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Monolayers and multilayers of chlorophyl *a* on a mercury electrode

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Abstract

A novel experimental technique used to investigate chlorophyll films on a hanging mercury drop electrode is described. Two different procedures are employed to prepare self-assembled chlorophyll monolayers and multilayers on the mercury electrode. Upon illuminating the chlorophyll *a* (Chl)-coated mercury electrode with an appropriate light source, the photocurrents generated by the Chl aggregates are measured under short-circuit conditions in the absence of photoartefacts. The preliminary results obtained by this novel technique are presented. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chlorophyll *a*; Self-assembled film; Mercury; Photocurrent

1. Introduction

An important biomolecule is chlorophyll *a* (Chl), a pigment present in the thylacoid membranes of higher plants that plays a fundamental role in the photosynthesis, whereby solar energy is converted to the stored free energy of carbohydrates. It consists of a porphyrin ring that chelates Mg(II) and contains a network of conjugated double bonds that absorb light; its long hydrocabon side chain imparts to chlorophyll a high affinity for lipids. In the thylakoid membrane, chlorophyll is present in the antenna complex, which consists of hundreds of chlorophyll molecules that absorb light and transfer their energy to a special pair of chlorophyll molecules. These in turn transfer the excited electron to plastoquinone, a molecule very similar to ubiquinone, which is reduced to the corresponding quinol and shuttles electrons to another protein incorporated in the thylakoid membrane.

The aim of the present research is to investigate self-assembled chlorophyll films on a mercury electrode using a novel experimental method, which has been recently developed in our laboratory to study the function and properties of photoactivatable compounds. Self-assembled Chl monolayers and multilayers supported by a hanging mercury drop electrode were formed by two different procedures. Upon illuminating the Chl-coated mercury electrode, the photocurrents generated by the Chl aggregates were measured under short-circuit conditions in the absence of photoartefacts. The preliminary results obtained by this novel technique are presented.

2. Experimental

2.1. Chemicals

The water used was obtained from light mineral water by distilling it once, and then by distilling the resulting water from alkaline permanganate, while discarding the heads. Merck reagent grade KCl was baked at 500 °C before use to remove any organic impurities. All inorganic salts were purchased from Merck. All measurements were carried out in 0.1 M KCl at pH=8.5 (1.2×10^{-3} M NaOH and 5×10^{-3} M H_3BO_3). Chlorophyll *a* was extracted from the cyanobacterium *Spirulina gelteri* and purified according to the method described in Ref. [1].

2.2. Experimental set-up

The experimental set-up employed in the present measurements is shown in Fig. 1. The whole set-up was contained in a water-jacketed box D, thermostated at 25 °C±0.1. The electrolytic solutions in the two cells A and C were deaerated by purging with high purity argon for no less than 20 min. The home-made hanging mercury drop electrode (HMDE) used in the measurements is described elsewhere [2–4]: in the present measurements, a surface

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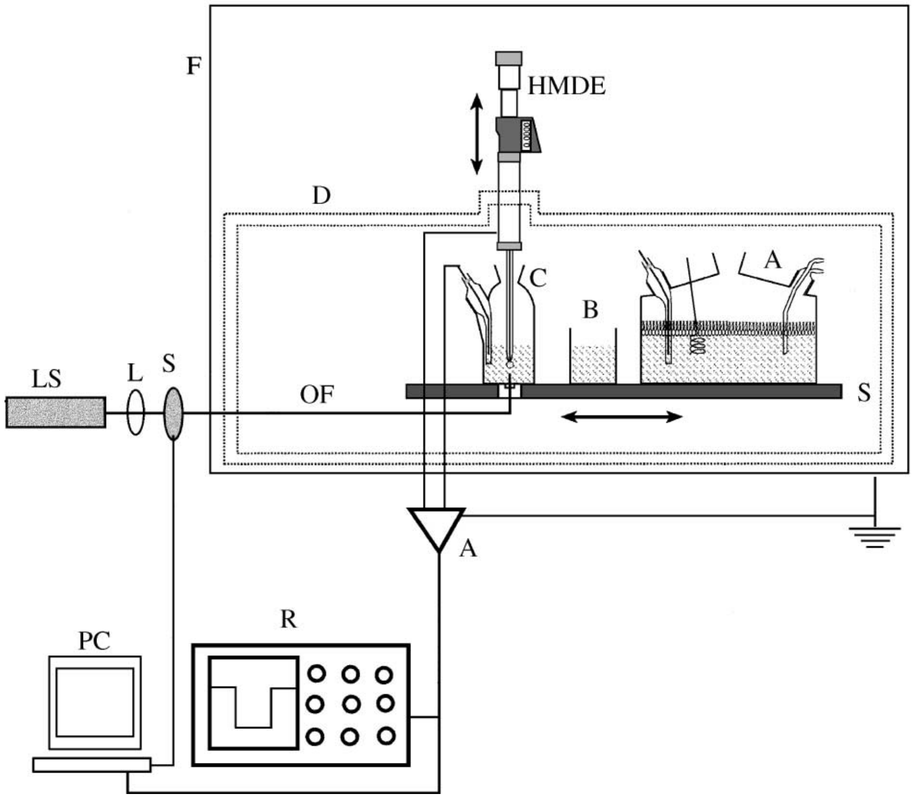

