SOLUBILIZATION OF CHLOROPLAST MEMBRANES BY ZWITTERIONIC DETERGENTS

Effect on photosystem II activity

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1. Introduction

Much of our knowledge of the function of photochemical reaction centers in plants and bacteria has come from biochemical and biophysical examinations of purified fractions containing the photochemically active pigments. For plants much is known about the photosystem I reaction center, but little unequivocal data are available on the photosystem II component largely because of a lack of a highly enriched and active reaction center preparation; much of what is already known has been derived from indirect examination. A number of laboratories have put much effort into solubilizing photosystem II from thylakoid membranes in an active form; disruption of membranes by sonication [1] or by passage through a French pressure cell [2] as well as solubilization with detergents have been used in an attempt to achieve this goal. By far the greatest success in preserving photosystem II activity on solubilization has been with the nonionic detergents digitonin [3–8] and Triton X-100 [8–11]. Although a number of other detergents have been tested [8,10] they have been less successful at solubilizing photosystem II in a photochemically active form. This report describes the use of two previously unexamined zwitterionic detergents, Deriphat 160 and Miranol S2M-SF, for solubilization of higher plant chloroplast membranes. The solubilized material contained photosystem II activity as measured by reduction of a Hill electron acceptor by photosystem II electron donors and by the presence of chlorophyll variable fluorescence.

Abbreviations: DPC, 1,5-diphenylcarbazide; DCIP, 2,6-dichloroindophenol; LDAO, lauryldimethylamine oxide; TES, *N*-tris-(hydroxymethyl) methyl-2-aminoethanesulfonic acid; SDS, sodium dodecylsulfate; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethyl urea

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2. Materials and methods

Leaf material was collected from 10 day old seedlings of maize (*Zea mays* L.) and barley (*Hordeum vulgaris* L.). Young leaves were collected from tobacco (*Nicotiana tabacum* L.). All plants were grown in a greenhouse with available light.

The preparation of washed thylakoid membranes was as in [12]. A solution containing 6.2 mM Tris, 48 mM glycine (pH 8.3) and 1% (w/v) detergent was added to the pelleted membranes to give a ratio of detergent to chlorophyll (w/w) of 10:1. Following homogenization the mixtures were centrifuged at 40 000 × *g* for 10 min. The green supernatant fractions were decanted for analysis. Less than 1% of the chlorophyll remained in the supernatant fraction if the membranes were not treated with detergent.

The concentration of chlorophyll and the chlorophyll *a*/*b* ratios in samples extracted with 80% (v/v) acetone were determined spectrophotometrically [13]. All absorption spectra were recorded on an Aminco DW-2 spectrophotometer.

The photochemical activity of photosystem II was assayed by monitoring the reduction of DCIP at 590 nm. Actinic light was supplied at a 90° angle to the measuring beam with a Bausch and Lomb Xenon

lamp and high intensity monochrometer equipped with a manual shutter. Light at 430 nm was passed through Corning 7-59 blue glass. The reference cuvette was shielded from actinic light, and the photomultiplier tube was covered with Corning 4-69 and 2-73 filters which allowed the 590 nm measuring beam to pass, but blocked the actinic light and chlorophyll fluorescence. Sampling containing 10–20 μg chlorophyll were assayed in a 1.0 ml reaction mixture of: 100 mM sucrose, 30 mM TES (pH 7.2), 25 mM hydroxylamine, 10 mM NaCl, 5 mM $MgSO_4$, and 25 μM DCIP. In some experiments DPC (5 mM) was used as a photosystem II electron donor in place of hydroxylamine. Rates of electron transfer in the dark were subtracted from the rates of the illuminated samples.

The % of variable chlorophyll fluorescence of solubilized membranes was also used to measure photosystem II activity. Chlorophyll (10–20 μg) was suspended in 2.5 ml containing 6.2 mM Tris (pH 8.3), 48 mM glycine, 200 μM DPC and 36 μM $K_3Fe(CN)_6$. After dark adaptation, the solution was excited with light from a 6.5 V, 2.75 A lamp passed through a photographic shutter and a Corning 5-57 filter. Fluorescent emission was measured at 90° to the actinic beam through a Corning 2-64 filter with an EMI 9558 photomultiplier. A Pacific Photometric Instrument's high-voltage power supply and picoammeter was used. The yield of fluorescence was recorded over ~20 s on a Tekronix 214 storage oscilloscope.

SDS and *N*-lauroyl sarcosine were purchased from Sigma Chemical Co., St Louis, MO. The following were generous gifts: LDAO from Onyx Chemical Co., Jersey City, NJ; Deriphat 160 from the Chemical Division of General Mills, Kankakee, IL; Miranol S2M-SF from the Miranol Chemical Co., Irvington, NJ.

