FORMATION OF BLACK LIPID MEMBRANES (BLM) FROM EXTRACTS OF SPINACH LEAVES

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Bilayer or black lipid membranes (BLM) have been produced from a wide variety of materials and their physical properties extensively investigated (Haydon and Taylor, 1968; Henn and Thompson, 1968; Howard and Burton, 1968; Lauger and Richter, 1968; Ohki and Goldup, 1968). A review of the work on BLM has recently appeared (Tien and Diana, 1968).

In order to study membrane-associated phenomena relevant to photosynthesis and related processes, we decided to form BLM from photosynthetic pigments and lipids which hitherto have not been used alone in the BLM formation. This communication describes detailed procedures for the preparation of lipid solutions from spinach chloroplasts for BLM formation. In addition, a summary of the measured physical properties of these new BLM is reported.

Materials and Methods

Materials. Fresh spinach leaves were obtained from local food markets. Chlorophylls and xanthophylls purchased from commercial sources were also used (K and K Rare Chemicals, Inc. and Sigma Chemical Co.). Laboratory distilled water was re-distilled in an all-glass apparatus before use. Other reagents included aliphatic hydrocarbon solvents, petroleum ether (30-60^o b.p.), butanol, and NaCl. They were of reagent grade wherever possible, and were used without further purification.

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Extraction of pigments from spinach leaves and preparation of BLM-forming solution. A number of methods for extracting chloroplast pigments from fresh spinach leaves were tried. The following three procedures have been found to yield extracts suitable for BLM formation.

(A) The first successful procedure used is that described by Strain, et al (1960). The steps are as follows:

1. 100 gm of spinach leaves (free of midribs) are washed twice in cold distilled water.
2. The wet leaves are dropped into 2 liters of vigorously boiling water for 60 seconds.
3. Cool the content rapidly with ice and filter.
4. Press leaves dry; first between stacks of paper towels in hand and then between more paper towels underfoot.
5. Soak the press-dried leaves with 150 ml of absolute methanol plus 30 ml petroleum ether with occasional agitation.
6. After 10 minutes, decant.
7. Repeat Steps 5 and 6 two more times.
8. Combine the three extracts in a 1000ml separatory funnel and wash with 150 ml of saturated NaCl solution.
9. The lower aqueous layer, containing the methanol, some dissolved ionic lipids and proteinaceous precipitate, is discarded.
10. The petroleum ether layer is filtered through 2 layers of filter paper and dried in a flash evaporator.
11. The residue which is completely soluble is dissolved in n-octane to 5% concentration. This solution may be used directly in the experiment.

(B) This is a simplified procedure of (A) which has been found to be suitable. 50 gm of washed and press-dried spinach leaves are chopped with 150 ml methanol: petroleum-ether mixture (2:1, v/v) for one minute in an electric blender at low speed. The mixture is then strained through 4 layers of cheese cloth and washed with an additional 50 ml of petroleum ether. The upper layer is carefully pipetted off and filtered to remove any suspended material. The filtrate is evaporated to dryness and dissolved in n-octane as before (steps 10 & 11 of procedure A).

(C) The third procedure, which is the simplest, has been found to give more stable and larger black membranes. The scheme for the extraction of pigments and preparation of BLM-forming solution is shown in Fig. 1.

BLM formation and methods of study. Black lipid membranes (BLM) in aqueou- solution were formed by techniques described in detail elsewhere (Mueller, Rudin, Tien, and Wescott, 1963; Tien and Diana, 1968). The following physical

