Photoelectric Effects in Thin Chlorophyll Films

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The primary processes of photosynthesis in green plants take place in the thylakoid membrane which has a thickness of about 100 Å and consists of proteins, lipids, and pigment molecules in a more or less ordered arrangement. The first chemical step after the absorption of a light quantum is presumably a redox reaction, in which the excited chlorophyll molecule exchanges an electron with its environment. With respect to this hypothesis studies with artificial lipid membranes containing chlorophyll are of great interest [1–4]. In the following, we describe some experiments with bimolecular *(black)* membranes formed from a mixture of lecithin and chlorophyll a. In the presence of the reducing agent *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) very pronounced photoelectric effects are observed with these membranes, indicating that a redox process occurs after the optical excitation of a pigment molecule in the membrane.

The membranes were formed in the usual way on a teflon frame which was submersed in an aqueous solution and had a circular hole of 3 mm diameter [5]. The composition of the lipid solution was 0.5% (w/v) dioleoyl lecithin + chlorophyll a in *n*-decane with a molar ratio lecithin : chlorophyll of 5 : 1. Membranes formed from this solution have a final thickness of about 50 Å and contain approximately 10^{13} chlorophyll molecules per square cm [4]. In most experiments, white light from a 250 W tungsten lamp was used which gave an intensity of about 0.1 W/cm² in the plane of the membrane.

The basic experimental observations are represented in figs. 1 and 2. The membrane is formed in aqueous Tris chloride. After the membrane is in the *black* state, the aqueous phase on one side is replaced by a TMPD solution. This results in the development of a membrane potential of 50 – 70 mV, the TMPD side being negative. When the light is turned on, the membrane potential increases by about 40 mV. The rise time of the photovoltage is about 3 sec under the conditions of this experiment. The rise time is approximately equal to the electrical time constant $\tau_m = R_m C_m$ of the membrane, where $R_m \cong 10^7$ ohm·cm² is the membrane resistance and $C_m \cong 4 \cdot 10^{-7}$ F/cm² the membrane capacitance. The sign of the photovoltage U_{ph} does not depend from which side the membrane is illuminated, i. e., the TMPD solution becomes always negative with respect to the TMPD-free solution. Superimposed to the normal rise of the photovoltage with $\tau = R_m C_m$, a slow drift of U_{ph} toward higher values is also observed. This slow process leads to a steady level of U_{ph} after a period of 10 – 30 min of continuous illumination. Besides this, the magnitudes of the dark and light voltage vary somewhat from one membrane to the other. However, for a given membrane the photoeffect is completely reversible in the steady state, as fig. 1 shows.

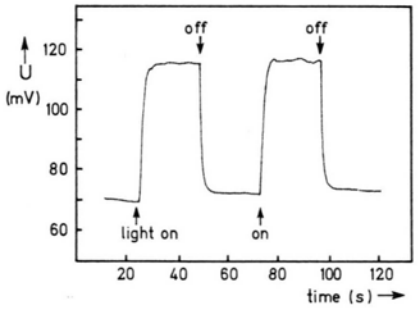

Fig. 1. Photovoltage of a lecithin/chlorophyll membrane. 15 mM TMPD, pH 7.0 on one side, 10 mM Tris chloride, pH 7.0 on the other side. U is the electrical potential of the TMPD-free solution with respect to the TMPD solution.

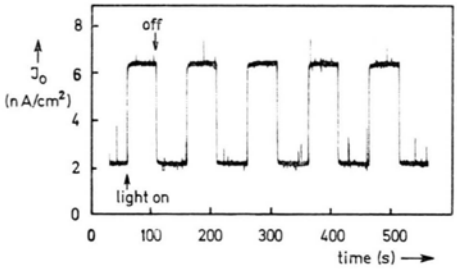

Fig. 2. Photocurrent J_0 of a lecithin/chlorophyll membrane under short circuit. 10 mM TMPD, pH 6.0 on one side, 30 mM Tris, pH 6.0 on the other. $J_0 > 0$ for current across the membrane from the TMPD side to the TMPD-free side.

The corresponding photocurrent is represented in fig. 2. Under short-circuit, a dark current of about 2 nA/cm² flows through the membrane from the TMPD side to the TMPD-free side. After the light is switched on, a fast increase in the current is observed. The photocurrent has a much shorter rise time than the photovoltage. From the oscilloscope trace (fig. 3) a time constant of ~ 2 ms is obtained. However, it can be shown that this rise time is determined by a second electrical time constant of the system, which depends on the input resistance of the oscilloscope amplifier. Thus, the value of 2 ms has to be considered as an upper limit.

