloroplasts. The presence of insoluble platinum on the platinized-chloroplast filter paper composition was determined by X-ray fluorescence analysis after the filter paper was rinsed in 2 L of continuously stirred distilled water for 1 h. Insoluble platinum was positively identified in this way for platinized chloroplasts prepared by precipitation from either $[Pt(Cl)_6]^{2-}$ or $[Pt(NH_3)_4]^{2+}$. However, only $[Pt(Cl)_6]^{2-}$ yielded photoactive material.

Electrical contact with the platinized chloroplasts was achieved by contacting the surface of the filter paper-entrapped material with a fine-mesh platinum gauze. A fine-mesh silver gauze, placed on the opposite side of the filter paper, was used as the counter electrode. All of the components were held in close proximity in a lucite sandwich-like assembly with nylon compression screws. Platinum lead wires were used to make electrical contact with the platinum and silver gauzes. These leads were connected to the input of an appropriate digital multimeter for current and voltage measurements. All electrical and gas evolution measurements were performed in a helium atmosphere. Figure 1 is a schematic illustration of the entrapped platinized chloroplast electrode assembly. Based on the data presented below, it is believed that the platinum gauze electrode on the chloroplast side makes pressure contact with the reducing end of the photosystem I via the platinum colloid particles that are formed by *in situ* precipitation. The counter-electrode, is in contact with the electrolyte-impregnated filter paper. Both silver and platinum gauze screens were used for these electrodes.

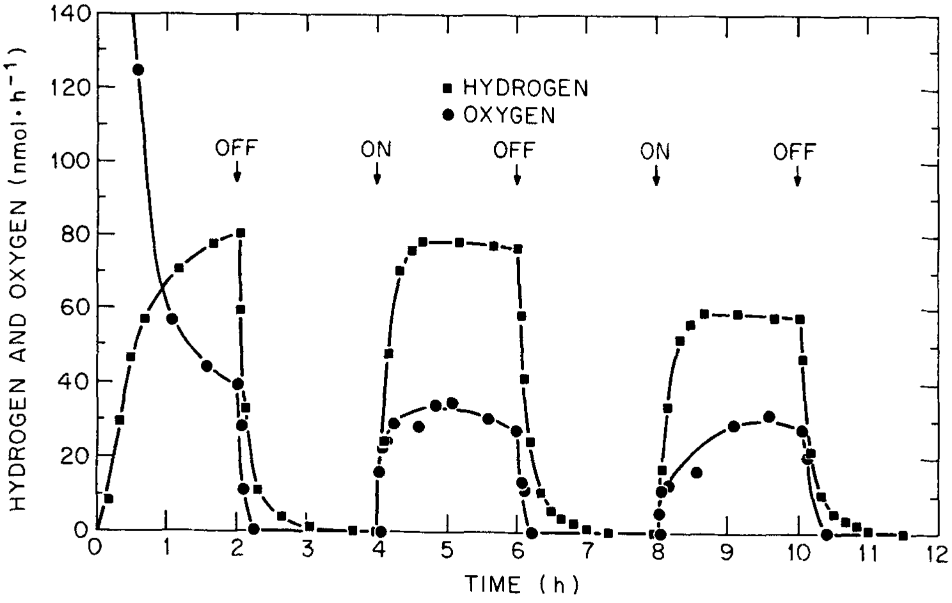

Fig. 2. Simultaneous photoproduction of hydrogen and oxygen by moistened platinized chloroplasts entrapped on filter paper. The ELH projector lamp, providing saturating illumination was turned on at T = 0 and alternated with 2-h on-off cycles, as indicated. The peak oxygen rate was 7 μmol/h. The area of the disk was 10.2 cm^2. The activity of the platinized chloroplasts was still measurable after 2–3 d of irradiation cycles at 10–20% of the initial rates (*from* ref. 3).

