Treatment of the Thylakoid Membrane with Surfactants

ASSESSMENT OF EFFECTIVENESS USING THE CHLOROPHYLL a ABSORPTION SPECTRUM

JOHN P. MARKWELL AND J. PHILIP THORNBER
Department of Biology, University of California, Los Angeles, California 90024

ABSTRACT

Treatment of higher plant (Nicotiana tabacum L. var. Samsun) chloroplast thylakoid membranes with surfactants results in a shift of the chlorophyll a absorption maximum in the red spectral region from its in vivo value of 678.5 nanometers to shorter wavelengths. The magnitude of this shift is correlated with membrane disruption, and is not necessarily due to the release of pigment from pigment-protein complexes present in the membrane. Membrane disruption has been measured by the amount of pigment in the supernatant fraction after centrifugation of surfactant treated membranes. For an equivalent amount of disruption, the extent of the blue-shift is influenced by the ionic nature of the surfactant: anionic surfactants cause small shifts, cationic surfactants cause the largest (~10 nanometers) shifts, and nonionic surfactants produce intermediate shifts. The wavelength of maximum absorbance of chlorophyll a in the red region is a convenient criterion for assessing the potential utility of different surfactants for studies on the structure, composition and function of higher plant thylakoid membranes.

Much of our knowledge about the supramolecular organization of photosynthetic membranes and the components within them is derived from the use of surfactants as membrane solubilizing and fractionating agents. However, surfactants cannot generally be used in a reasonably predictable manner with biological systems. This has necessitated an empirical approach to their efficacy. Furthermore, the traditional method of ascertaining the ability of a surfactant to disrupt membrane structure—centrifugal fractionation of "soluble" and "insoluble" fractions—is cumbersome and time-consuming. The minimum time required is usually 2 or more h (including sample treatment, centrifugation, and analysis). The capacity of the centrifuge rotor limits the number of samples that can be treated at one time and requires considerable amounts of experimental material. Investing so much time and sample in preliminary experiments to determine the optimal concentration or stoichiometry of surfactant to be added to the membrane sample is often a serious disadvantage. It would thus be highly desirable to have a readily measured biochemical criterion with which to assess the effectiveness of a surfactant using minimal quantities of sample.

We have recently explored the usefulness of surfactants previously unused for biochemical research (10, 12). During this work it became apparent that the shift of the Chl a red absorption maximum to shorter wavelength values might be a convenient criterion to assess the efficacy of the various surfactants for disrupting the thylakoid membrane. Chl a has major absorption bands in the blue and red region of the electromagnetic spectrum (17, 19). The red band of Chl a in organic solvent is narrower and at shorter wavelengths than that observed for Chl a in vivo (7). The in vivo broadening occurs because the red peak is a composite of several slightly different spectral forms of Chl (7), resulting from individual Chl molecules residing in different environments. The thylakoid membrane pigments occur in specific pigment-protein complexes (4, 22). It is believed that pigment-pigment and pigment-protein interactions within individual holocomplexes, and, to a lesser extent, the membrane milieu produce the majority of the different spectral forms observed. Addition of surfactants can result in disruption of the membrane and possible denaturation of pigment-protein complexes, thereby altering the special environments of the pigment molecules and causing long wavelength forms to absorb at shorter wavelengths (7). The net effect is to shift the composite red absorption peak to shorter wavelength values. These surfactant-induced shifts have been known for 40 years (21). They were systematically studied by Sauer and Park (18) who found that the magnitude of the wavelength change was not correlated with a loss of photosynthetic activity as measured by the Hill reaction. The present report will suggest that at least a portion of the wavelength shift on addition of surfactant results from disruption of the thylakoid membrane structure and that the ionic nature of the surfactant used is directly related to the effect observed.

RESULTS AND DISCUSSION

The organism used for the reported studies was tobacco (Nicotiana tabacum L. var. Samsun) although experiments with maize (Zea mays L.) or barley (Hordeum vulgare L.) gave similar results. Tobacco plants were grown in a soil-vermiculite mixture for 4 to 6 weeks in a greenhouse.

Leaves were harvested, washed in cold water, and deribbled. All further steps were carried out at 0 to 5°C. The tissue was homogenized in a precooled Waring Blender containing 400 mm sorbitol, 10 mm NaCl, and 20 mm Tricine-NaOH (pH 7.6). The brei was filtered through two layers of Miracloth (Chicopee Mills, Inc., Milltown, NH) and the filtrate centrifuged at 3,000g for 5 min. After centrifugation, the chloroplast-containing pellet was suspended with the aid of a glass homogenizer in 50 mm Tricine-NaOH (pH 7.8), 1 mm EDTA, and the solution centrifuged at 17,000g for 10 min. This washing was repeated and an aliquot reserved for Chl determination. The final pellet was used directly for studies involving treatment with surfactants.