Fig. 1. Experimental set-up. F: Faraday cage; D: water-jacketed box; HMDE: hanging mercury drop electrode, A: three-electrode cell; B: vessel; C: two-electrode cell; S: movable support; OF: optical fiber; LS: light source; L: optical fiber coupler; S: shutter; A: current amplifier; R: recorder; PC: computer.

area of 1.4×10^{-2} cm^2 was adopted. The HMDE was housed in a water-jacketed sleeve on the top of the box, so as to permit the mercury reservoir to be effectively thermostated. The vertical movements of the HMDE through the argon–solution interface were realized by means of an oleodynamic system; this ensured the complete absence of vibrations while permitting an appreciable range of velocities. A second oleodynamic system S was used for the horizontal movements of the movable support, on which the two cells and the vessel were placed; this permitted the HMDE to be positioned above, and then lowered into, any of these vessels. The water-jacketed box was contained in a Faraday cage F to avoid electrical noise. A vibration-free table was also employed to prevent mechanical vibrations.

The glass cell A was used to form Chl films by the spreading procedure and contained a platinum counter-electrode, an SCE reference electrode and, when required, the working electrode HMDE. The differential capacitance *C* of the working electrode was measured in this cell by ac voltammetry with a Metrohm Polarecord E506 (Herisau, Switzerland), and was directly obtained from the quadrature component of the ac current. The ac signal had a 10-mV amplitude and a 75-Hz frequency. The system was calibrated with a precision capacitor. The vessel B contained the Chl solution in pentane and was used to form a Chl film by the soaking procedure. The Plexiglas cell C contained an Ag–AgCl (0.1 M KCl) reference electrode and, when required, the HMDE; it was provided with a quartz optical fiber (0.6 mm in diameter), whose tip was positioned on the cell bottom and was pointed vertically toward the HMDE for its illumination [5]. For a good alignment of the optical fiber with the mercury drop, the cell was mounted on an x–y slide. The monochromatic light source LS (red light laser, 670 nm, Electron, Model LA5-3.5G-670) was focused and collimated using an optical fiber coupler (Newport, Model F-915T). Light pulses were produced using an electromechanical shutter S (blade shutter and digital shutter controller, Model 845, Newport), which was controlled by the computer PC through a digital-to-analog converter (IOtech DAC488/2). The current generated by illuminating the Chl adsorbed on the mercury drop under short-circuit conditions was amplified (Current Amplifier, Keithley 428), recorded (16-bit analog-to-digital converter, IOtech ADC488/8SA), visualized (Oscilloscope, Tektronix TDS 340A) and stored (Power PC G3, Macintosh). Operation of the experimental set-up and data acquisition were carried out under computer control (GBIP interface, National Instruments board) using a home-made acquisition program written in LabView environment. To increase the signal to noise ratio, current versus time curves were stored upon averaging no less than 50 current signals. The signal was usually sampled at 200-μs intervals over a 120-ms time window.

Chl absorption spectra were determined by a UV–visible spectrophotometer (Cary Varian 3). Because of the high sensitivity of chlorophyll to the blue and red components of visible light, all measurements were carried out under green light conditions.

2.3. Formation of chlorophyll films

Films of chlorophyll were transferred on a mercury drop by using two different procedures:

(a) Spreading procedure: the Chl coating was obtained by spreading an amount of Chl corresponding to 6–8 monolayers, dissolved in a suitable solvent (pentane or hexane), on the surface of an electrolytic solution, allowing the solvent to evaporate and immersing the mercury electrode into the solution through the Chl film;
(b) Soaking procedure: the Chl coating was obtained by immersing the mercury drop in a solution of Chl in pentane or other organic solvent for 15–20 s; the Chl-coated mercury drop was then rinsed with the pure solvent to remove unadsorbed chlorophyll and immersed into the electrolytic solution.