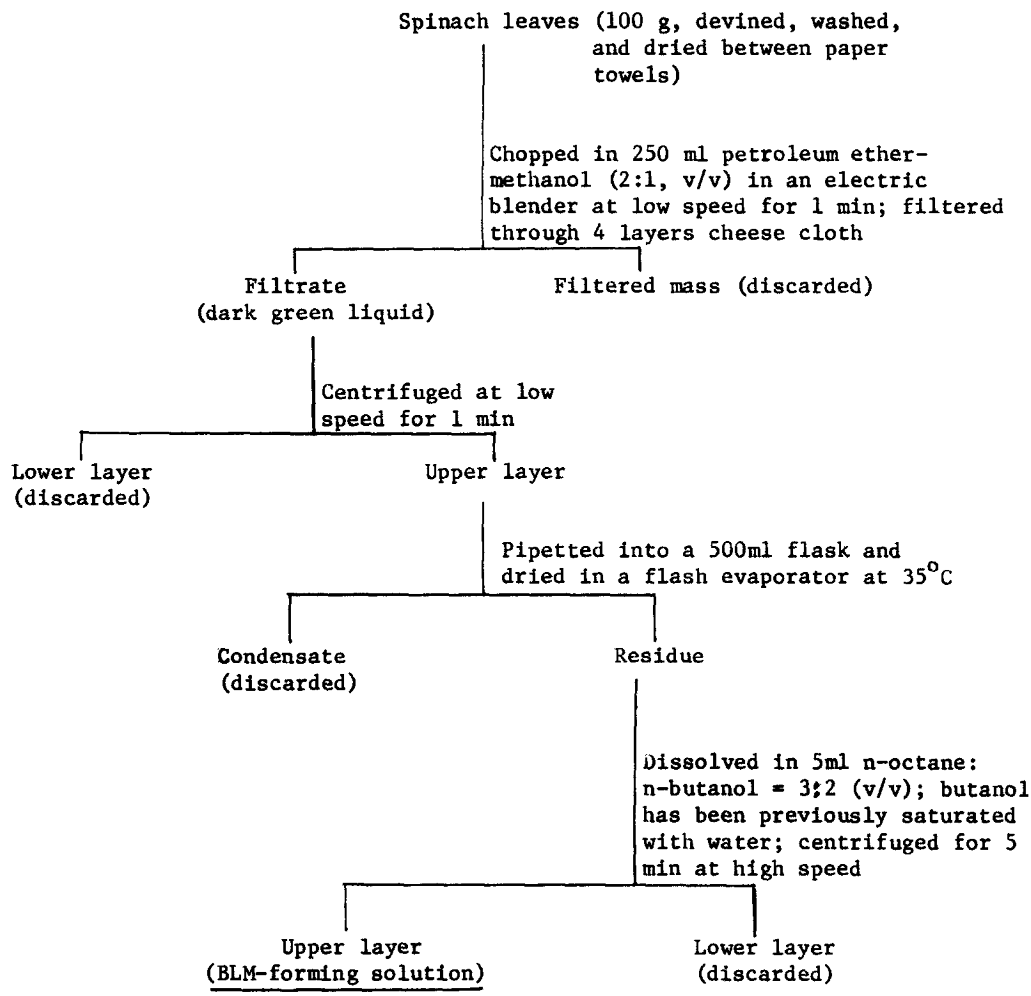

Fig. 1. Scheme for extraction and preparation of BLM-forming solution from spinach leaves.

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properties of this new class of BLM were investigated: the thickness, the bifacial tension, the permeability to water, and electrical parameters, using the methods reported previously (Tien and Diana, 1968).

Results and Discussion

Apart from some variations noted below, the formation characteristics of the BLM produced from different preparations were essentially very similar. Preparations (A) & (B) tended to give membranes of smaller area than Preparation (C). Also the membranes formed from Preparations (A) & (B) sometimes exhibited rigid interference pattern. Unless a minute quantity of lipid solution was used, the solution (i.e., in the form of a thin lipid layer) would not thin spontaneously in the aperture of the Teflon support. The time required for thinning to the black state varied a great deal ranging from a minute or so to as much as 30 minutes. In contrast, Preparation (C) gave very fluid membranes that thinned down to black membranes spontaneously. The thinning time was usually less than 2 minutes. In all preparations, the lifetime of the black membrane was not predictable. However, the lipid solutions from Preparation (C) gave the most consistent results with membranes lasting usually 15-30 minutes and longer. The main results of some physical properties of these new BLM examined in the present investigation are summarized in Table 1.