RESULTS AND DISCUSSION

Gas Evolution Measurements

In the work reported here, colloidal platinum was precipitated directly onto photosynthetic membranes in an aqueous suspension. The resulting chloroplast-colloidal platinum composition was then entrapped on filter paper. As indicated in Fig. 2, this moistened material was capable of sustained simultaneous photoevolution of hydrogen and oxygen when irradiated with visible light. This experiment, performed without electrodes, has been reported previously (3). Since no electron relay was added to the system and the overall reactions occurred in an immobilized matrix, it was concluded that the precipitated colloidal platinum directly contacts the reducing end of photosystem I in such a way that electron flow occurs across the biological membrane-metal-colloid interface with preservation of charge continuity and catalytic activity. In addition to the special photocatalytic properties of platinized chloroplasts, the specific ionic species used to prepare the material yield information on the physicochemical properties of the photosystem I reduction site on the thylakoid membrane surface. The data in Fig. 2 were obtained by preciptiating platinum from the hexachloroplatinate(IV) ion, $[Pt(Cl)_6]^{2-}$. Platinized chloroplasts prepared by precipitating platinum from the tetraamineplatinum(II) ion, $[Pt(NH_3)_4]^{2+}$, resulted in no hydrogen activity and only a transient oxygen gush. The presence of insoluble platinum in the filter paper-entrapped composition was determined by X-ray fluorescence analysis.

The time profiles of photoactivity are presented in Fig. 2 for three light-dark cycles of 2 h each. Figure 2 shows that the first irradiation period has a time profile that is qualitatively different from those of the subsequent two periods. In the first cycle, the oxygen profile underwent a transient gush (peaking at 7 nmole, nanomole of O_2/h) before settling down to steady state. The hydrogen rate climbed monotonically to steady state. The time required to reach 50% of steady state in the first cycle was ~25 min, whereas the corresponding times for the second and third cycles were 5–8 min, respectively.

These patterns may be explained as follows. The initial transient oxygen gush represents the filling of reducible pools on the reducing side of photosystem II. Molecular oxygen is capable of oxidizing the components in the electron transport chain linking the two light reactions of photosynthesis, including the plastoquinone pool (4). The platinized chloroplasts were exposed to air during the filtration process and during the time interval before insertion in the reaction chamber of the apparatus used for measuring photoactivity (3). Therefore, the initial oxygen gush is believed to represent the filling of the oxidized pool as well as other reducible species in the preparation.

As indicated above, the time for the hydrogen evolution rate to reach steady state was longer for the first interval of irradiation than for the subsequent two intervals, whose time profiles were determined by the response time of the apparatus.* Since the hydrogen-evolving catalyst in this preparation was elemental platinum, the observed kinetics of the first irradiation interval was interpreted to represent the reduction of the oxide layer formed on the metallic platinum during the period when the platinized chloroplasts are exposed to air, during insertion into the reaction chamber. Because the platinized chloroplasts remained anaerobic for the two subsequent irradiations, neither oxygen transients nor hydrogen delays were observed.

A novel aspect of this material is the absence of an added electron relay (such as ferredoxin or methyl viologen) to transport electrons from the reducing end of photosystem I to the hydrogen-evolving catalyst. Two control experiments were performed that emphasize this point. In the first experiment, the order used in adding chloroplasts and precipitation was reversed. That is, the colloidal platinum was prepared in the absence of chloroplasts (by precipitation from $[Pt(Cl)_6]^{2-}$, and then the chloroplasts were added to the aqueous phase. This mixture was then entrapped on fiberglass filter paper. In spite of the physical contact between

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*The response time of the apparatus was determined by step functions of hydrogen and oxygen provided by the electrolysis cell and a constant current source.

platinum colloid particles and chloroplasts, no photocatalytic activity was observed, except for a brief oxygen transient.