The system has the following further properties: 1. The photocurrent is proportional to the light intensity up to at least 0.1 W/cm². 2. The action spectrum of the photocurrent measured with a series of narrow-

[1] H. P. TING, W. A. HUEMOELLER, S. LALITHA, A. L. DIANA, and H. T. TIEN, Biochim. biophysica Acta [Amsterdam] **163**, 439 [1968].
[2] H. T. TIEN, J. physic. Chem. **72**, 4512 [1968].
[3] H. T. TIEN, Nature [London] **219**, 272 [1968].
[4] N. ALAMUTI and P. LÄUGER Biochim. biophysica Acta [Amsterdam], in press.
[5] P. LÄUGER, W. LESSLAUER, E. MARTI, and J. RICHTER, Biochim. biophysica Acta [Amsterdam] **135**, 20 [1967].

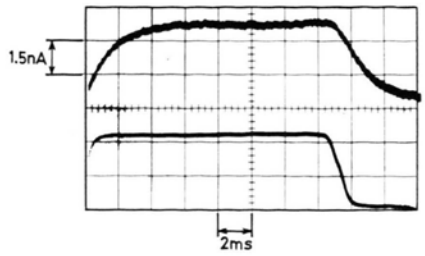

Fig. 3. Rise time of the photocurrent. A photographic shutter was used as a light switch. Lower trace: light intensity (recorded with a phototransistor); upper trace: photocurrent.

band interference filters has a peak in the vicinity of 410 nm and a second peak near 680 nm. The similarity between the action spectrum and the absorption spectrum of chlorophyll a indicates that the primary act in the generation of the photocurrent is the optical excitation of a chlorophyll molecule. 3. The photocurrent rises with increasing TMPD concentration. In the absence of TMPD, no photoeffect is observed. 4. The photocurrent rises with increasing pH and goes through a maximum at pH $\cong$ 7.

It is interesting to compare the above results with a recent study of ULLRICH and KUHN [6] on photoelectric effects of *black* membranes in the presence of cyanine dyes. In the cyanine system, a transient potential spike is observed when the membrane is illuminated. In contrast to this, the chlorophyll/TMPD system develops a stationary photovoltage under continuous illumination. This indicates that the mechanism of charge separation is quite different in both systems.

[6] H.-M. ULLRICH and H. KUHN, Z. Naturforsch. **24b**, 1342 [1969].

Phosphorus-32 Uptake from Deuterated Medium

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Earlier workers have shown that for optimum growth in a fully deuterated medium algae require a nutrient balance different from that needed in a protiated medium [1]. They also noted a higher yield of deuterated *Scenedesmus obliquus* in a high phosphate medium. In yeast, depressed rate of growth when cultured in D_2O has been attributed to the inhibition of glucose and phosphate uptake; the retarded uptake being possibly due to direct isotope effects on transport processes [2]. In higher plants, impaired mineral absorption has been suggested as a possible cause for poor growth in deuterated media [3]. Information on mineral uptake from D_2O by higher plants is lacking. We have here investigated, the uptake of ^{32}P from H_2O and D_2O by intact bean plants.

Experimental

Kidney beans (*Phaseolus vulgaris* L.) were obtained from a local seed company. Phosphorus-32 as carrier free H_3PO_4 in dilute HCl solution and heavy water (99.8% D_2O) with low tritium content were obtained from the Isotope Division and the Chemical Engineering Division respectively of the Bhabha Atomic Research Center.

The seedlings were germinated in sterilized vermiculite, in the dark, at 25 $\pm$ 2 °C. Modified Hoagland's medium (pH 5.6) containing chelated trace elements served as the nutrient. On the eighth day, the cotyledons were removed and the plants were placed under continuous illumination. After 2 days plants of about the same height (15 cm) and leaf area (10 — 15 sq. cm) were selected and their roots freed of adhering vermiculite, sterilized with 10% Ca-hypochlorite solution for 1 min and then thoroughly rinsed with sterile distilled water. Each plant was then transferred to a polythene container with the roots dipping into 25 ml of 0.1 M $CaCl_2$ solution in H_2O or D_2O, containing 0.1 or 1.0 μCi of ^{32}P as orthophosphate. The pH of the medium was 5.4 for H_2O and 5.8 for D_2O. After 2 hours of absorption under diffuse light, and continuous aeration, the plants were removed and their roots wiped between folds of filter paper. They were then divided into two batches, one for direct ^{32}P determination and the other kept in $CaCl_2$ solution in H_2O/D_2O without ^{32}P, for a further period of 2 hours. Radioactivity leached out in the post treatment medium was also assayed. Transpiration rate was determined by measuring the water lost from the plants during the experiment.