With both procedures, Chl solutions of different concentration in various solvents were employed.

3. Results and discussion

Owing to the peculiar structural characteristics, chlorophyll may easily form different aggregates in solution, i.e. monomer, dimer, oligomer or polymer, depending both on the concentration of chlorophyll and on the solvent nature. These various species present different properties and reactivity, as a result of the specific interactions between the aggregate and the surrounding medium.

To determine the structural organization, purity and concentration of the Chl samples, the absorption spectra in diethylether were recorded. The typical spectrum of chlorophyll *a* in this solvent presents two sharp peaks at λ=428 and 660 nm, which are characteristic of the monomeric form of chlorophyll *a* [6]. If the oligomeric form is present, an additional peak at $\lambda \sim 745$ nm is observed [7,8]. The Chl adsorption spectrum in pentane shows that chlorophyll is present as a monomer at low concentration (2×10^{-6} M). At concentration higher than 4×10^{-5} M, the oligomeric form is also present, as can be deduced from the appearance of the corresponding peak in the adsorption spectrum.

The differential capacities of the Chl films prepared by the spreading and soaking procedures were determined by ac voltammetry. The differential capacity measurements were carried out in 0.1 M KCl at pH=8.5 since chlorophyll *a* presents its maximum stability at this pH [9]. The differential capacity C versus potential E curves were usually recorded over a potential region ranging from −0.2 to −0.9 V (SCE). In the case of Chl films formed by the spreading procedure, C has a value of 2.7–3.3 μF cm^{-2}. We also observed that, as the mercury electrode was repeatedly immersed through the argon–solution interface, C progressively decreased and finally attained a constant value of 0.3 μF cm^{-2}, as shown in the inset of Fig. 2. We may explain the behavior of C by assuming that a low value of the differential capacity corresponds to the formation of a Chl multilayer on the mercury electrode and that chlorophyll is

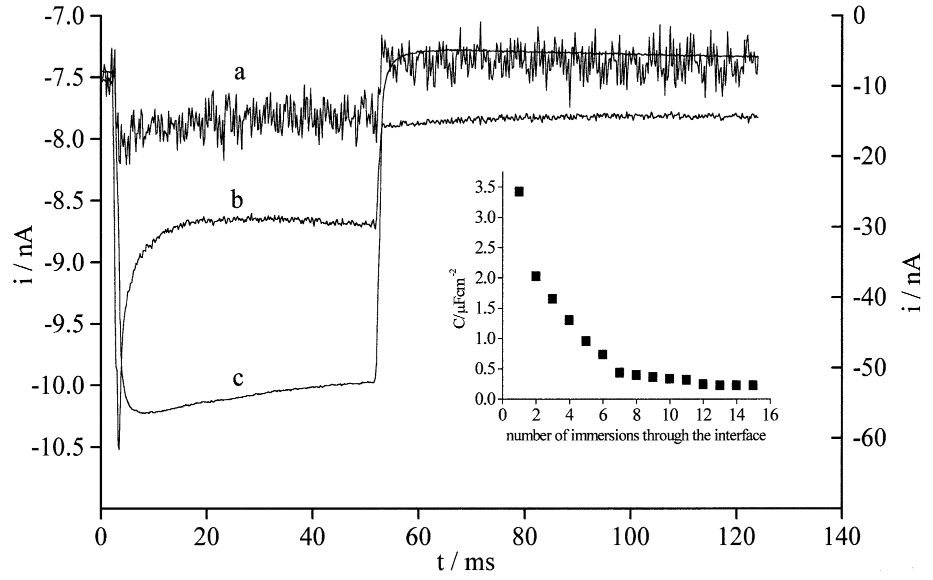

Fig. 2. Photocurrents generated by Chl films supported by a hanging mercury drop electrode. Photocurrents a and b are obtained from Chl films formed by the spreading procedure after 2 and 11 immersions of the mercury electrode through the argon–solution interface, respectively. Photocurrent c is obtained from a Chl film formed by the soaking procedure. The left *y*-axis refers to curves a and b, while the right *y*-axis refers to curve c. The inset shows the plot of the differential capacity of Chl films obtained by the spreading procedure as a function of the number of immersions of the mercury electrode through the argon–solution interface.

mainly present as a monomer at the argon–solution interface. The differential capacity of Chl films obtained by the soaking procedures is much lower and equal to 0.6±0.1 μF cm^{-2}. If the Chl-coated mercury electrode was then rinsed with the pure solvent, *C* increased up to 1.1±0.2 μF cm^{-2}.