In the second experiment, the filtration step was omitted. The 6-mL aqueous suspension of platinized chloroplasts was stirred in the photoreactor of the assay system with a Teflon stirrer. Hydrogen and oxygen photoproduced in the aqueous phase equilibrated with the carrier gas in the headspace above the liquid. The hydrogen rate peaked at 550 min and then decayed monotonically with a first half-life of 5110 min, a time significantly shorter than the corresponding value for the filter paper-entrapped platinized chloroplasts of Fig. 2. The loss of activity was presumably associated with the separation of chloroplast and platinum colloid particles at the photosystem-I reducing site.

Electrical Measurements

The simultaneous photoevolution of hydrogen and oxygen strongly suggests that the chemically precipitated platinum physically contacts the reducing side of photosystem I on the thylakoid membrane surface. Since considerably more platinum was precipitated than is necessary for catalytic activity, electrical contact with the photosynthetic election transport chain can be achieved by pressing the platinized chloroplast-filter paper composition against a gauze electrode. The data of Fig. 3, showing the short-circuit photocurrent for consecutive ON-OFF light cycles, indicate that this is indeed possible.

There are several noteworthy aspects of the data in Fig. 3. First, the platinized chloroplasts that give rise to the photocurrent are unoriented and presumably electrically noncontiguous. This implies that only a fraction of the functional chloroplasts actually contribute to the observed photocurrent. The schematic illustration in Fig. 4 suggests that, although the chloroplasts are unoriented, the platinum is precipitated on the external surface of the thylakoid membranes. This external surface also corresponds to the reducing end of photosystem I. The network of colloidal platinum, platinized chloroplasts, and platinum gauze forms an isopotential surface that gives rise to the directionality of current shown in Fig. 3.

Note also that the sign of current flow indicates that electrons flow from the platinum electrode to the silver/silver chloride electrode through the external circuit. This directionality of current flow agrees with the generally accepted vectorial model of photosynthesis, in which the external surface of the thylakoid membrane is driven to negative potentials under the influence of light.

The current-vs-time profile of the first cycle shown in Fig. 3 is qualitatively different from those of the second and third cycles. The relatively slow increase of current in the first cycle is interpreted as meaning that a major fraction of the electric current is performing electrochemical processes. When the light is turned on the second and third times, the