For measuring the distribution of the ^{32}P absorbed, each plant was divided into shoot, hypocotyl and root, which were then placed in separate Erlenmeyer flasks and dried overnight at 90 °C. After weighing, the samples were digested by boiling with 40 ml of conc. HNO_3. Following digestion, the volume was made upto 50 ml and ^{32}P activity was counted in a liquid counting Geiger tube. Corning or equivalent glassware, previously saturated with Na_3PO_4 solution were used throughout. Each treatment consisted of five plants and the experiments were repeated thrice.

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[1] H. L. CRESPI, S. M. CONRAD, R. A. UPHAUS, and J. J. KATZ, Ann. New York Acad. Sci. **84**, 648 [1960].

[2] R. T. O'BRIEN, Proc. Soc. expt. Biol. Med. **117**, 555 [1964].

[3] C. R. BHATIA and H. H. SMITH, Planta **80**, 176 [1968].

Results

Phosphorus-32 uptake by the plants both in D_2O and H_2O is shown in Table 1. Radioactivity measurements obtained after 2 hours of absorption indicate inhibition of ^{32}P uptake by D_2O. This inhibition was statistically significant. Post treatment in $CaCl_2$ solution, without ^{32}P, for a further period of 2 hours, caused some leaching of the absorbed ^{32}P from the roots. However, the net uptake remained significantly lower in D_2O than in H_2O.

Expt. No.	^{32}P supplied per plant [μCi]	Medium	[Counts/mg/mt] $\pm$SE	
			Without post treatment	With post treatment (2 hours)
I	0.1	H_2O	101.4 $\pm$ 8.1	55.6 $\pm$ 7.2
		D_2O	52.2 $\pm$ 4.6	18.0 $\pm$ 4.3
II	1.0	H_2O	461.0 $\pm$ 23.9	164.5 $\pm$ 15.2
		D_2O	148.2 $\pm$ 13.4	61.0 $\pm$ 6.8

Table 1. Uptake of ^{32}P by bean plants with and without post treatment in $CaCl_2$.

Expt. No.	^{32}P supplied per plant [μCi]	Medium	Without post treatment		With post treatment		
			Shoot	Root	Shoot	Root	Leached out
I	0.1	H_2O	1.7	98.3	2.3	80.6	17.1
		D_2O	4.6	95.4	12.6	58.9	28.5
II	1.0	H_2O	1.6	98.4	3.9	58.7	37.4
		D_2O	4.8	95.2	15.4	39.5	46.0

Table 2. Percent distribution of ^{32}P in shoot * and root with and without post treatment. * Leaves and hypocotyl.

The distribution of the absorbed ^{32}P in shoot and the root is shown in Table 2. After 2 hours in protiated medium 1.7 to 3.4% of the absorbed ^{32}P was translocated to the shoot whereas in deuterated medium 4.6 to 7.1% was translocated. It should be noted that while D_2O inhibited ^{32}P uptake by the roots, it promoted translocation of the absorbed phosphorus to the shoot. In case of the plants that were post treated with $CaCl_2$ solution, the data indicate a greater leaching of ^{32}P from the roots in D_2O environment, and also confirms the promoting effect of D_2O on translocation.

Increased translocation of ^{32}P in deuterated medium is contrary to the expectations. Most of the vital biological processes, so far investigated, are retarded in D_2O. The increased amount of ^{32}P in the shoot of the D_2O plants could not be accounted for by the rate of transpiration. Water lost by the plants was initially slightly lower in D_2O, but at 4 hours, the amount transpired was the same for D_2O and H_2O plants.

It is reported that in young barley plants after absorption, phosphate is extensively esterified in the roots. However, it appears to be transported to the shoot as inorganic phosphate accompanied by only a small amount of a single organic compound. 2:4-dinitrophenol (DNP), which is a respiratory inhibitor reduces the uptake of phosphorus and also the amount of esterification, and hence causes accelerated upward movement to the shoot [4]. D_2O is known to act as a metabolic inhibitor [5], and slows down respiration of seeds [6]. It is quite probable therefore that D_2O in this case acts as a inhibitor like DNP and causes increased transport to the shoot inspite of reduced total uptake. Whether this is caused by less phosphorus being converted to organic forms is under investigation.

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[4] B. C. LOUGHMAN and R. SCOTT RUSSEL, J. expt. Bot. [London] **8**, 280 [1957].

[5] J. F. THOMSON: Biological Effects of Deuterium, Pergamon Press, New York 1963, p. 133.

[6] S. BHATTACHARYA (nee BOSE), M. K. BHANDARKAR, and B. K. GAUR, Physiol. Plantarum [Copenhagen] **22**, 1025 [1969].