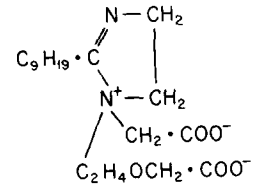
3. Results

A variety of detergents were tested to identify those which would solubilize the maximum amount of chlorophyll-containing material from thylakoid membranes with the minimum alteration of native associations of pigments and proteins (table 1). A decrease in the red wavelength maximum of in vivo chlorophyll can be attributed to an alteration in the environment of some or all of the chlorophyll molecules (cf. [14–16]). The material solubilized by the two zwitterionic detergents, Miranol S2M-SF and Deriphat 160 (fig.1), have red absorption maxima which are shifted very little from that of the untreated membranes, but % solubilization is slightly less than with the other detergents. SDS and LDAO cause the largest shift in the red peak (6 and 9 nm) but extract the largest % of the chlorophyll. *N*-Lauroyl sarcosine extracts an intermediate amount of pigmented material and causes an intermediate spectral shift.

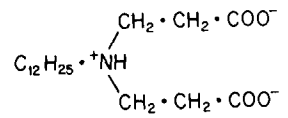
Table 1
Properties of soluble fractions obtained after treatment of tobacco chloroplasts with various detergents

Treatment	% Chlorophyll solubilized	Red absorption maximum (nm)
Unfractionated	–	678
SDS	98	672
Miranol S2M-SF	72	677
Deriphat 160	84	676
N-Lauroyl sarcosine	92	675.5
LDAO	98	669

Detergent extracts were assayed for photosystem II activity. Measurement with DCIP as electron acceptor and hydroxylamine as donor showed that Miranol S2M-SF and Deriphat 160 extracts retained the photochemical activity (table 2). The reduction of DCIP was dependent upon light intensity and chlorophyll concentration in the different species tested. DPC could replace hydroxylamine as donor although a



MIRANOL S2M-SF



DERIPHAT 160

Fig.1. Chemical structures of Deriphat 160 and Miranol S2M-SF.

Table 2
Photosystem II electron transport of chloroplast detergent extracts measured using hydroxylamine as electron donor

	μmol DCIP reduced/ h/mg chlorophyll		
	Tobacco	Barley	Maize
Untreated thylakoids	1270	480	1200
SDS extracts	0	0	0
Miranol S2M-SF extract	870	280	650
Deriphat 160 extract	730	180	1040

lower rate of DCIP reduction occurred in both untreated chloroplasts and in the extracts. With either electron donor, the reaction was quantitatively (>99%) inhibited by the presence of 10 μM DCMU or by heat treatment (100°C, 30 s) of the pigmented extracts. SDS extracts have no photochemical activity (table 2) and similar results were obtained (not presented) for LDAO and lauroyl sarcosine. Additionally, if equal aliquots of Deriphat 160 or Miranol S2M-SF extracts were mixed with an SDS extract, no photosystem II activity could be measured.

A second criterion used to substantiate the presence of photosystem II reaction centers in the extract was the preservation of time-dependent fluorescence induction [17]. The IDP changes of the slow (seconds) phase were monitored [18]. The variable fluorescent component of unextracted thylakoids from the three higher plant species was 50–90% of the invariable component. The variable component in the Deriphat 160 and Miranol S2M-SF extracts had 62–130% of the intensity observed in the unextracted thylakoids; no variable component was observed in the SDS extracts. As observed in [18,19] addition of 10 μM DCMU eliminated the variable fluorescent component.

The solubilized photosystem II was relatively stable at 4°C, losing ~25% of the initial activity in 24 h; approximately as much as unextracted thylakoid membranes stored similarly.

4. Discussion

In order to analyze the pigments and proteins of photosystem II, especially those associated with the reaction center, it is necessary to first solubilize the photosynthetic membranes. Once solubilized, it is possible to further fractionate and purify the required components. An additional requirement is that this be accomplished without eliminating photosystem II activity. Prior to this report, some success had been achieved by using either Triton X-100 or digitonin. Now, two zwitterionic detergents (Miranol S2M-SF and Deriphat 160) have also been found to be successful; no attempt was made to optimize the conditions for preserving the photochemical activity of the extracts.

These two detergents are preferable to Triton X-100 which causes a larger shift in the red absorption maximum of extracted material indicating that it has a deleterious effect on the longer wavelength forms of chlorophyll in the photochemical apparatus [5]. A special advantage of the two zwitterionic detergents over digitonin is the considerable cost of the latter. A further general advantage of ionic detergents is that they can be more easily removed from detergent–protein complexes than nonionic detergents [20]. It should be pointed out that not all zwitterionic detergents were useful; lauroyl sarcosine is zwitterionic but proved ineffective for the stated goal. We have shown here that Miranol S2M-SF and Deriphat 160 are useful additional tools to those already available to solubilize higher plant photosynthetic membranes without loss of photosystem II activity. We anticipate that fractionation of these zwitterionic detergent-extracts will lead to a better understanding of the biochemical composition and processes involved in the function of this photochemical system.

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