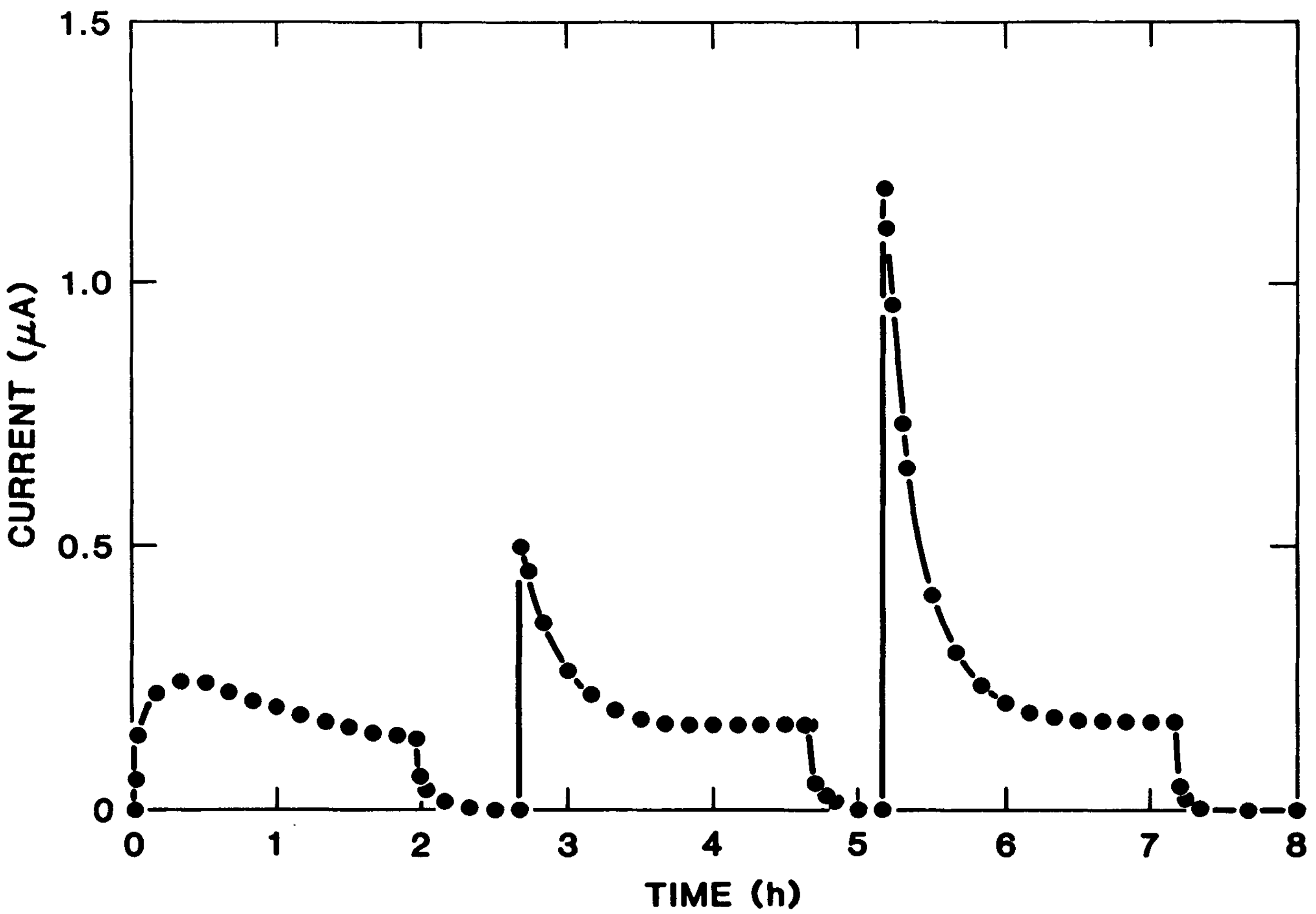

Fig. 3. Plots of the short-circuited photocurrent generated by the entrapped platinized chloroplasts contained in the compression assembly of Fig. 1. The transients observed at the beginning of the second and third photocycles represent a capacitative interfacial charging. The steady-state, direct current component eventually reached in each cycle is interpreted as photocurrent generated by the light reactions of photosynthesis flowing in an external circuit maintained by the electrical contacts within the compression assembly.