The Chl films formed by the spreading and soaking procedures were photoexcited with red light. The resulting photocurrents were strongly dependent on the procedure used in preparing the film, as shown in Fig. 2. As a rule, Chl films obtained by the spreading procedure yield very low photocurrents of about 0.4–0.5 nA (curve a). As the differential capacity decreases progressively, the photocurrent increases up to a maximum value of 1–2 nA (curve b). On the other hand, Chl films obtained by the soaking procedure yield much higher photocurrents (curve c), which usually range from 10 to 40 nA (see left *y*-axis).

The photocurrents generated by the Chl films on mercury may be qualitatively explained in the following way. First of all, we have to consider that chlorophyll in the excited state is both a strong reductant, with a redox potential of about -1 V/NHE when it releases the excited electron, and a strong oxidant, with a redox potential of about 1 V/NHE, when it takes up an electron to fill the hole left by the excited electron. Considering the photocurrents shown in Fig. 2, we observe that the photocurrents have a negative sign, which corresponds to the transport of negative charge from the mercury electrode toward the aqueous solution [5]. Therefore, the photocurrent observed is actually a reduction photocurrent. This cathodic photocurrent is due to the transfer of electrons from the mercury electrode to the chlorophyll molecules, which in turn release their excited electron to the water molecules with hydrogen evolution.

4. Conclusion

Self-assembled chlorophyll films supported by a mercury electrode can be conveniently investigated using the novel experimental set-up described in this report. Our preliminary results show that a photocurrent can be measured upon shining the Chl-coated mercury drop with red light. This photocurrent can be qualitatively explained in terms of a Chl-mediated electron transfer from mercury to water with hydrogen evolution. Further characterization of the Chl films on mercury is underway.

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References

[1] J.G. Brace, F.K. Fong, D.H. Karweik, V.J. Koester, A. Shepard, N. Winograd, Stoichiometric determination of chlorophyll *a* — water aggregates and photosynthesis. Symbiotic roles of the magnesium atom and the ring V cyclopentanone group in the structural and photochemical properties of chlorophyll *a* monohydrate and dihydrate, J. Am. Chem. Soc. 100 (1978) 5203–5207.

[2] M.R. Moncelli, L. Becucci, R. Guidelli, The intrinsic pK_a values for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in monolayers deposited on mercury electrodes, Biophys. J. 66 (1994) 1969–1980.

[3] M.R. Moncelli, L. Becucci, The intrinsic pK_a values for phosphatidic acid in monolayers deposited on mercury electrodes, J. Electroanal. Chem. 385 (1995) 183–189.

[4] M.R. Moncelli, L. Becucci, A novel model of the hanging mercury drop electrode, J. Electroanal. Chem. 433 (1997) 91–96.

[5] A. Dolfi, F. Tadini Buoninsegni, M.R. Moncelli, R. Guidelli, Photocurrents generated by bacteriorhodopsin adsorbed on thiol/lipid bilayers supported by mercury, Biophys. J., submitted for publication.

[6] G.R. Seely, R.G. Jensen, Effect of solvent on the spectrum of chlorophyll, Spectrochim. Acta 21 (1965) 1835–1845.

[7] A. Agostiano, Chlorophyll *a* in aqueous organic solvents, spectroscopic and electrochemical investigation of the factors effecting molecular aggregation and photoreactivity, Trends Photochem. Photobiol. 3 (1994) 371–382.

[8] A.G. Volkov, M.I. Gugeshashvili, G. Munger, R.M. Leblanc, The light-dependent oxygen reduction by monolayers of hydrated chlorophyll *a* oligomer, Bioelectrochem. Bioenerg. 29 (1993) 305–314.

[9] C. Mingotaud, J.-P. Chaveut, L.K. Patterson, Stability of chlorophyll *a* at the gas–water interface in pure and mixed monolayers. An evaluation of interfacial pH, J. Phys. Chem. 100 (1996) 18554–18561.