Mixed Monomolecular Films of Chlorophyll and Cytochromes

P. Chin and S. S. Brody

Department of Biology, New York University, New York

(Z. Naturforsch. **31 c**, 44–47 [1976]; received February 3/October 3, 1975)

Cytochrome, Chlorophyll, Monolayers Photosynthesis

Mixed monomolecular films of cytochrome (Cyt) and chlorophyll (Chl) were spread at a nitrogen-water interface. A large interaction is observed between reduced Cyt f and Chl a in a mixed film. Oxidized Cyt f and Chl a in a mixed film show little evidence for interaction. Mixed monomolecular films of Chl a with either reduced or oxidized Cyt c appears to result in denaturation of the protein at the surface.

A light reaction is observed only in mixed films of reduced Cyt f and Chl a.

Introduction

Cytochrome f (Cyt f) appears to occupy a key position between the electron transport system and photosystem I. It appears that there is a light-driven electron transfer from Cyt f to chlorophyll (Chl) of system I [1] in photosynthesis.

The electron transport system is located in the thylakoid membrane of the chloroplasts. This membrane also contains the pigments necessary for photosynthesis. According to models of the thylakoid membrane, the pigments exist as a monolayer at a lipid-protein interface [2, 3]. Pigment monolayers on an aqueous surface serve as a useful model to study various aspects of the physical and chemical properties of the *in vivo* state.

The properties of monomolecular films of oxidized and reduced Cyt c and Cyt f were reported elsewhere [4]. Evidence for an interaction between Cyt c and Chl in a mixed monolayer was reported by Aghion *et al.* [5].

The present study is concerned with the interaction of Chl a with oxidized and reduced Cyt c and Cyt f in mixed monomolecular films. The effect of light on these films will also be examined.

Materials and Method

Studies of mixed monomolecular films are carried out using a Wilhelmy plate surface balance housed in an environmental chamber; the latter has provision for evacuation and flushing with nitrogen. A constant temperature of 15 °C is maintained using a thermostatically controlled water cooling system. The sensitivity of the balance is ± 0.2 dyn/cm.

Surface potential measurements are made as previously described [6]. The accuracy of surface potential measurements is ± 10 mV.

The area/molecule, A_π and the surface potential ΔV_π are measured at a surface pressure $\pi = 6$ dyn/cm.

The concentration of cytochrome or chlorophyll in the spreading solution is determined spectrophotometrically (Cary, Model 14R). The spreading solution for chlorophyll is benzene, and for cytochrome, water. Chlorophyll is spread on the surface, using a Hamilton microliter syringe. The film of chlorophyll is compressed and expanded several times while the chamber is flushed with nitrogen to insure even spreading and evaporation of the benzene solvent. Surface isotherms are measured repeatedly until they are reproducible. After the cytochrome is added to the surface, the mixed monolayer is compressed and expanded several times in order to facilitate mixing. Surface isotherms are again measured repeatedly in the dark, until they are reproducible. The mixed monomolecular film, maintained at a constant area, is irradiated for fifteen minutes using a 100 W, low pressure Hg arc lamp (GE H100-A4). The intensity of light on the surface is 2×10^3 ergs/cm^2. After this period of irradiation, several isotherms are measured in the light.

The number of molecules of Chl in the spreading solution is determined using a molar extinction coefficient of 7.95×10^4 (at 670 nm). The molar extinction coefficient used for Cyt c reduced and oxidized is 2.9×10^4 and 0.84×10^4, respectively. For reduced Cyt f 2.6×10^4 is used and for the oxidize form 1.1×10^4.

Crystalline chlorophyll a is prepared and stored according to Aghion *et al.* [5]. Cyt f is a generous gift from Prof. N. Bishop of the University of Oregon, Corvallis. Horse heart Cyt c type II is obtained from

Requests for reprints should be sent to Prof. Dr. S. S. Brody, New York University, Department of Biology, 952 Brown Building Washington Square, *New York*, N. Y. 10003.

Sigma Chemical Co., St. Louis, Mo. To fully reduce the cytochromes, excess sodium ascorbate is added (Nutritional Biochemical Corp., Cleveland, Ohio). Excess potassium ferricyanide (Fisher Chemical Co. N.J.) is used to oxidize the cytochromes. The benezene used for spreading Chl is obtained from Mallincroft (St. Louis, Mo.).

Phosphate buffer, ionic strength 0.6 and pH 7.8 is used as the substrate for all experiments. A nitrogen atmosphere is maintained (Matheson, Rutherford, N.J., 99.995%) above the films at all times.

A theoretical area, THEOR, is determined from the following equation: $\mathrm{THEOR} = A_1 n_1 + A_2 n_2$, where A_1 and A_2 refer to the area/molecule of individual films of Cyt and Chl, respectively, and n_1 and n_2 refer to the mole fraction. The experimentally measured area of the mixed monomolecular film on the surface is designated as EXPER.