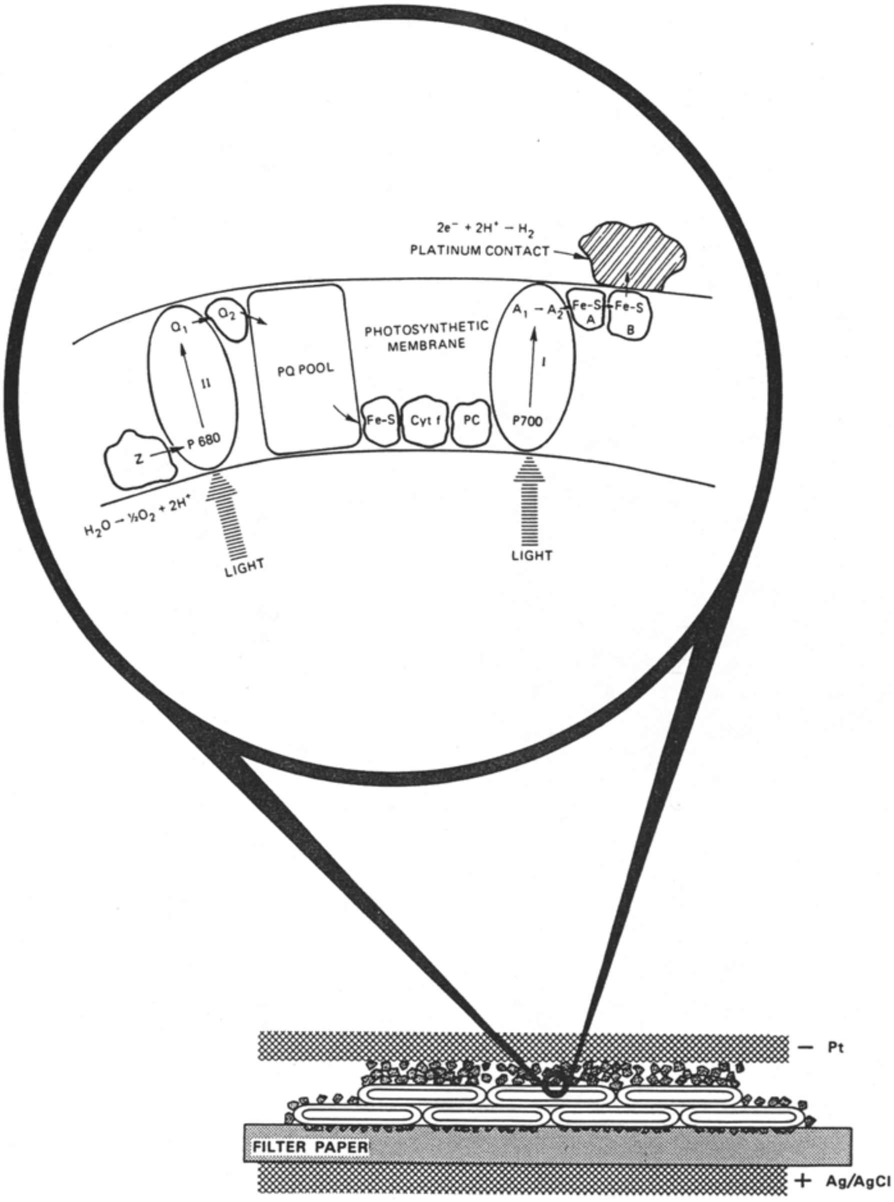

Fig. 4. Illustration detail of outer membrane interface. Colloidal platinum is precipitated onto the external surface of the photosynthetic membranes. For clarity of illustration, only one platinum colloid particle is shown in contact with the reducing end of Photosystem I.

current-vs-time profile shows two distinct features: a capacitative, interfacial-charging component and a long-term, steady-state, direct current (DC) component. The presence of this long-term DC component demonstrates electrical contact with the electron transport chain of photosynthesis.

The flow of photocurrent can be understood as follows. In Fig. 4, electrons that emerge from the outer surface of the photosynthetic membrane make electrical contact with the precipitated platinum. A certain fraction of these platinum colloid particles make direct metal-to-metal contact with the platinum gauze electrode in the compression assembly. The direction of flow of photocurrent indicates that electrons flow from the platinum gauze electrode to the silver/silver chloride electrode. As indicated in Fig. 5, the following reaction takes place at the silver/silver chloride electrode: $AgCl + e^- \rightleftharpoons Ag + Cl^-$. Movement of chloride ions takes place in the potassium chloride solution impregnated filter paper because of the local positive electrical potential, as indicated in Fig. 6, that is generated in the intrathylakoid space of the photosynthetic membranes. It is well known that biological membranes are permeable to chloride ions.

The electrical measurements performed in this paper represent a new approach to the successful work of earlier researchers. For example, Barsky et al. (*5*) measured electrical responses with a proteoliposome-photosystem I system. Haehnel et al. (*6*) have described a light-driven galvanic cell that was constructed making use of the photosynthetic activity of isolated chloroplasts. In this aqueous-phase system, chemical mediators were used to transfer electrons from the chloroplasts to the platinum electrodes in each of the joined half-cells. Ochiai et al. (*7*) have reported anodic photocurrents using entrapped chloroplast electron transport systems with polyvinyl alcohol. It is clear from these early studies, as well as from subsequent research (*8–13*), that the interaction of chloroplasts and subcomponents with external electrodes can be achieved in a variety of ways. Of particular interest is the work of Trissl and Kunze (*14*), who constructed a capacitative cuvet for measuring the primary electrogenic reactions in chloroplasts by picosecond flash-induced dielectric polarization. In their work, the photovoltaic signal originated from the combination of the light gradient in the chloroplast suspension and the anisotropic excitation from the primary charge separation in the reaction centers of the photosystems.