The relevance of formation of bimolecular lipid membranes (BLM) from chloroplast pigments and lipids may be viewed in relation to the postulated structural organization of the constituent molecules in the chloroplast. Although at present different interpretations about the ultrastructure (grana) have been given by different groups of workers (Moor & Muehlethaler, 1963; Menke, 1966; Park & Pon, 1963; Weier & Benson, 1966; Muehlethaler, 1966; Branton & Park, 1967), they seem to agree that there is a highly organized array of lipids, pigments, and proteins in the chloroplast. In particular, Muehlethaler (1966) has suggested that bimolecular lipid layers are involved in the chloroplast lamellae. Earlier, Calvin (1959) and others have suggested that for efficient energy utilization in photosynthesis, the pigment molecules in the photosynthetic apparatus must be close-packed and quasi-crystalline. In view of this background, the present membrane system (aqueous-solution/BLM/aqueous-solution) is a useful model, and of immediate interest for testing hypotheses such as energy transduction. In fact, using

Table 1 Some physical properties of bilayer lipid membranes (BLM) produced from extracts of spinach leaves.

Property	Value	Remarks
Thickness	105 ± 5A	Using an optical method[c]
Bifacial tension[a]	3.8-4.5 dynes/cm	The bulging method[c]
Osmotic water permeability	51 μ/sec	NaCl as the solute
Electrical resistance (D.C.)	2-3 x 10^5 ohms-cm^2	Ohmic up to 150 mV
Dielectric breakdown voltage	100-200 mV	Lower value for commercial pigments
Activation energy (water permeation)	4.2 Kcal/mole	Temp range 17.5-34°
Entropy of activation (water permeation)	0.9 cal/deg/mole	Calculated using the theory of absolute rates
Enthalpy of activation (water permeation)	3.6 Kcal/mole	See above
Energy of activation[b] (electrical conduction)	15.9 ± 0.3 Kcal/mole	Temp. range: 16-30°C

[a]Obtained by Miss S. Lalitha [b]Obtained by Dr. A. L. Diana
[c]For a detailed discussion, see Tien (1968a)

the BLM reported here, these membranes have been found to exhibit both photovoltaic effect and photoconductivity (Tien, 1968b).

Summary and Conclusions

Black or bilayer lipid membranes (BLM) of about 100A in thickness have been formed in aqueous solution. These new BLM were constituted from chloroplast extracts and from commercial pigments. Detailed procedures are given for the extraction and preparation of BLM-forming solutions. Some physical properties of the membranes are summarized. It is suggested that these new BLM, resembling in certain aspects the postulated thylakoid membrane, are useful model systems for testing hypotheses such as energy transfer processes that are important in photosynthesis.

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Literature Cited

Branton, D., and Park, R. B., J. Ultrastruct. Res., 19, 283 (1967)

Calvin, M., Rev. Mod. Phys., 31, 147 (1959)

Haydon, D. A., and Taylor, J. L., Nature, 217, 739 (1968)

Henn, F. A., and Thompson, T. E., J. Mol. Biol., 31, 227 (1968).

Howard, R. E., and Burton, R. M., J. Am. Oil Chemists' Soc., 45, 201 (1968)

Lauger, P., Richter, J., and Lesslauer, W., Ber. Bunsenges. Physik. Chem., 71, 906 (1968).

Menke, W., in Goodwin, T. W., ed., Biochemistry of Chloroplasts, Vol. 1, Acad. Press, New York, (1966) p. 3.

Moor, H., and Muehlethaler, K., J. Cell Biol., 17, 609 (1963).

Muehlethaler, K. in Goodwin, T. W., ed., Biochemistry of Chloroplasts, Vol. 1, Acad. Press, New York, (1966) p. 49.

Mueller, P., Rudin, D. O., Tien, H. T., and Wescott, W. C., J. Phys. Chem., 67, 534 (1963).

Ohki, S., and Goldup, A., Nature, 217, 458 (1968).

Park, R. B., and Pon, N. G., J. Mol. Biol., 6, 105 (1963).

Strain, H. H., Thomas, M. R., Crespi, H. L., Blaker, M. I., and Katz, J. J., N. Y. Acad. Sci., 84, 617 (1960).

Tien, H. T., J. Gen. Physiol., 52, part 2, 125 (1968a).

Tien, H. T., Nature, 219, 272, (1968b); J. Phys. Chem., 72, No. 12 (1968b).

Tien, H. T., and Diana, A. L., Chem. Phys. Lipids, 2, 55 (1968).

Weier, T. E., and Benson, A. A., in Goodwin, T. W., ed., Biochemistry of Chloroplasts Vol. 1, Acad. Press, New York, (1966) p. 91.