Results and Discussion

The Chl a used in the present study had an A_6 of 107 Å^2 at pH 7.8, ionic strength 0.6. This area is about 7% larger than that reported by Aghion *et al.*[5] and Bellamy *et al.*[7]. The difference in A might be related to the higher ionic strength buffer used in the present work. Isotherms of Chl a are constant and reproducible; even after remaining on the surface for one hour.

Monomolecular films of oxidized Cyt c are stable with time while monomolecular films of reduced Cyt c are not[4]. The addition of Chl to films of oxidized Cyt c make these films unstable also, indicating that there is an interaction between oxidized Cyt c and Chl. The instability is shown by a time dependent increase in area at constant π (Fig. 1). Perhaps Chl may be promoting the reduction of Cyt c in the dark to the unstable reduced form. Illumination has no effect on the film properties (Fig. 1).

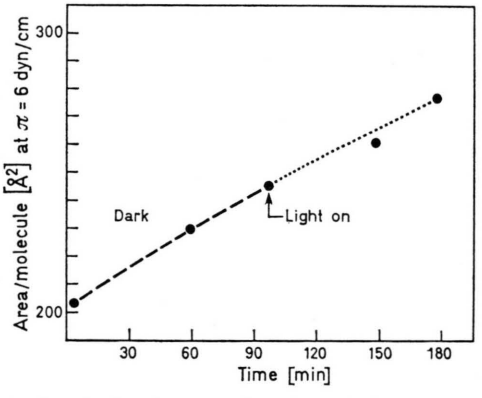

Fig. 1. Area/molecule, as a function of time of a mixed film of oxidized cytochrome c and Chl a at a ratio of 1:1. The area increases at the same rate both in dark (dashed line) and in the light (dotted line). The aqueous subphase contained phosphate buffer at pH 7.8, ionic strength of 0.6 and the temperature was maintained at 15 °C. All measurements were made in a nitrogen atmosphere.

Mixed monomolecular films of oxidized Cyt f and Chl exhibit a slight increase in the difference (EXPER – THEOR) as a function of mole fraction (Fig. 2). However, this slight increase is not considered significant enough to show an interaction between Chl and oxidized Cyt f. This slight increase in area may arise from the presence of small amounts of reduced Cyt f (the reduced form shows a large interaction with Chl – see below). Irradiation of the mixed film for fifteen minutes produced no change in the film area.

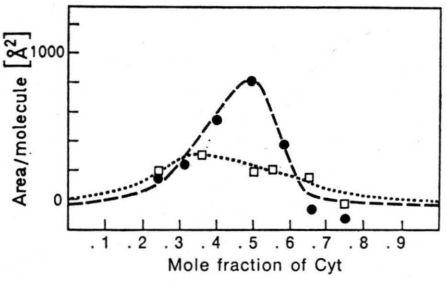

Fig. 2. The (EXPER–THEOR) areas of a mixed monomolecular film of Cyt f and Chl a as a function of the mole fraction of Cyt f. Measurements were made at a surface pressure of 6 dyn/cm. The data for reduced Cyt f is shown by solid circles (dash line). The data for oxidized Cyt f is shown by open squares (dotted line). Aqueous phase as described in Fig. 1. All measurements were made in a nitrogen atmosphere and in the dark.

Mixed monomolecular films of reduced Cyt f and Chl show a large increase in the difference (EXPER – THEOR), indicating an interaction. The maximum interaction occurs at a mole ratio of 1 : 1 (see Fig. 2).

The large increase in (EXPER – THEOR) observed with mixed films of reduced Cyt f and Chl a in the dark, originates primarily from Cyt f. The relatively small size of Chl a compared to Cyt f could not account for the large increase observed for (EXPER – THEOR). Conformational changes in the structure of Cyt f seems to be induced by interaction with Chl a. Oxidized Cyt f ($A_6 = 2600$ Å^2) is smaller than reduced Cyt f ($A_6 = 2900$ Å^2)[4]; therefore, the increase in (EXPER – THEOR) would argue against the possibility of reduced Cyt f being oxidized in the presence of Chl.

Irradiation of a mixed film of reduced Cyt f and Chl (mole ratio of 1 : 1) for fifteen min results in a decrease in area (Fig. 3). This decrease in A indicates a light-sensitized reaction between reduced Cyt f and Chl. Such a decrease in A is to be expected if a redox reaction occurs, since oxidized Cyt f is smaller than reduced Cyt f.