CONCLUSIONS

This paper describes recent research focusing on a new method for chemically modifying the surface of thylakoid membranes and making electrical contact with the electron transport chain of photosynthesis. The primary light reaction occurs in a molecular structure that is 5–10 nm in size. Moreover, the characteristic switching time of the primary process

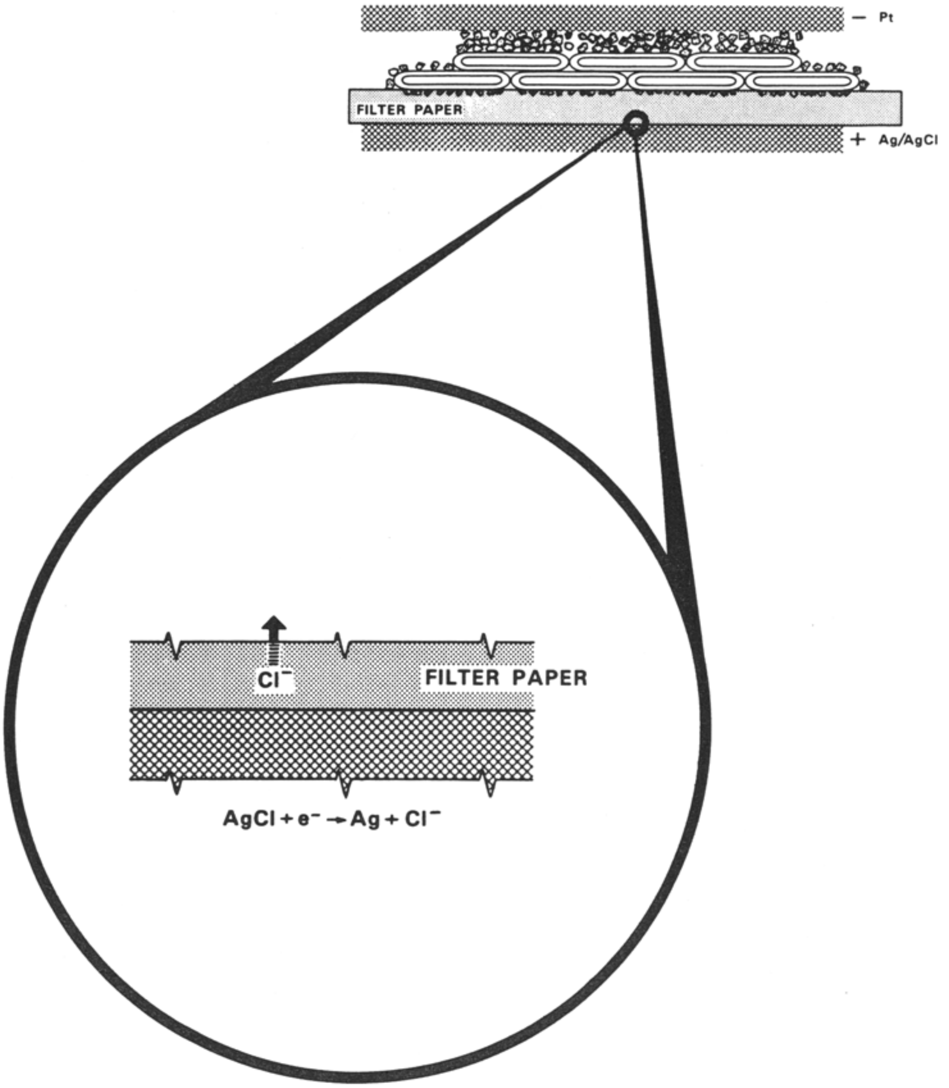

Fig. 5. Schematic representations of silver/silver chloride electrode-filter filter paper interface. Electrons are derived from the platinum cathode that is in pressure contact with the platinized chloroplasts.