For each mg of Chl (Chl a + b) in the pellet, 1 ml of cold surfactant solution at the concentration indicated was added. The pellet was suspended and homogenized with the aid of a glass homogenizer. The solution was allowed to incubate on ice for 10 to 20 min prior to centrifugation. Centrifugation at 100,000g was performed in a Beckman L-2 ultracentrifuge at 2°C. Solutions of

---

1 Supported by the National Science Foundation, Grant PCM 78-15835, and by the Science and Education Administration of the United States Department of Agriculture under grant number 5901-0410-8-0170-0 from the Competitive Grants Office.
surfactants were prepared on a per cent (w/v) basis in a buffer consisting of 6.2 mm Tris and 48 mm glycine (pH approximately 8.3). Solutions of nonionic surfactants were always freshly prepared to minimize the production of any oxidizing agents (6).

The concentration of Chl in samples extracted in 80% (v/v) acetone was determined spectrophotometrically using Arnon's equations (2). All absorption spectra were taken with an Aminco DW-2 spectrophotometer using a bandpass of 1.5 nm. Samples of treated and untreated membranes that were too concentrated to have their absorption spectrum determined directly were diluted in 6.2 mm Tris, 48 mm glycine (pH approx. 8.3). To determine the exact wavelength of the Chl red absorption maximum, the recorder scale was expanded to 1 nm/cm and the spectrum in the red region was scanned at a speed of 0.2 nm/s.

Sorbitol, Tricine, Tris, glycine, SDS, sodium N-lauroylsarcosine, Triton X-100, Triton X-165, and cetyltrimethylammnonium bromide were purchased from Sigma. The Tris salt of dodecylsulfate was prepared by treating a 10% aqueous solution of SDS with Amberlite IR-120 (H⁺ form) and neutralizing the solution with solid Tris base. Sodium dioctylsulfosuccinate, octyl glucoside, and EDTA were purchased from Eastman Kodak Co., Calbiochem, and J. T. Baker Chemical Co., respectively. Nonidet P-40 was purchased from Particle Data Laboratories, Ltd., Elmhurst, IL. Sodium dodecylbenzene sulfonate was purchased from Research Organic/Inorganic Chemical Corp., Sun Valley, CA. The following surfactants were generously provided by the indicated manufacturers: magnesium dodecylsulfate (Sipon LM) from Alcolac, Inc., Baltimore, MD; Deriphat 160 from the Henkel Corporation, Minneapolis, MN; Surfonic N-95 from the Jefferson Chemical Co., Inc., Houston, TX; Arquad 12-50 (dodecyltrimethylammonium chloride) from Armak Industrial Chemicals Division, Chicago, IL; dodecyltrimethylammonium bromide from Bios Laboratories, Inc., New York, NY; and BTC-50 and dodecylmethyldiamine oxide from the Onyx Chemical Co., Jersey City, NJ. Deriphat 160 was neutralized to pH 8 with added Amberlite IR-120 (H⁺ form) prior to use.

RESULTS

Effect of Surfactant Concentration. Treatment of thylakoid membranes with surfactants shifts the wavelength maxima of

![Fig. 1. Treatment of tobacco thylakoid membrane fragments with various concentrations of two surfactants.](https://plantphysiol.org)

peaks in the spectral regions attributed to the absorbance of the Chl chromophore, and disrupts the membrane structure in a process which is often referred to as “solubilization.” We have examined both the absorption changes and solubilization as a function of increasing surfactant concentration. Typical data for two of the surfactants tested are shown in Figure 1. As the amount of surfactant added to a thylakoid membrane sample was increased, the amount of Chl containing material that remained in the supernatant fraction following centrifugation at 100,000 g for 1 h (i.e. amount solubilized) increased as expected. Concomitant with this increase, and in agreement with Sauer and Park (18) we observed that the red wavelength maximum shifted to shorter wavelengths. The amount of Chl containing material liberated by a given surfactant appeared to be generally correlated with the extent of the shift of the absorption maximum irrespective of whether the added surfactant was anionic, nonionic, cationic or zwitterionic; that is, the greater the amount of surfactant added, the greater the shift.

Effect of Centrifugation Time. Centrifugation of a sample of surfactant-treated thylakoid membranes for increasing lengths of time at 100,000 g yielded supernatant fractions in which the amount of Chl-containing material steadily decreased (Fig. 2B). We assume that as the centrifugation time increased, the mean size of the particles remaining in the supernatant fraction would steadily decrease. Concomitant with this is a shift toward the blue of the supernatant fraction’s Chl red wavelength maximum (Fig. 2A). The extent of the shift is greater than that observed for aliquots incubated for equal times without centrifugation (i.e. no fractionation of large and small particles). This phenomenon was observed for an anionic (sodium dodecylbenzenesulfonate), nonionic (Nonidet P-40), zwitterionic (sodium N-lauroylsarcosine) and cationic (BTC 50) surfactant, although the shift reached its maximum more rapidly in the latter case. Thus, the blue-shift of absorbance upon addition of surfactant appears to be correlated
with the degree of disruption of the thylakoid membrane induced by the surfactant.