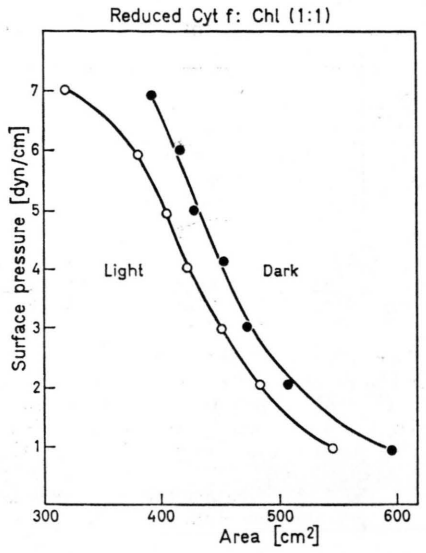

Fig. 3. Surface isotherms of a monomolecular film of Chl and reduced Cyt f (mole ratio 1:1) in a nitrogen atmosphere. The isotherm in the dark is shown by solid circles, the isotherm after illumination is shown by open circles. The aqueous phase is as described in Fig. 1.

The difference in interaction of Chl a with the reduced and oxidized form of Cyt f may be significant as it relates to photosynthesis. It appears that complex formation with Chl in a monolayer is a property of the reduced form of Cyt f but not the oxidized form. Such specificity would conform to the proposal that the reaction center of system I contains reduced Cyt f and Chl [8]. The primary photoreaction of photosynthesis involves the oxidation of Cyt f by reaction center I Chl. The sequence of events as usually proposed is that Chl undergoes a photooxidation by an electron acceptor and is then reduced to its original state by Cyt f [1]. A strong interaction between Chl a and reduced Cyt f in the dark could facilitate such a restorative reaction.

It is not yet clear what substance is the oxidant in the photoreaction between Chl and reduced Cyt f in the monolayer system. If the oxidant is the trace amount of oxygen in the nitrogen environment then the monolayer reaction is analogous to the *in vivo* reaction. However, if the oxidant is Chl, then Chl may be photoreduced, by Cyt f, rather than photooxidized.

If indeed there is a photoreaction between chlorophyll and reduced Cyt f, as well as between chlorophyll and plastocyanin [6] in the monolayer models, it would tend to support the schemes proposed by Hind and Olsen [9], and Knaff and Arnon [10]. Essentially, an electron may be transferred to chlorophyll of system I by either of two light reactions involving Cyt f in one reaction, and plastocyanin in another.

The results reported here with monolayers may be contrasted with those obtained in colloidal systems of Chl and Cyt c. A light catalyzed reaction between Chl, Cyt c and TMQ (trimethyquinone) was reported previously [11, 12]. It was proposed that Chl in the excited states reacts with TMQ to formed reduced TMQ which in turn reduces Cyt c in a dark reaction. A slow photoreduction of Cyt c by Chl in ethanol and aquenus systems was also reported [13, 14]. The hydrogen donor was presumed to be some unknown impurity. When the system contained reduced Cyt c and Chl there was no reaction.

In the monolayer system a pH more alkaline than that used in solution studies was used to avoid pheophytinization of Chl. Also with Cyt c films the major reaction appears to be denaturation. To what extent pheophytinization of Chl and denaturation of Cyt c occur in the colloidal system was not reported. In any event these two reactions might in part account for the difference in photoreaction observed in monolayer and colloidal systems.

Another important difference in the two systems is the relative orientation of Chl and Cyt. In the monolayer system the Chl and Cyt are in the same phase (the film), whereas in the colloidal system Cyt is in the aqueous phase and Chl is associated with alcohol.

[1] R. P. F. Gregory, Biochemistry of Photosynthesis, pp. 132, Wiley & Sons, London 1971.
[2] M. Calvin, Brookhaven Symposium Biol. **11**, 160 [1959].
[3] W. Kreutz, Adv. Bot. Res **3**, 53 [1970].
[4] P. Chin and S. S. Brody, Biochemistry **14**, 1190 [1975].
[5] J. Aghion, S. B. Broyde, and S. S. Brody, Biochemistry **8**, 3120 [1969].
[6] S. S. Brody, Z. Naturforsch. **28 c**, 397 [1973].

[7] W. D. Bellamy, G. L. Gaines, and A. G. Tweet, J. Chem. Phys. **39,** 2528 [1963].
[8] W. Butler, Arch. Biochem. Biophys. **93,** 413 [1961].
[9] G. Hind and J. M. Olsen, Ann. Rev. Plant Physiol. **19,** 249 [1968].
[10] D. B. Knaff and D. I. Arnon, Proc. Nat. Acad. Sci. U.S. **64,** 715 [1969].
[11] L. P. Vernon and E. R. Shaw, Biochemistry **4,** 132 [1965].
[12] B. Ke, L. P. Vernon, and E. R. Shaw, Biochemistry **4,** 137 [1965].
[13] A. A. Krasnovsky, Dokl. Acad. Nauk., SSSR **103,** 283 [1955].
[14] L. P. Vernon, Acta Chem. Scand. **15,** 1645 [1961].