of photosynthesis is in the picosecond range, suggesting that discreet molecular devices based on direct electrical contact with photosynthetic reaction centers may offer an interesting area of research for the development of very fast molecular electrooptical devices.

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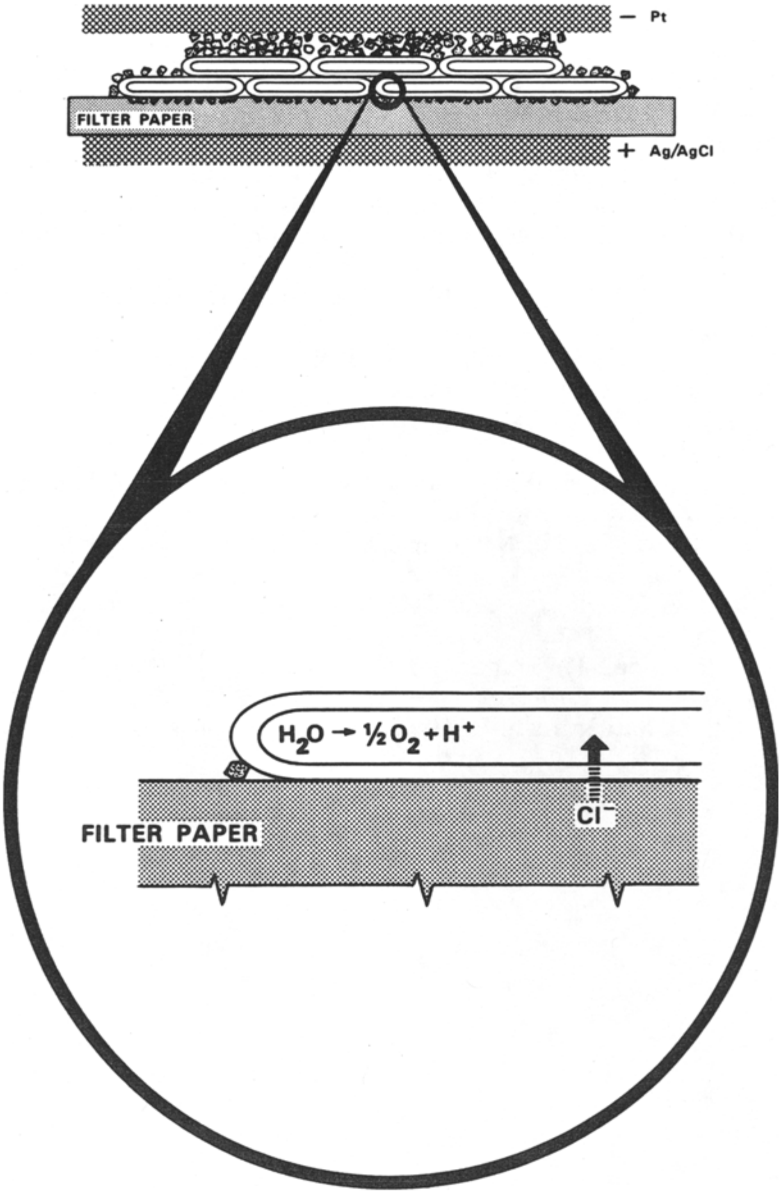

Fig. 6. A model of the intrathylakoid (lumenal) region of chloroplasts. The photosynthesis water splitting reaction produces a local increase in the concentration of hydrogen ions. This results in the creation of a local positive electrical potential that is neutralized by the movement of chloride ions.

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