**Effect of Surfactant’s Ionic Nature.** From the above data, it seemed that the extent of wavelength shift observed for a given amount of membrane disruption may be strongly influenced by the ionic nature of the surfactant used. To test this, aliquots of thylakoids were examined as above with arbitrary concentrations (generally around 1%) of a number of different surfactants (Fig. 3). The data demonstrate that there apparently is a correlation between the ionic class of the surfactant and the extent to which the Chl absorption peak is shifted for approximately equal amounts of material liberated from the membrane. The Chl peak is blue-shifted to a greater extent by cationic and nonionic surfactants, than by anionic surfactants. Because of differences in experimental design, the data in Figure 2 are not strictly comparable with the data presented in Figure 3.

**DISCUSSION**

Different types of biological membranes can have extremely different lipid and protein compositions, and hence properties. Therefore, a surfactant may vary in its efficacy between different membrane systems. For example, difference in bilayer fluidity has a profound effect on the ability of a surfactant to solubilize membrane components (15). Most researchers rely on generalizations when selecting which surfactants to use. Thus, it is a commonly held belief that nonionic surfactants (e.g. Triton X-100) cause less denaturation of protein components than ionic surfactants (e.g. SDS) (8). However, this dictum originated from studies on water-soluble proteins and the conclusion may not be valid for all proteins (14), particularly those in membrane systems, or for all concentrations of surfactant. For effective use of surfactants, it is important to take into consideration the specific characteristics of the membrane system being studied (e.g. lipid composition, pI, surface charge density, ionic strength and nature of ions in the system).

Regardless of the particular reason for treatment of a membrane fraction with surfactant, it is usually necessary to monitor the resulting dispersal of the constituent components. Traditionally, the extent of membrane disruption has been monitored by centrifugation. For many biochemists this has resulted in operational definitions of soluble and insoluble for the supernatant and pellet fractions, respectively, following some arbitrary centrifugation regimen (e.g. 100,000g for 1 h). The data presented in this report demonstrate a reasonable correlation between the wavelength maximum of Chl-containing material liberated from the membrane, and the amount solubilized by the above definition, as well as the sizes of the fragments resulting from surfactant treatment.

The different susceptibilities of the Chl absorption spectrum to the various ionic classes of surfactants probably represent specific interaction of the pigment-protein complexes with the surfactant molecules. The thylakoid membrane has a pI of approximately 4.3 and at physiological pH values has a net negative surface charge (13). Because not all interaction of surfactant molecules with the membrane is hydrophobic in nature (16), this negative charge will have a profound influence on the ability of surfactants to interact electrically with membrane components. Comparison of the binding of a monomeric surfactant with a charge similar to the membrane, approximated by the Langmuir-type adsorption isotherm (9), with that of a nonionic surfactant leads to the prediction that the former surfactant would be less binding; a surfactant of opposite charge would have increased binding. Interaction of the membrane with surfactant micelles would also show a charge effect due to electrical properties of the Gouy-Chapman diffuse layer and the Stern layer (3, 16). Of course this view of the situation is an oversimplification. In reality, the membrane surface charge is microheterogenous. However, the major class of proteins in the thylakoid membrane, the pigment-protein complexes, all have pH values between 4 and 5 (20), indicating that they are negatively charged at the pH values used in these experiments.

For approximately equivalent disruption of the membrane (i.e. the same percentage of solubilization), the absorption maximum is shifted less by anionic surfactants than by cationic or nonionic surfactants as expected from the foregoing discussion. The large wavelength changes caused by cationic surfactants (e.g. Fig. 2) at concentrations which do not appreciably disrupt the membrane structure (as monitored by the liberation of Chl-containing components) probably then reflect dissociation of intracomplex, in addition to intercomplex, associations of the pigment-protein holocomplexes. We tested a number of zwitterionic surfactants and found them to cause amounts of blue-shifting equal to or less than that of the nonionic surfactants (data not shown). However, these data are difficult to interpret since the net charge on some of these surfactants was not neutral due either to the pH used (e.g. N-lauroylsarcosine) or to the unequal numbers of anionic and cationic groups in their structures (e.g. Deriphat 160).

Our observations could have an alternative explanation: the same data would be obtained if solubilized PSII components are inherently smaller than the longer wave-length-absorbing components of PSI. We feel that this alternate explanation is unlikely for several reasons: First, the relationship between wavelength shift and extent of membrane disruption was observed to take place for all surfactants examined. This was the case even for

---

**Fig. 3.** Treatment of tobacco thylakoid membranes with arbitrary concentrations of a number of surfactants. Percent of Chl in the supernatant fraction following centrifugation at 100,000g for 1 h is plotted versus the red wavelength of maximum absorbance in this fraction for each surfactant. A, Anionic surfactants: (1), 0.9% Mg dodecylsulfate; (2), 1.5% Na dodecylsulfate; (3), 1.5% Tris dodecylsulfate; (4), 1.5% Na dodecylbenzenesulfonate; (5), 1.0% Na dioctylsulfosuccinate. B, Cationic surfactants: (1), 1.5% dodecyltrimethylammonium bromide; (2), 1.5% Arquad 12-50; (3), 1.3% sodium dodecyltrimethylammonium bromide; (4), 1.0% BTX 50; (5), 0.75% octyltrimethylammonium bromide. C, Nonionic surfactants: (1), 1.0% Surfonic N-95; (2), 1.0% Nonidet P-40; (3), 1.5% Triton X-100; (4), 1.0% octylglucoside; (5), 1.0% Triton X-165.
those surfactants of low or moderate effectiveness in which the relative amounts of PSI and PSII components liberated from the thylakoid membrane probably varied for each surfactant used. Second, particles of PSI liberated by Triton X-100 (23) or digitonin (1) are reported to be larger, not smaller, than those of PSII. Third, the wavelength of maximum absorbance for purified PSI components has been shown to be blue-shifted by added surfactants (5); that is, they are not inherently more resistant to wavelength shifts than other components. And fourth, it is well-documented (7, 17, 19) that individual Chl chromophores are greatly influenced by their immediate environment through the mechanism of solvation. The wavelength of maximum absorption for the spectrum of pure Chl is directly dependent on the refractive index and dielectric strength of the solution and therefore it seems reasonable to postulate that similar effects can occur within individual pigment-protein complexes when surfactant treatment produces changes in intercomplex and intracomplex structure.

One could also argue that these observed shifts in wavelength are primarily the effect of denaturation of pigment-protein complexes with the consequent production of a surfactant-Chl complex which absorbs at shorter wavelengths than Chl molecules in vivo (i.e. increased surfactant interaction produces more ‘free’ pigment). We think this explanation is also unlikely; we have previously shown (11) that treatment of thylakoid membranes with SDS at concentrations equivalent to those used here, which liberates a large percentage of the pigment-protein complexes, does not produce any significant amount of free Chl. Furthermore, Sauer and Park (18) showed that surfactant-induced shifts of the Chl a absorption peak were not correlated with either loss of the Hill reactions or increases in Chl a fluorescence. This would tend to confirm that the shift in wavelength of the Chl a red absorption peak is reflective of general changes in the photosynthetic membrane as a whole, rather than indicative of the state of one or a few specific components.

Researchers must, however, be aware that specific results of the type presented in this paper are valid only under the conditions employed for the studies. Changes in ionic strength, pH, protein concentration, etc. from those employed in the initial studies may markedly affect the ability of a given surfactant to liberate individual membrane components. Furthermore, photosynthetic membranes from different species may not be strictly comparable due to differences in the specific protein and lipid components present, or to the ratio of these two classes of components. While an awareness of the effect of surfactants on the Chl a absorption spectrum will not obviate the need for an empirical approach to the use of surfactants in studies on the photosynthetic membrane, we suggest that it can provide a rapid and convenient criterion for evaluating a number of these compounds.

Acknowledgments—We are grateful to Dr. Kimiyuki Satoh and Dr. Merri Skrilda for assistance with some of the experiments reported in this paper. We also wish to acknowledge many stimulating discussions about this material with Dr. John Bennett.

This work would not have been possible without the surfactant samples generously donated by their manufacturers.

LITERATURE CITED

7. FRENCH CS 1971 The distribution and action in photosynthesis of several forms of chlorophyll. Proc Natl Acad Sci USA 68: 2895–2897
8. HELENIUS A, K SIMONS 1975 Solubilization of membranes by detergents. Biochim Biophys Acta 415: 29–79
11. MARKWELL JP, JP THORNBER, RT BOGGS 1979 Evidence that in higher plant chloroplasts all the chlorophyll exists as chlorophyll-protein complexes. Proc Natl Acad Sci USA 76: 1233–1235
14. NIELSON CA 1971 The binding of detergents to proteins. I. The maximum amount of dodecyl sulfate bound to proteins and the resistance to binding of several proteins. J Biol Chem 246: 3895–3901